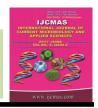


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### **Original Research Article**

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## Effect of UV- C Light Treatment on Physicochemical and Bioactive Compounds in Apple and Pineapple Juices

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#### ABSTRACT

### Keywords

Ultraviolet treatment, Apple juice, Pineapple Juice, pH, TSS, Vit-C.

#### **Article Info**

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The effect of ultraviolet (UV-C) on physicochemical (viz. pH, total soluble solids (TSS), Absorbance, total color difference), bioactive component (viz. ascorbic acid, total phenolic content, antioxidant activity) of apple and pineapple were studied during this research work. The process conditions for ultraviolet treatment were treatment time (5, 10, 15 min) and distance of sample from lamp source (8.6, 13.7, 18.6 and 22.8 cm) at 1 mm sample thickness. The results obtained from this study showed that the ultraviolet treatment (UV) doesn't have any significant effect on pH, TSS of apple and pineapple juices. However, the UV treatment conditions had significant effect on vitamin-C content. The color parameters (viz. L\*, a\* and b\*) were slightly affected by ultraviolet treatment. The bioactive components of apple and pineapple juices were affected by the treatment conditions. The Antioxidant activity of both the juices showed a decreasing trend with respect to an increase in dosage level. The obtained results suggested that, ultraviolet treatment conditions slightly affect the quality parameters of apple and pineapple juices. However, the changes were found to be minimum as that of thermal treatments in literature.

### Introduction

Fruit juices are good sources of vitamins and minerals and are associated with many health benefits. The major component of the fruit juice is water. The other most common constituent is carbohydrates which comprise sucrose, fructose, glucose and sorbitol. Also, limited amount of protein and minerals are found in fruit juices. However fruit juice contains no fat or cholesterol. It was believed that fruit juices are safe from contamination due to their acidity however fruit juices can

support the growth of several types of microorganisms, such as bacteria, yeasts and molds that are primarily responsible for causing the spoilage of these products.

The contamination and growth of pathogenic bacteria such as *Escherichia coli* (*E. coli*) O157:H7 in acidic food products, fruit juices and fruit-based drinks has caused great concern. Several outbreaks of illness caused by the consumption of fruits or fruit juices

contaminated with *Salmonella*, *E. coli* O157:H7 and *Cryptosporidium* have been reported throughout the world.

Conventional thermal pasteurization is the most common technique employed for pasteurization of fruit juices. It is the best known technique in order to reduce the number of pathogenic organisms like *E. coli* O157:H7, *Cryptosporidium parvum* in various types of juices (Tandon *et al.*, 2003). The thermal pasteurization of apple juice and pineapple juice involves 80-82°C for 2-3 seconds and 90-95 °C for15-30 s respectively.

Pasteurization is an effective technology in order to reach safety requirements. However, it has many limitations like cost of the equipment is prohibitive especially for small operations, adverse effects of thermal pasteurization on the food quality and increased demand for the fresh-like juice products.

Alternative methods are addition of micro biocidal agents, high pressure application, pulsed electric field, irradiation, and aseptic packaging.

However there are some disadvantages of these techniques. Opstal *et al.*, (2006) reported the loss of ascorbic acid in the peroxidase applied fruit juices.

In another study it was shown that PEF treatment causes brightness in the color of orange juice (Min *et al.*, 2003) and its application to industry is limited due to its high cost. Also, as light browning in the color of white grape juice was observed after the high pressure treatment (Daoudi *et al.*, 2002). Considering these limitations of other techniques, UV-C radiation can be used as an alternative to other preservation techniques. This process does not produce chemical residues (Canitez, 2002). Besides, it is a low-

cost operation and effective against many microorganisms (Bintsis *et al.*, 2000). UV-light is the part of electromagnetic spectrum with wavelengths ranging from100-400nm. UV-light is traditionally subdivided into the following categories: UV-A range from315-400nm and this range is responsible for Changes in human skin.

UV-B range from 280 to 315nm and can cause skin burning and has the potential to cause skin cancer.

UV-C ranges from 200 to 280nm and is very well known for its antimicrobial effect. It used for the decontamination of medical equipment, water treatments, drinking water, water for swimming pools, and surface disinfection of different fruits and other processing equipment. Application of UV light on various liquid foods like apple cider, orange juice, grape juice, milk and honey have been developed recently.

UV-C radiation germicidal properties are due to the DNA absorption of the UV-light which causes crosslinking between the neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand (Miller *et al.*, 1999).

Due to this, the DNA transcription and replication is blocked, which compromises cellular functions and leads to cell death (Miller *et al.*, 1999).

Application of UV light in liquid food products has shown positive results. Different modes of UV treatments can be applied to food products such as continuous mode, pulsed mode.

The present experiment was aimed to study the effect of ultraviolet treatment on physicochemical and bioactive compounds of apple and pineapple juices.

#### **Materials and Methods**

### Sample preparation

### Apple juice

Fresh apples of approximately same size were purchased from local market IIT Kharagpur. Surface of apples was properly cleaned with distilled water followed by 1% sodium hypochlorite sanitize solution (Walter et al., 2009). Since sodium hypochlorite is harmful for health, hence the apples again properly cleaned with distilled water. After cleaning apples, seeds were removed and pulp portion was sliced and separated. The pulp was then mixed thoroughly to remove any lumps. Cut pieces were put in fruit juicer (maximum RPM: 6000) for 5 minutes and homogenized in dispenser (3000 RPM) for 4-5 minutes. This was queezed for apple juice using a filter paper. The samples were then immediately stored and frozen at-25°C and were thawed to room temperature before treatment.

### Pineapple juice

Whole Pineapples were procured from local market at IIT Kharagpur. Pineapples were washed with distilled water followed by NaOCl solution (5 ppm). Skin, eyes, core was removed and cut into small pieces. The small pieces were put in fruit Juicer (maximum RPM: 6000) for 5 minutes, and homogenize in dispenser (3000 RPM) for 4-5 minutes. This puree was squeezed for clear juice using a filter paper. The samples were then immediately stored and frozen at -25 °C and were thawed to room temperature before treatment.

### **Chemicals and reagents**

All the chemicals and reagents used in the study were analytical grade and procured from Merck, India and Sigma-Aldrich, Germany.

## Ultraviolet treatment of apple and pineapple juices

Apple and pineapple juices were processed using batch type UV-C apparatus designed and fabricated (REF) at IIT Kharagpur. The system was designed such a way that, the distance of sample from lamp source can be varied. An 18 W low pressure mercury vapor UV lamp which emits the UV-C light continuously in the wave length ranges from 200-300 nm were mounted at the top of treatment chamber. Time of UV-C exposure was controlled using a manually operated control switch.

Measured quantity of apple and pineapple juices were poured in 100 mm standard size petri plates and placed at the center of holder platform (used for maintain different distances between sample and lamp source). 1 mm sample thickness was maintained throughout the experiment. To prevent the exposure of UV light to human skin, a cover was placed in front of the system.

### **Experimental design**

Full factorial design was used in this experiment with two independent variables (viz. treatment time and distance of sample from lamp source) of 3 and levels respectively. Responses such as pH, TSS, Absorbance. Color, Vit-C, Antioxidant activity and Total phenolic content were measured before and after the experiment. Analysis of variance (ANOVA) test was conducted using Design expert version 7.0.0 software (State-Ease Inc., Minneapolis, USA) to evaluate the significance (at 95% confidence level) of the effect of independent variables and their interactions on the responses.

## Measurement of physicochemical properties of apple and pineapple juices

## **Determination of pH**

Measurement of pH values of the samples were carried out by using a bench top pH meter (make: Toshiba, model: CL-46 Plus) at room temperature.

### **Determination of TSS (Total soluble solids)**

A handheld Refracto meter was used to determine the brix levels of the juice samples.

A few drops of the sample were put into the reading cell and the results at 20 °C were recorded.

## Determination of absorbance of juice samples

Absorbance values of juice samples were determined using Spectrophotometer (Model: UV-1700 Pharma, Make: Shimadzu, Japan) at 254nm. Different dilution factors were applied (1:10, 1:25, 1:50, 1:100, 1:250, 1:500 and 1:1000). Absorbance coefficient was estimated from the slope of absorbance versus sample concentration plot.

### Color measurements of juice samples

Color parameters of juice samples were detected employing a portable colorimeter (Make: BYK Gardener, Germany; Model: Spectro-guide 45/0gloss). CIE tristimulus L\* (brightness-darkness), a\* (redness-greenness), and b\* (yellowness-blueness) values were obtained before and after the UV exposure. Also total color difference (ΔE) was calculated according to the following formula (1).

$$\Delta E^* = \sqrt{(L_0^* - L_1^*)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2}.....(1)$$

# Measurement of bioactive components of apple and pineapple juices

#### **Measurement of vitamin- C content**

Vitamin – C content was measured by 2, 6-dichlorophenolindophenol method. A blue substance called 2, 6-dichlorophenolindophenol (or DCPIP for short) acts as an indicator. It changes from blue to red with acids but loses its colour in the presence of certain chemicals, one of which is ascorbic acid (vitamin C). DCPIP solution was used to estimate the amount of vitamin C in foods.

A sample of 105mg of Na<sub>2</sub>CO<sub>3</sub> was mixed with 500 ml of hot glass distilled water using a magnetic stirrer. 125mg of DCPIP was mixed with this solution and stirring was done until the solution came to room temperature. Dye solution was then filtered and stored at 4 °C. Also, Dye factor was found by titration.

A 500ml solution of HPO<sub>3</sub> was prepared and this was used for dilutions of ascorbic acid solution. A 100 ml solution of ascorbic acid (100 mg/100ml) was prepared and was diluted 10 times using HPO<sub>3</sub> solution. This was used as the working solution. 2 ml of juice sample was mixed with 18 ml of HPO<sub>3</sub> and was titrated against the dye solution.

### Measurement of anti-oxidant capacity

The phenolic compounds present in sample were solubilized in 80% v/v ethanol made in ultra-pure water. A five gram sample was mixed with 20 mL of solvent and kept in a shaker for 3 h at 25 °C.

The supernatant obtained after centrifugation at 12,000 rpm at 4 °C was considered as the extract for measuring both total phenolic content (TPC) and antioxidant capacity.

DPPH solution made was using approximately 15 mg DPPH in 100 ml methanol. Its absorbance at 517 nm was adjusted to unity and it was kept in the dark at -20 °C till further use. The change in color of the DPPH solution from purple to yellow, resulting from the addition of different quantities of ethanolic extract of juices or gallic acid (GA) standard (20 to 200 µl) was measured at 517 nm after allowing the solution to stand in the dark for 20 min. The decrease in absorbance of DPPH after 20 min was calculated and expressed as mg of GA equivalents antioxidant capacity (GAEAC) per 100 g.

## **Measurement of Total phenolic content** (TPC)

TPC in the sample extract was determined by the Folin-Ciocalteu method. Equal volume of the sample extract and Folin-Ciocalteu reagent (FCR) (0.25 mL each) was mixed with 750 µL of 20% sodium carbonate solution and it was diluted to 5 mL by double distilled water followed by a vortex. After incubation at 25 °C in the dark for 1.5 h, the absorbance of blue color was measured at 750 nm in a UV-VIS spectrophotometer (Model: UV-1700 Pharma Spec, Make: Shimadzu, Japan). The comparison was made with a calibrated curve made of different gallic acid (Sigma-Aldrich, Germany) concentrations values and TPC were expressed as gallic acid equivalent (GAE) per 100 g sample.

### **Results and Discussion**

# Physicochemical and bioactive components of raw samples

The physicochemical properties of raw apple and pineapple juices were measured with three replications and the results were presented in table 1. From obtained results, we can be observed that the pH values for apple juice is not very low indicating that the juice is less acidic compared to pineapple juice. The absorbance values indicate that the penetration of UV-light in apple juice is higher than that in pineapple juice. The Vitamin-C contents indicate that it is of significance in pineapple juice while it is not of much importance in apple juice.

### Effect of UV-C treatment on pH

The pH of the samples after treatments at different dosage levels is represented in the table 2. It can be inferred from the results that there is no impact on the pH levels of both apple juice and pineapple juice for any of the treatments.

#### Effect of UV-C treatment on TSS

The TSS of the samples after treatments at different dosage levels is represented in the table 3. It can be inferred from the results that there is no impact on the TSS levels of both apple juice and pineapple juice for any of the treatments.

#### Effect of UV-C treatment on color

Effect of UV-C on color components of apple is presented in fig.1, 2, and 3. The obtained results showed that, there is no significant effect of UV-C treatment on a\* values of apple. However, there is significant decrease in L\*, b\* values with increasing in UV dosage. Further, the total color difference of pineapple juice during UV - C treatment is presented in figure 4. From the results we can observed that, there is very little colour deviation (<  $2.5 \Delta E^*$ ) in any of the treatments. However at higher dosages the colour has changed considerably compared to the lower dosages. This can be observed from the fact that the treatments at the closer distances to the lamp and the higher time of exposure have impacted the colour significantly compared to other treatments.

## Effect of UV-C treatment on the vitamin-C content

The effect of UV treatment on Vitamin-C content of pineapple juices has been represented in the Figure 5. The obtained results showed that, the Vitamin-C content of apple and pineapple juices were significantly affected by treatment conditions. It is inferred that the Vitamin-C content is dependent on the UV-C dosage rather than the distance from lamp source. Vitamin C is a light sensitive Vitamin degrades in presence of UV light. It is inferred that the Vitamin-C content is dependent on the UV-C dosage rather than the distance from lamp source. There was a reduction of up to 18% at the higher dosage levels while the lower dosages had minimal or no effect on the Vitamin C content of the juice. A test runs of 30 minutes exposure at the minimum distance from lamp source (8.64 cm) resultedina40% reduction of Vitamin-C This experimentation was not conducted for apple juice as it was found that the Vit-C content of the apples juice made was insignificant (1 mg/100 ml).

## Effect of UV-C treatment on the antioxidant capacity

The impact on antioxidant activity at various levels of treatment for apple and pineapple juice is represented in Fig. 6 and Fig. 7. respectively. The antioxidant activity of UV treated apple juice was lower than the control sample but with no significant difference. A decreasing trend was observed increasing dosage levels. At the highest dosage applied there was a decrease of only 8% in AAC in apple juice. The reason for this decrease in AAC could be the light sensitive anti-oxidant components like ascorbic acid, carotenoids, phenols etc present in the pineapple juice which can easily undergo photolysis in the presence of UV light. The same decreasing trend was observed in pineapple juice.

**Table.1** Physico chemical properties of control samples

Property	Apple	Pineapple
pН	4.28 ± 0.02 @ 26.7 °C	$3.46 \pm 0.02$ @ $26$ $^{0}$ C
TSS	$14.3 \pm 0.01^{\circ} Brix$	$11.3 \pm 0.1$ <sup>0</sup> Brix @ 26 <sup>0</sup> C
Color	$L^* = 17.82 \ a^* = 6.41$ $b^* = 16.64$	$L^* = 30.48 \ a^* = -1.41$ $b^* = 16.00$
Titrable Acidity	$0.42\pm0.04$	$0.58 \pm 0.04$
Total Phenolic Content (mg GAE/100 ml)	$48.68 \pm 2.1$	29.6 ± 1.7
Antioxidant Activity (mg GAE/100 ml)	$21.3 \pm 0.8$	$14.6 \pm 1.1$
Vitamin C (mg/100 ml)	1.5 mg/ 100 ml	16.9 mg/100 ml

Values are presented as mean  $\pm$  standard deviation (n = 4)

Table.2 Effect on pH on application UV Dosage at various levels of treatments

Distance	Exposure time	Apple Juice pH	Pineapple juice pH
(cm)	(min)		
8.6	5	$4.32 \pm 0.01$	$3.44 \pm 0.01$
8.6	10	$4.32 \pm 0.01$	$3.45 \pm 0.01$
8.6	15	$4.32 \pm 0.01$	$3.44 \pm 0.01$
13.7	5	$4.29 \pm 0.01$	$3.42 \pm 0.01$
13.7	10	$4.29 \pm 0.01$	3.43 +/- 0.01
13.7	15	$4.29 \pm 0.01$	$3.45 \pm 0.01$
18.6	5	$4.29 \pm 0.01$	$3.43 \pm 0.01$
18.6	10	$4.30 \pm 0.01$	$3.43 \pm 0.01$
18.6	15	4. $30 \pm 0.01$	$3.43 \pm 0.01$
22.8	5	$4.30 \pm 0.01$	$3.43 \pm -0.01$
22.8	10	$4.29 \pm 0.01$	$3.44 \pm 0.01$
22.8	15	$4.29 \pm 0.01$	$3.43 \pm 0.01$

Values are presented as mean  $\pm$  standard deviation (n = 4)

Table.3 Effect of TSS over application of UV Dosage at various treatment conditions

Distance	Exposure time	Apple Juice TSS ( <sup>o</sup> Brix) Pineapple juice TSS	
(cm)	(min)		(°Brix)
8.6	5	$14.3 \pm 0.01$	$11.3 \pm 0.1$
8.6	10	$14.3 \pm 0.01$	$11.2 \pm 0.1$
8.6	15	$14.5 \pm 0.01$	$11.5 \pm 0.1$
13.7	5	$14.3 \pm 0.01$	$11.3 \pm 0.1$
13.7	10	$14.3 \pm 0.01$	$11.4 \pm 0.1$
13.7	15	$14.4 \pm 0.01$	$11.4 \pm 0.1$
18.6	5	$14.3 \pm 0.01$	$11.3 \pm 0.1$
18.6	10	$14.3 \pm 0.01$	$11.3 \pm 0.1$
18.6	15	$14.4 \pm 0.01$	$11.4 \pm 0.1$
22.8	5	$14.3 \pm 0.01$	$11.3 \pm 0.1$
22.8	10	$14.3 \pm 0.01$	$11.3 \pm 0.1$
22.8	15	$14.4 \pm 0.01$	$11.4 \pm 0.1$

Values are presented as mean  $\pm$  standard deviation (n = 4)

Fig.1 Effect of UV-C radiation on L\* values of apple juice

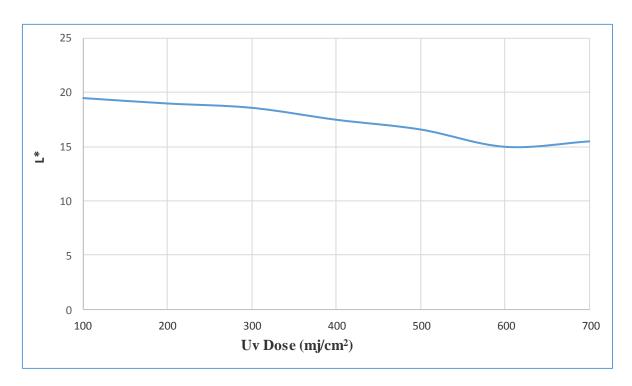


Fig.2 Effect of UV-C radiation on a\* values of apple juice

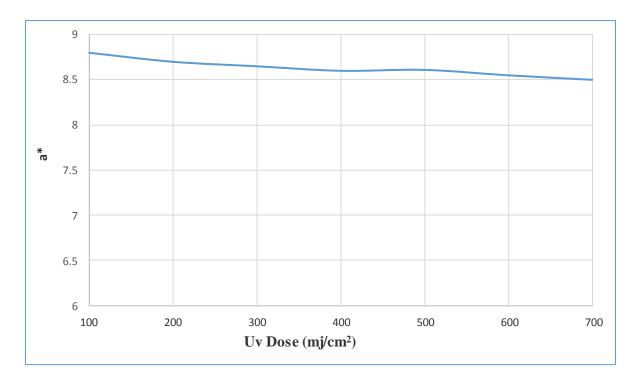


Fig.3 Effect of UV-C radiation on b\* values of apple juice

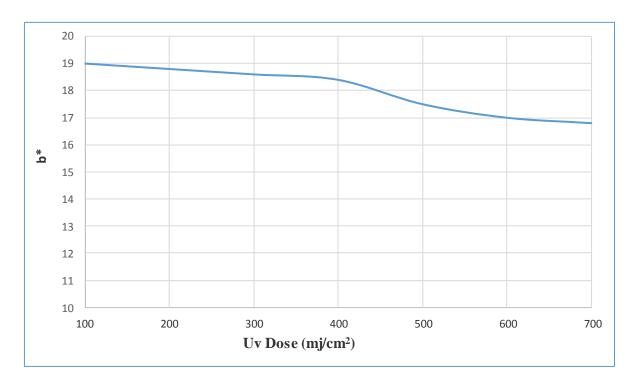


Fig.4 Impact of UV Dosage on colour of Pineapple juice

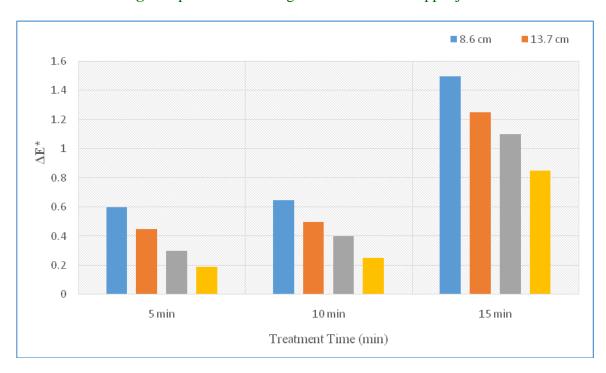


Fig.5 Impact of UV Dosage on the Vitamin-C content of pineapple juice

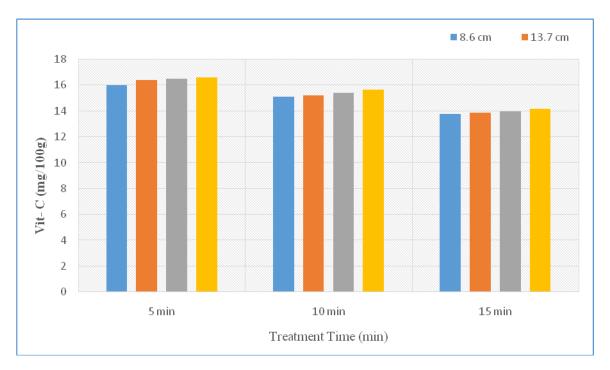


Fig.6 Impact of UV treatment on AAC of apple juice

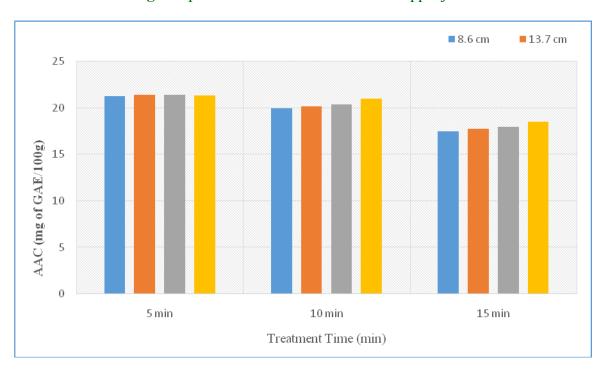


Fig.7 Impact of UV treatment on AAC of pineapple juice

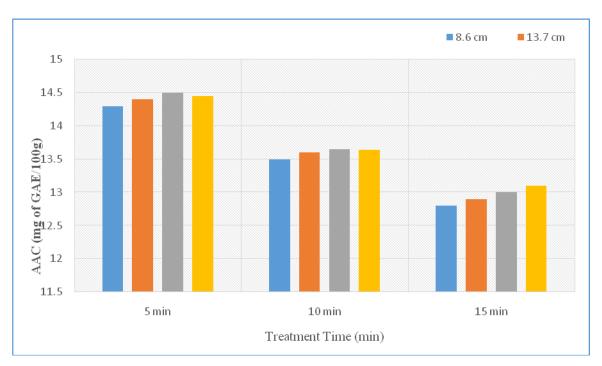
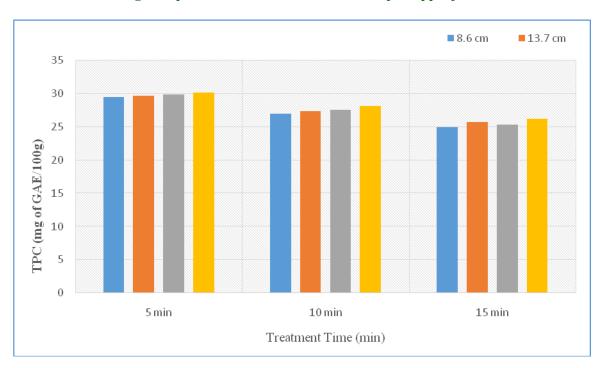


Fig.8 Impact of UV treatment on TPC of pineapple juice



# Effect of UV-C treatment on the total phenolic content

The effect of treatment conditions on TPC on pineapple juice has been represented in the Fig 8.

The total phenolic content of UV treated samples have shown an overall decreasing trend with increasing UV dosage but not significant as compared to the control sample. At the highest dosage applied there was a decrease of only 8% in TPC.

Effect of UV-C treatment on apple and pineapple juices was studied during this research work. The results obtained from this study showed that the ultraviolet treatment (UV) doesn't have any significant effect on pH, TSS of apple and pineapple juices. However, the UV treatment conditions had significant effect on vitamin-C content. The color parameters (viz. L\*, a\* and b\*) were slightly affected by ultraviolet treatment. The bioactive components of apple and pineapple juices were affected by the treatment conditions.

The Antioxidant activity of both the juices showed a decreasing trend with respect to an increase in dosage level. The obtained results suggested that. ultraviolet treatment conditions slightly affect the quality parameters of apple and pineapple juices. However, the changes were found to be minimum as that of thermal treatments in literature. Hence we can conclude that, the UV-C treatment is effective method to processing apple and pineapple juices.

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