

#### Journal

J. Biol. Chem. Environ. Sci., 2018 Vol. 13(3): 21-43 http://biochemv.sci.eg

# EFFECT OF ULTRA VIOLET (UV-C) AND COLD STORAGE ON APPLE JUICE QUALITY

Bazaraa<sup>\*1</sup>, W. A.; Shahinaz<sup>1</sup>, A. Helmy; H. A. Eissa<sup>2</sup> and R. M. Abd-Elhafez<sup>2</sup>

<sup>1</sup>Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt <sup>2</sup>Food Technology Department, National Research Centre, Cairo, Egypt.

#### **ABSTRACT**

The effect of the UV-C treatment on physico-chemical characteristics (pH, titratable acidity, TSS, color and ascorbic acid content), polyphenol oxidase activity (PPO), as well as microbial count of apple juice, compared to fresh juice was studied. The juice samples were irradiated using UV-C at 254 nm for different exposure times (15, 30, 45 and 60 min) and stored at  $4\pm1$  °C for 30 days. Treatment didn't significantly affect pH values, titratable acidity and TSS (%) in both fresh and stored samples. A negative correlation between exposure time and L\* values was noted for fresh and stored samples. On the other contrary, positive relations were observed with a\* and b\* values. Ascorbic acid content decreased as a result of increasing exposure time where it was completely lost after 5 days for samples treated for 30, 45 and 60 min., 10 days for samples treated for 15 min. and 15 days for control one. PPO was partially deactivated by either treatments (76.8 % loss of its initial activity after 60 min. of UV exposure) or during storage (at slower rate). Total bacterial count as well as yeast and mold numbers were negatively affected by increasing the UV exposure time. It was obvious, the higher UV resistance of yeasts and molds than bacteria. The sensory characteristics, i.e. odor, color, taste, consistency and overall acceptability didn't change as a result of UV treatment up to 30 min. and significantly decreased thereafter.

**Key words:** apple juice, cold storage, polyphenol oxidase, sensory characteristics.UV-C.

#### INTRODUCTION

Fruit juices are important trade commodities in most countries (Vasavada 2003). Fruit juices provide nutrients that are beneficial for human health and are in high and continually increasing demand (Ashurst 2005). Apple juice is consumed by people around the world, mainly in single-strength, reconstituted or concentrated form, in blends for new flavor, in beverages as well as other products (Noci et al. 2008). Generally, the shelf life of fresh apple juice is restricted by enzymes and microbial activities. Spoilage of fruit and vegetable juices is mainly due to the presence of osmophillic microflora (Tahiri et al. 2006). These microflora (mostly yeasts) cause fermentation and produce a buttermilk-like off-flavour (Tournas et al. 2006). Presently, thermal pasteurization is considered the most effective technology in inactivating microorganisms and enzymes to extend product's shelf life (Noci et al. 2008). However, the used temperature can affect the overall quality of juice by changing its nutritional and biochemical properties (Sanchez-Vega et al. 2009). In response to this limitation, ultraviolet (UV) irradiation, which is a non-thermal technology, was introduced to overcome the problems associated with heating and to obtain a product that has similar quality attributes to the fresh juice (Mertens and Knorr1992).

Ultraviolet (UV) radiation covers a small part of the electromagnetic spectrum, which also includes radio microwaves, infrared radiation, visible light, X- rays and  $\gamma$ - radiation (**Diffey 2002**). Ultraviolet radiation involves the use of radiation from the electromagnetic spectrum from 100 to 400 nm. The UV radiation is categorized to three areas: UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm) as mentioned by Guerrero-Beltrán and Barbosa-Cánovas (2004). UV radiation technology has been utilized in food industry for decades to disinfect water and effectively destroy microorganisms on surfaces using 254 nm at low temperature (Bintsis et al. 2000). The other advantages associated with UV-C treatment are: no known toxic by-products are formed during the treatment, certain organic contaminants may be removed, no off flavor or off odor is formed and it requires very little energy compared to thermal pasteurization processes (Koutchma et al. 2009).

Shortwave UV irradiation (200-280 nm) induces the crosslinking of neighboring pyrimidine nucleoside bases in the same DNA strand,

blocking DNA transcription and replication and eventually causing cell death (Guerrero-Beltrán and Barbosa-Canovas 2004). In addition to microbial spoilage, reactions facilitated by inherent enzymes such as polyphenol oxidase can also adversely affect shelf life and consumer acceptance (Tomás-Barberán and Espín 2001). Polyphenol oxidase (PPO) is a copper containing enzyme, which occurs in many fruits (Falguera et al. 2012). The enzyme catalyzes the oxidation of various phenolic substrates, whose polymerization leads to the formation of undesirable brown pigments and therefore has to be inactivated by processing to enhance the shelf life of the juice (Chisari et al. 2011).

Most of the studies concerning UV irradiation of fruit juices have been carried out in order to assess its effect on microbial inactivation (**Falguera** *et al.* **2012**). Nevertheless, there are few references about the influence of UV on some enzyme activities that are important in this kind of fruit derivatives (**Jiang** *et al.* **2004**; **Gayan** *et al.* **2013**; **Falguera** *et al.* **2014**). Similarly, little is known about the storage quality of UV treated fruit juices (**Forney** *et al.*, **2004**; **Franz** *et al.* **2009**; **Koutchma** *et al.*, **2009**; **Lu** *et al.* **2010**; **Fredericks** *et al.* **2011**). Therefore, the aim of this work was to evaluate the effect of UV-C treatment at 254 nm at room temperature for different exposure times and storage (30 days at  $4 \pm 1^{\circ}$ C) on the quality of apple juice which includes microbial load, pH, TSS, titratable acidity, color characteristics, ascorbic acid content, polyphenol oxidase activity (PPO) and sensory criteria.

#### MATERIALS AND METHODS

#### 1-MATERIALS

Apple fruits (red delicious variety) at maturity stage, were purchased from local market during the fall and winter seasons of 2016 – 2017. The fruits were stored at 4°C until needed. Plate count agar (PCA), potato dextrose agar (PDA), were obtained from Sisco Research Laboratory, catechol, 2,6-dichlorophenolindophenol sodium salt, sodium hydroxide, ascorbic acid were purchased from Sigma Chemical Company (St. Louis, Missouri, USA).

#### 2-METHODS

#### a-Preparation of apple juice

Apple fruits were rinsed with tap water, sectioned to longitudinal slices and juice was extracted using a juicerator (Braun, model K700, Germany) and filtered through a stainless filter. Apple juice was collected in a beaker containing 0.5 g ascorbic acid / 1000 ml juice with stirring. The added ascorbic acid was to prevent browning (**Liao** et al. 2018).

#### b-Ultra violet (UV-C) treatment and storage conditions

The UV-C treatments were carried out in a stainless steel chamber (50 x 35 x 30 cm), equipped with one UV-C germicidal lamp (254 nm), mercury low pressure (Phillips, model T8, Holland) as shown in **Fig.(1)**. In each experiment, a 200 mL of fresh apple juice was placed in an in covered glass petri dish (27 x 2 cm) forming a film thickness of 5-7 mm under continuous stirring. Temperature was controlled at  $25 \pm 1^{0}$ C. The distance between the surface of the juice and the lamp was 7 cm and the exposure was for the periods of 15, 30, 45, and 60 min. After irradiation process, the samples were placed in sterile capped test tubes (25 mL) and stored at  $4\pm 1^{\circ}$ C. Samples were randomly withdrawn for each dose at 0, 5, 10, 15 and 30 days for analysis.

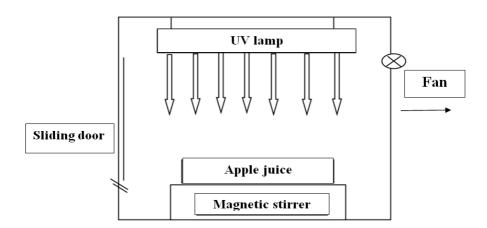


Fig. (1): Diagram of UV chamber

#### c-pH

The pH values of apple juice samples were measured using a digital pH-meter (HANNA, HI 902 meter, Germany).

#### d-Total soluble solids (°Brix)

The percent of total soluble solids (TSS), expressed as <sup>o</sup>Brix, was determined with a hand refractometer (ATAGO, Japan). Measurements were performed at 20 °C as recommended by Ranganna (1986).

#### e-Titratable acidity (TA)

Juice sample (10 ml) was titrated with 0.1 N sodium hydroxide to the end-point in the presence of phenolphthalein indicator. Results were expressed as milligrams of malic acid per 100 ml apple juice (**Tung-Sung** *et al.***1995**).

#### f-Ascorbic acid

Ascorbic acid content was determined according to **AOAC** (2005) using 2,6 dichlorophenolindophenol sodium salt by titration.

#### g-Color measurement

The color of juice samples was measured using Hunter Lab color system (Hunter, Lab Scan XE - Reston VA, USA). The instrument was calibrated using a white tile ( $L^*=92.46$ ;  $a^*=-0.86$ ;  $b^*=-0.16$ ). Color values were expressed as  $L^*$  (lightness or brightness/ darkness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness) according to Feng *et al.* (2013).

#### h-Polyphenol Oxidase (PPO) activity

One ml of apple juice (diluted when needed) was mixed with 2 ml of catechol solution (0.2 M) prepared in a phosphate buffer solution (pH 7, 0.2 M). PPO activity was determined by spectrophotometrically measuring the increase in absorbance at 420 nm at 25°C. The absorbance values were recorded every 30 s for 3 min. The enzyme activity was calculated on the basis of the slope of the linear portion of the curve plotted of A420 against time. One unit of PPO activity was defined as 0:001  $\Delta A_{420}$  / min under the assay conditions (**Ozoglu and Bayndrl 2002**).

#### i-Microbiological analysis

Total plate count as well as total yeasts and molds were determined using pour plate technique on plate count agar and potato dextrose agar, respectively (**Hatcher** *et al.* 1992). Juice samples were serially diluted using sterile saline (0.85% Nacl) and the appropriate dilution was then plated. The plates for total count were incubated for 48 h at 35 °C, whereas the plates for yeasts and molds were incubated for 5 days at 28 °C. Colonies were then counted and expressed as  $\log_{10}$  colony forming unit per milliliter of juice ( $\log_{10}$  CFU.ml<sup>-1</sup>).

#### j-Sensory analysis

Juice samples were given three digit codes and organoleptically evaluated by 10 untrained panelists from Food Technology Department, National Research Centre. All samples were evaluated for color, odor, taste, consistency and overall acceptability on a ten point's hedonic scale on which score of ten represented attributes most liked, 5 represented attributes at an unacceptable margin and 1 represented attributes most disliked. Samples (20 ml, each) were served in clear plastic cups at room temperature and compared with a fresh control. Water and neutral wafers were also served for cleaning palate between samples.

#### k-Statistical analysis

Data were statistically analyzed using one-way analysis of variance ANOVA (**Silva and Azevedo, 2009**). All data were the averages of 3 experiments unless otherwise stated.

#### RESULTS AND DISCUSSION

#### a-Effect of UV-C and cold storage on the pH of apple juice

The value of pH is one of the important quality characteristics that describe the stability of bioactive compounds in fruit juices (Sanchez-Moreno et al. 2006). Table (1) shows the effect of UV at different exposure times and storage on the pH values of apple juice. Results indicated no significant changes in pH values of the untreated juice compared to the treated one. At zero time, the pH values were 4.31, 4.29, 4.28, 4.26 and 4.20 for samples exposed for different times (0, 15, 30, 45 and 60 min., respectively). Obtained results are in agreement with those of Bhat et al. (2011), Caminiti et al. (2012) Feng et al. (2013) and Shamsudin et al. (2014), who reported no

significant changes in pH values of different types of fruit juices treated with different of UV dosages. Similarly, no statistical differences in pH values were noticed during storage for 30 days at 4°C for all treatments. These results are also in the same line with a study of **Del Caro** et al. (2004), who indicated no significant changes in pH of orange juice during 15 days of storage at 4°C and values ranged from 3.34 (zero time) to 3.43 (after 15 days). In contrast, **Cortes** et al. (2008) reported a significant increase in pH value of fresh orange juice during storage for 4 weeks at 2°C. However, **Unluturk and Atilgan** (2015) reported a significant decrease in pH value during storage of grape juice.

Table (1): Effect of UV-C treatment and storage (30 day, 4°C) on pH values of apple juice

Exposure time (min.)	Storage (days)						
	0	5	10	15	30		
0	*4.31 <sub>a</sub> <sup>A</sup> ±0.004	4.26 <sub>a</sub> A±0.006	4.22 <sub>a</sub> A±0.001	4.25 <sub>a</sub> <sup>A</sup> ±0.005	4.28 <sub>a</sub> A±0.010		
15	4.29 <sub>a</sub> A±0.005	4.27 <sub>a</sub> <sup>A</sup> ±0.006	$4.23_a^A \pm 0.005$	$4.25_{a}^{A}\pm0.004$	4.25 <sub>a</sub> A±0.040		
30	4.28 <sub>a</sub> A±0.005	$4.26_a^{A} \pm 0.005$	$4.23_a^A \pm 0.005$	$4.27_a^{A} \pm 0.003$	$4.23_a^A \pm 0.010$		
45	$4.26_{a}^{A}\pm0.005$	4.24 <sub>a</sub> <sup>A</sup> ±0.006	4.23 <sub>a</sub> A±0.001	4.21 <sub>a</sub> A±0.003	$4.20_{a}^{A}\pm0.010$		
60	4.20 <sub>a</sub> A±0.005	4.23 <sub>a</sub> A±0.005	4.20 <sub>a</sub> A±0.005	4.21 <sub>a</sub> A±0.004	4.22 <sub>a</sub> A±0.060		

<sup>\*</sup>Means followed by different superscripts (within columns) and different subscripts (within rows) are significantly different ( $p \le 0.05$ ).

### b-Effect of UV-C and cold storage on titratable acidity of apple juice

The obtained results for the effect of UV-C treatment on titratable acidity are summarized in the following points. The titratable acidity of fresh apple juice was 0.336 mg malic acid.100 ml<sup>-1</sup> juice. No significant change in such value was noted in juices treated at the different UV exposure times. Similarly, the storage for 30 days at 4°C didn't significantly affect the titratable acidity value obtained at zero time. Obtained results are in agreement with those of Chisari et al. (2011); Pala and Toklucu (2011); Caminiti et al. (2012); Shamsudin et al. (2014) and Kaya and Ünluturk (2015), who

reported that UV didn't significantly affect the titratable acidity of treated juices during refrigerated storage.

#### c-Effect of UV-C and cold storage on TSS of apple juice

Total soluble solids (TSS) are routinely employed to measure the approximate sugar levels in fruit juice (**Bhat**, **2016**). Therefore, TSS was measured for all treated samples compared with control sample and the results are presented in **Fig.** (2). Data revealed that TSS values for control and the UV- treated samples at zero time ranged between 12 to 12.8 with no significant differences between them. Such values persisted with no changes during storage (30 days, 4°C). These results are in harmony with those of **Noci** *et al.* (2008), **Bhat** *et al.* (2011); **Feng** *et al.* (2013) and **Falguera** *et al.* (2013) where they reported no significant differences in the TSS% of fruit juice samples treated with UV irradiation.

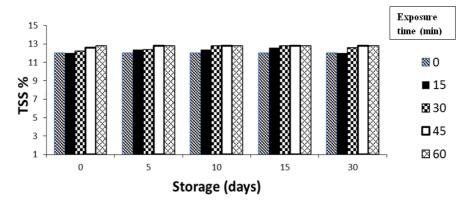


Fig. (2): Effect of UV-C treatment and storage ( 30 days, 4°C) on TSS (Brix) of apple juice

#### d-Effect of UV-C and cold storage on the color of apple juice

The color of the juice is an important visual criterion which has received top attention in food industries and consumers. Color changes in fruit juices can serve as an indicator of microbiological and enzymatic qualites as well as bad processing (**Flaguera** *et al.* **2011a**). Therefore, color characteristic of apple juice samples were measured to determine the effect of different UV tretments as well as cold storage time at 4°C up to 30 days on it. The results are summarized in **Table** (2). The L\* value measures the lightness and ranges from 0 (black) to 100 (white). The a\* value measures the redness and ranges

from  $-a^*$  (greenness) to  $+a^*$  (redness). Lastly,  $b^*$  measures the yellowness and ranges from  $-b^*$ (blueness) to  $+b^*$  (yellowness). Data revealed that the  $L^*$  values were inversely proportional to exposure time. Where  $L^*$  values recorded 30.07, 28.86, 26.87, 22.85 and 21.50 for samples treated for 0, 15, 30, 45 and 60 min., respectively. On the other hand,  $a^*$  values were directly proportional to UV exposure time, where, it recorded -2.43,

-1.13, 0.98 and 3.98, respectively, compared to -2.98 of the control. Similar to a\* values, b\* values showed the same trend where values significantly increased by increasing UV exposure time and the recorded data were 14.92, 17.84. 20.05 and 25.75 for samples treated for 15, 30, 45 and 60 min, respectively, compared to 13.59 of the control sample before storage.

Obtained results are similar to those reported by Manzocco et al. (2009); Falguera et al. (2013) and Müller et al. (2014), who confirmed the reduction in L\* values (lightness) of apple and grape juices and the increase in a\* and b\* values of the UV treated juices. Therefore, the apple and grape juices became darker, redder and more yellow. Müller et al. (2014) reported that both a\* and b\* values of untreated apple juice significantly increased from day 3 during the 18 days of storage and such changes were refered to the activity of PPO.

Regarding storage for 30 days at 4°C, data are summarized in **Table** (2). In control and treated apple juice, an increase in 'L' value with significant differences (p≤0.05) was observed from zero time to 30 days. The color of the apple juice became lighter during storage for 30 days. Similar trend was also observed with a\* and b\* values during storage for 30 days at 4°C. For example, a\* and b\* values of fresh apple juice was -2.98 and 13.59 for control samples, while it was -1.03 and 22.35 after 30 days of storage, respectively. **Donahue** *et al.* (2004) reported a decrease in L\* values of apple cider and referred such results to the enzymatic and non-enzymatic browning.

<b>▲</b> CP	Ex.time	Storage (days)					
	(min)	0	5	10	15	30	
	0	•30.07 <sub>d</sub> A±0.01	34.05 <sub>c</sub> <sup>A</sup> ±0.05	34.83 <sub>c</sub> <sup>A</sup> ±0.06	$35.46_b^A \pm 0.02$	36.33 <sub>a</sub> A±0.04	
	15	$28.86_{c}^{B}\pm0.04$	$28.42_c^B \pm 0.20$	$30.89_b^B \pm 0.08$	$31.15_a^B \pm 0.07$	$31.24_a^B \pm 0.03$	
L*	30	$26.87^{\text{C}}_{\text{c}}\pm0.30$	$27.83_b^B \pm 0.40$	28.47 <sub>a</sub> C±0.40	$28.77_a^c \pm 0.20$	28.80 <sub>a</sub> C±0.10	
_	45	$22.85_d^{D} \pm 0.10$	$22.51_d$ C $\pm 0.08$	23.07 <sub>c</sub> D±0.20	$23.70_b^{D}\pm0.10$	$25.92^{D}_{a}\pm0.01$	
	60	$21.50_d^{E}\pm0.01$	$21.85_d^{D} \pm 0.04$	$22.52^{D}_{c}\pm0.10$	$23.86_b^D \pm 0.02$	24.14 <sub>a</sub> E±0.03	
	0	-2.98 <sub>d</sub> D±0.01	-1.45 <sub>c</sub> D±0.07	-1.16 <sub>b</sub> D±0.02	-1.12 <sub>b</sub> C±0.07	-1.03 <sub>a</sub> E±0.05	
	15	-2.43 <sub>e</sub> D±0.06	$1.90_d^{C} \pm 0.06$	4.24 <sub>c</sub> C±0.05	$5.05_b^B \pm 0.03$	$6.24_a^D \pm 0.02$	
a*	30	-1.13 <sub>d</sub> C±0.09	$3.30_{c}^{B}\pm0.06$	$5.31_b^B \pm 0.25$	$6.33_a^{A}\pm0.03$	$6.60_a^{C} \pm 0.05$	
a	45	$0.98_{d}^{B}\pm0.06$	$5.31_{c}^{A}\pm0.06$	$6.03$ <sub>b</sub> $^{A}\pm0.03$	$6.84_b^{A}\pm0.13$	$7.61_a^B \pm 0.05$	
	60	$3.98_d^A \pm 0.01$	$5.85_{c}^{A}\pm0.05$	$6.00_b^{A}\pm0.08$	6.63 <sub>b</sub> A±0.09	$8.87_a^{A\pm}0.05$	
	0	13.59 <sub>d</sub> E±0.08	20.65°D±0.05	20.71 <sub>c</sub> C±0.20	$21.37_b^B \pm 0.30$	22.35 <sub>a</sub> C±0.10	
	15	14.92 <sub>e</sub> D±0.08	$22.96_d^{C}\pm0.10$	24.48 <sub>c</sub> B±0.20	$25.37_b^{A}\pm0.09$	$26.34_a^B \pm 0.06$	
b*	30	$17.84_d^{C} \pm 0.10$	$24.51_c^B \pm 0.10$	$24.49_b^B \pm 0.30$	$25.81_b^{A} \pm 0.10$	27.38 <sub>a</sub> A±0.20	
	45	$20.05_b^B \pm 0.07$	$24.52_a^B \pm 0.10$	$24.37_a^B \pm 0.20$	$25.54_a^A \pm 0.10$	$27.72_a^A \pm 0.20$	
	60	$25.75_b^A \pm 0.03$	25.30 <sub>c</sub> A±0.10	25.30 <sub>b</sub> A±0.09	25.00 <sub>b</sub> A±0.40	27.57 <sub>a</sub> A±0.08	

Table (2): Effect of UV-C treatment and storage (30 day, 4°C) on color characteristics of apple juice.

### e-Effect of UV-C and cold storage on ascorbic acid content of apple juice

Ascorbic acid was determind in apple juice samples immediately after UV-C treament and during storage time for 30 days at  $4\pm1^{\circ}$ C and the results are presented in **Fig.** (3). The obtained results indicated that ascorbic acid recorded 2.75 mg.100 ml<sup>-1</sup> in control (zero time). Meanwhile, it decreased by 81.8% after exposure to UV-C (254 nm) for 15 min. where, it recorded 0.5 mg.100 ml<sup>-1</sup>. Also, it decreased to 0.25 mg.100 ml<sup>-1</sup> as a result of increasing the exposure time to 30 min, then it persisted at 45 and 60 min of exposure. These results are in harmony with those found by **Bradshaw** *et al.* (2001) and **Falguera** *et al.* (2014) who indicated a significant decrease in ascorbic acid content as a result of exposure to UV-C irradiation in pear juices.

A complate degradation of ascorbic acid was noted after 5 days of storage for samples treated for 30, 45 and 60 min., 10 days for samples treated for 15 min., while 15 days were needed for the control. This result is in harmony with that observed by **Feng** *et al.* (2013) who stated that no ascorbic acid remained in watermelon juice

<sup>&</sup>lt;sup>▲</sup>Color parameters and <sup>■</sup>Exposure time.

<sup>\*</sup>Means followed by different superscripts (within coulmns) and different subscripts (within rows) are significantly different at the  $(P \le 0.05)$ , L\* (lightness), a\* (redness) and b\* (yellowness).

by the end of storage period at 4°C. The degradation of ascorbic acid during storage has been attributed to atmospheric oxygen (**Odriozola-Serrano** *et al.* **2008**). Also, various factors such as light exposure, hydrogen peroxides, storage temperature, type of processing packaging materials, and enzymes present such as ascorbate oxidase and peroxidase could affect the degradation of ascorbic acid (**Davey** *et al.* **2000** and **Ayhan** *et al.* **2001**).

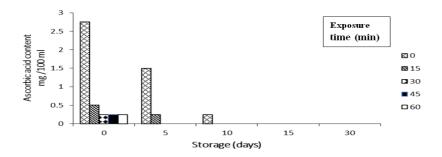


Fig (3): Effect of UV exposure time and storage at 4 °C for 30 days on ascorbic acid content of apple juice.

#### f-Effect of UV-C and cold storage on PPO activity of apple juice

Enzymatic browning causes color change in fruits and vegetables and such changes in color result from the action of polyphenol oxidase (PPO) on the phenolic compounds in fruits and vegetables in the presence of oxygen (Manzocco et al. 2009).

**Table (3)** indicates the effect of UV exposure time on the PPO activitiy in apple juice stored up to 30 days at 4°C. Results indicated a significant decrease ( $p \le 0.05$ ) in PPO activitiy by increasing exposure time. The activity was 54.67 (u.ml<sup>-1</sup>) for the control sample then gradually decreased to be 12.67 (u.ml<sup>-1</sup>) after 60 min. of exposure to UV light with 76.8% reduction of the total activity. However, the 45 min. treatment resulted in higher PPO activity than the 60 min, it is of interest to report that there was no significant difference between both of them and therefore, the 45 min. treatment was recommended. Accordingly, similar trend in the PPO activity reduction was noticed during storage for 30 days at 4 °C, but with a slower rate. On the other hand, almost constant values (with no significant differences,  $p \le 0.05$ )) were obtained in case of the 45 and 60 min UV-C treatments.

Enzymes deactivation was reported to occur generally due to photo-oxidation *via* two routes: direct absorption of radiation by protein or bound chromophore or *via* indirect oxidation by singlet oxygen (**Davies and Truscott, 2001**).

Barbosa-Cánovas and Guerrero-Beltrán (2006) and Falguera et al. (2011b) reported a reduction of PPO activity in different juices after treatment with UV at 25°C. Manzocco et al. (2009) ascertained the need for a longer treatment in case of juices than model system due to the low transmittance of UV-C light through the suspended and soluble solids in juice.

Table (3): Effect of UV-C treatment and storage ( 30 day,  $4^{\circ}$ C) on PPO activity (u.ml<sup>-1</sup>) in apple juice.

Exposure	Storage (days)						
time (min)	0	5	10	15	30		
0	*54.67 <sub>a</sub> A*	28.33 <sub>b</sub> <sup>A</sup>	15.33 <sub>c</sub> <sup>A</sup>	17.33 <sub>c</sub> <sup>A</sup>	13.33 <sub>c</sub> <sup>A</sup>		
15	44.67 <sub>a</sub> B	16.67 <sub>b</sub> B	13.33 <sub>b</sub> <sup>A</sup>	$16.67_{\rm b}{}^{\rm AB}$	$12.00_{\rm b}{}^{\rm A}$		
30	28.00 <sub>a</sub> <sup>C</sup>	$14.67_{\rm b}{}^{\rm BC}$	$13.67_{\rm bc}{}^{\rm A}$	$14.67_{\rm b}{}^{\rm AB}$	$13.05_{c}^{A}$		
45	15.67 <sub>a</sub> D	$12.67_a^{BC}$	11.33 <sub>a</sub> A	12.00 <sub>a</sub> BC	$12.00_a{}^A$		
60	12.67 <sub>a</sub> D	9.67 <sub>a</sub> <sup>C</sup>	10.67 <sub>a</sub> A	8.67 <sub>a</sub> <sup>C</sup>	$10.00_{a}^{A}$		

<sup>\*</sup>Means followed by different superscripts (within columns) and different subscripts (within rows) are significantly different at the ( $p \le 0.05$ ).

### g-Effect of UV-C and cold storage on microbial quality of apple juice

UV-C radiation is routinely used in the food industry as nonthermal treatment for inhibiting microbial growth on surfaces, in air and in liquids. It is most effective at 254 nm since energy at that wavelength can induce formation of pyrimidine dimers that distort the DNA and block cell replication (Bintsis, 2000; Lado and Yousef 2002; Guerrero-Beltrán and Barbosa-Cánovas 2004).

The effect of both UV treatment and storage period (30 day at 4°C) on the total bacterial load in apple juice was indicated in **Table** (4). A negative correlation between UV exposure time and the total bacterial count was noted. At zero time, slight decrease with no significant differences were noticed in bacterial count between the control, 15 and 30 min. samples and the reduction was from 2.48 to 2.39 and 2.22 log<sub>10</sub>CFU, respectively. A significant reduction (31.7%)

in number was achieved upon using a treatment of 45 min. On the other hand, a 100% reduction was achieved when 60 min. exposure was used. The storage for 30 day at 4°C for the control resulted in significant increase in total bacterial count. No growth at all was noted in 60 min. treatment during the storage period. The highest increase was for the untreated sample and an increase of 0.92 log 10CFU was noted. The lowest increase was for the 45 min. treatment sample and an increase of 0.18 log<sub>10</sub>CFU was achieved. In all cases, the bacterial growth was very well controlled since the maximum obtained bacterial count was 3.4 log<sub>10</sub>CFU and such number in much lower than that listed as the onset of bacterial spoilage of 10<sup>6</sup> CFU.ml<sup>-1</sup> (Mirrazavi, 2011). Such low number was expected since the pH values of the juice didn't exceed 4.3 and at such low pH most of the bacterial genera can't grow (Hatcher *et al.* 1992).

The Saudi Arabian Standards (3081 / 1434) for fresh juices limited the total bacterial number to  $(5x10^3 \text{ CFU.ml}^{-1})$ . Therefore, the tested juice, even after 30 days of storage at 4°C is bacteriologically accepted.

Table (4): Effect of UV-C treatment and storage (30 day,  $4^{\circ}$ C) on the total plate count ( $log_{10}CFU.ml^{-1}$ ) of apple juice

Exposure time			Storage (day	rs)	
(min.)	0	5	10	15	30
0	*2.48 <sub>c</sub> <sup>A</sup>	2.91 <sub>b</sub> <sup>A</sup>	2.99 <sub>b</sub> <sup>A</sup>	3.25 <sub>a</sub> A	3.40 <sub>a</sub> A
15	$2.39_{c}^{A}$	$2.75_b^A$	$2.90_a^A$	2.95 <sub>a</sub> B	$3.00_a^B$
30	$2.22_d^{AB}$	$2.46_{c}^{B}$	$2.63_b^B$	$2.77_{ab}^{C}$	$2.85_a^B$
45	$1.79_b{}^B$	$1.74_{\rm b}{}^{\rm C}$	1.82 <sub>b</sub> <sup>C</sup>	1.92 <sub>a</sub> <sup>D</sup>	1.97 <sub>a</sub> <sup>C</sup>
60	$0.00_a^{C}$	$0.00_a^D$	$0.00_a^D$	$0.00_a^{E}$	$0.00_a^{D}$

\*Means followed by different superscripts (within columns) and different subscripts (within rows) are significantly different at the (p<0.05).

## h-Effect of UV-C and cold storage on total yeasts and molds of apple juice:

The effect of UV on the total yeast and mold counts in apple juice was indicated in **Table** (5). The increase in exposure time resulted in sharp decline in yeast and mold numbers where decreased from 2.61 (control) to 1.44 log<sub>10</sub> CFU.ml<sup>-1</sup> (60 min treatment) with the exception of the 15 and 30 min. treatments where no significant

differences were obtained. During storage, number of yeasts and molds persisted with almost no big differences. On the other hand, the untreated sample (control) was considered to be microbialy spoiled since total yeast and mold numbers exceeded the onset of spoilage of  $10^6$  after 13 days of storage at 4°C. All tested UV treatments controlled the growth of yeast and mold successfully. The Saudi Arabian Standards (3081 / 1434) specified 100 cells.gm<sup>-1</sup> as the maximum acceptable number of yeasts and molds in juice. Obtained results indicated that the 60 min. treatment succeeded to lower yeasts and molds number to meet with the Saudi Arabian Standards limits even after storage for 30 days at 4°C (1.85 log<sub>10</sub> CFU. ml<sup>-1</sup>).

It is of interest to report that in all cases the tested juices didn't exceed the microbial onset of spoilage of 10<sup>6</sup> CFU.ml<sup>-1</sup> (Mirrazavi 2011).

Unlike the effect of UV on bacteria, the 60 min treatment didn't completely destroy yeasts and molds and a surviving value of 56.% was obtained at zero time. The main reason for yeast and mold to exhibit higher UV resistance than bacteria could be attributed to their lower content of pyrimidine nucleosides where lower chance for the formation of thymine dimers is expected. Additionally, the chemical composition and thickness of the cell wall of yeast and mold are different than bacteria, which render them to be UV resistant (Miller et al. 1999).

Keyser *et al.* (2008), Feng *et al.* (2013), Unluturk and Atilgan (2015) and Bhat (2016) reported the effectiveness of UV-C in deactivation of bacteria, yeasts and molds in different juices. Such inhibition was intensity dependent. Chia *et al.* (2012) reported an improvement of 7 weeks in the shelf life of pineapple juice treated with UV (53.4 J.cm<sup>-2</sup>) and stored at 4°C. Kaya *et al.* (2015) extended the microbial shelf life of lemon-melon juice blend (4°C) by 28 days utilizing UV-C treatment at 2.461 J.ml<sup>-1</sup>. This treatment was successfully eliminated the entire microbial population present in the fresh form.

Exposure time (min.)			Storage (day	s)	
	0	5	10	15	30
0	*2.61 <sub>c</sub> A	4.02 <sub>c</sub> <sup>A</sup>	5.45 <sub>b</sub> <sup>A</sup>	6.32 <sub>a</sub> A	7.54 <sub>a</sub> A
15	$2.54_{c}^{A}$	2.59 <sub>c</sub> B	2.61 <sub>bc</sub> B	$2.80_{ab}^{B}$	2.85 <sub>a</sub> B
30	2.46 <sub>b</sub> A	$2.49_{b}^{B}$	2.58 <sub>ab</sub> B	2.61 <sub>ab</sub> B	2.71 <sub>a</sub> <sup>C</sup>
45	1.91 <sub>c</sub> B	2.06 <sub>b</sub> <sup>C</sup>	2.17 <sub>a</sub> <sup>C</sup>	2.18 <sub>a</sub> <sup>C</sup>	$2.17_{a}^{D}$
60	1.44 <sub>c</sub> C	$1.65_{\rm b}^{\rm D}$	1.72 <sub>b</sub> <sup>D</sup>	$1.77_{\rm h}^{\rm D}$	1.85 <sub>a</sub> E

Table (5): Effect of UV-C treatment and storage (30 day, 4°C) on yeast and mold counts (log<sub>10</sub>CFU.ml<sup>-1</sup>) in apple juice

#### i-Effect of UV-C treatment on sensory evaluation of apple juice:

From the consumer point of view, fruit juice color, odor, taste and consistency are very significant for determining its marketability. The effect of UV exposure times (15, 30, 45 and 60 min) on the sensory attributes (color, odor, taste, consistency and overall acceptability) of apple juice compared to control is indicated in **Table** (6). Results indicated that color scores didn't significantly ( $p \le 0.05$ ) changed compared with control sample until 30 min of exposure followed by significant decrease thereafter. Similar trend was observed for data obtained for both odor and taste scores.

On the other hand, statistical analysis revealed no significant differences( $p \le 0.05$ ) for consistency scores for all tested samples compared to control. Also, the highest overall acceptability scores were for the control and sample treated for 15 min. with no significant diffrences between them. The increase of exposure time up to 30 min. resulted in a signifficant decrease in overall acceptability.

Obtained results are in harmony with those reported by **Harrington and Hills (1968)**, who indicated that apple cider exposed to UV light for 120 sec. exhibited no significant differences in sensory criteria compared with fresh control apple cider. Also, our results are in the same line with those reported by **Donahue** *et al.* (2004) who used UV light (254.7 nm) as a low-cost alternative technique for pastarizing of apple cider. They ascertained that treatment with UV irradiation didn't affect the sensory attributes of apple ciders compared to control one.

<sup>\*</sup>Means followed by different superscripts (within columns) and different subscripts (within rows) are significantly different (p≤0.05).

Our results are also in agreement with those reported by Caminiti *et al.* (2012) who treated apple juice with different UV energy dosages (5.31 to 53.10 J.cm<sup>-2</sup>). They indicated that samples exposed to 5.31 and 10.62 J.cm<sup>-2</sup> exhibited acceptability of color, odor, flavor and overall acceptability while at higher dosage (26.55 J.cm<sup>-2</sup>), odor was negatively affected, meanwhile the dosage of 53.1 J.cm<sup>-2</sup> was needed to decrease color scores.

Therefore, it could be concluded that UV treatment up to 30 min didn't affect the sensory attributes of apple juice.

Table (6): Effect of UV-C treatment on sensory attribute of apple juice

Exposure time (min.)	Sensory criteria						
	color	odor	taste	consistency	Overall acceptability		
0	*8.10 <sup>a</sup> ±1.70	$8.20^{ab} \pm 1.40$	8.35a±1.50	8.30 <sup>a</sup> ±1.40	8.40a±1.70		
15	$8.10^a \pm 1.10$	$8.50^a \pm 0.85$	$8.40^{a}\pm1.07$	$8.35^{a}\pm1.40$	$8.50^a \pm 1.20$		
30	$7.10^{ab} \pm 0.88$	$7.50^{abc} \pm 0.97$	$7.30^{ab}\pm0.95$	$7.75^{a}\pm1.20$	$7.40^{ab}\pm1.07$		
45	$5.80^{b}\pm1.50$	$6.80^{bc} \pm 1.20$	$6.40^b \pm 1.50$	$7.00^{a}\pm1.80$	6.30bc±1.80		
60	$5.50^b \pm 1.60$	$6.00^{\circ} \pm 1.60$	$6.10^{b}{\pm}1.80$	$6.70^{a}\pm2.06$	$5.10^{c} \pm 1.85$		

<sup>\*</sup>Means followed by different superscripts within each columns are significantly different (p≤0.05).

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# تأثير الأشعه فوق البنفسجية قصيرة الموجة والتخزين تحت تبريد علي جودة عصير التفاح

 $^{2}$ وائل أحمد بازرعه $^{1}$  - شاهيناز أحمد حلمي - هشام أمين عيسى  $_{-}$  - رمضان محمد عبد الحافظ

أ قسم علوم الأغذية – كلية الزراعة – جامعة القاهرة – الجيزة – مصر  $^2$ قسم الصناعات الغذائيه – المركز القومي للبحوث – القاهرة – مصر

تم دراسة تأثير الأشعه فوق البنفسجية (UV-C) على الخصائص الفيزيوكيميائيه (pH) ، الحموضة ، المواد الصلبة الذائبة ، اللون ، محتوي حمض الأسكوربيك) وكذلك نشاط البولي فينول أوكسيديز (PPO) بالإضافه إلي العد الميكروبي في عصير التفاح مقارنة بالعصير الطازج تم تشعيع عينات العصير باستخدام الأشعة فوق البنفسجية (254 نانوميتر) 30 الأزمنة مختلفه (15، 30 ، 45 ، 60 دقيقة) ، وتم تخزين العينات على  $\pm 1^{\circ}$  م لمدة يوما لم تؤثر المعامله بالأشعه فوق البنفسجية بشكل كبير على قيم pH ، الحموضة ، المواد الصلبة الذائبة في كل من العينات الطازجة والمخزنة لوحظ وجود علاقة عكسية بين زمن التعرض وقيم \*L (درجة السطوع) للعينات الطازجة والمخزنة. على العكس من ذلك ، لوحظ وجود علاقة طردية بين زمن التعرض مع قيم \*a وقيم \*b. انخفض محتوى حمض الأسكور بيك بزيادة زمن التعرض حيث فقد بالكامل بعد 5 أيام للعينات المعاملة بالأشعة فوق البنفسجية لمدة 30 ، 45 ، 60 دقيقة و بعد 10 أيام للعينات المعاملة لمدة 15 دقيقة و 15 يومًا للعينة الطازجة. تم تثبيط نشاط إنزيم PPO جزئيا عن طريق المعاملة بالأشعة فوق البنفسجية (حيث تم فقد 76.8٪ من نشاطه بعد 60 دقيقة من التعرض للمعاملة) وكذلك أثناء التخزين (ولكن بمعدل أبطأ). تأثر عد البكتيريا الكليه وكذلك أعداد الخمائر والفطريات سلبا عن طريق زيادة زمن التعرض للأشعة فوق البنفسجية. وكان من الملاحظ أن الخمائر والفطريات كانت أكثر مقاومة للأشعة فوق البنفسجية من البكتيريا. كما لم تتغير الخصائص الحسية (الرائحة، اللون ، الطعم ، التجانس ، القبول العام) نتيجة المعاملة بالأشعة فوق البنفسجية لمدة تصل إلى 30 دقيقة، ثم انخفضت بعد ذلك بشكل معنوى.

الكلمات الدالة: الأشعة فوق البنفسجية ،عصير التفاح، البولي فينول أوكسيديز، الخصائص الحسية ، التخزين تحت تبريد.