International Food Research Journal 18(4): 1265-1268 (2011)

Impact of UV-C light on orange juice quality and shelf life

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Abstract: The effect of UVC on microbial inactivation and also quality of orange juice was studied. The shelf life was extended for 7 days after being exposed to UV light (254 nm). 18% reduction in Vitamin C content was measured by HPLC and titration methods which were similar to results retrieved from thermal sterilization. Unlike conventional methods no tangible relation between UV-C and Pectin methyl esterase enzyme deactivation was witnessed. Less energy was consumed by UV treatment in comparison to thermal methods. No significant alteration was observed in juice pH and color.

Keywords: UV-C, orange juice, APC, vitamin C, pectin methyl esterase

Introduction

Emerging non thermal techniques are applied as alternatives to conventional thermal processes in order to improve the safety of food products and also maintain their quality. One of these techniques which have recently drawn attentions is Ultra Violet treatment. UV light range is split in four subcategories based on their wavelength: UVA (320-400 nm), UVB (280-320 nm), UVC (200-280 nm) and UVV (100-200 nm) (Guerrero-Beltrán, 2004; Barbosa-Cánovas, 2004). UVC light has germicidal functionality as it penetrates the cell membrane and results in electron shifting and DNA structure breakdown (Shama, 1992, 1999). UVC is reported to have maximum effect on bacteria, virus, protozoa's, fungi and algae in the range of 250-260 nm (Shama, 1999; Bintsis *et al*., 2000). UV-C illumination plays a key role as a post harvest treatment which postpones horticultural products' ageing and subsequently decaying (Allende *et al*., 2006).

Micro organisms responsible for fresh orange juice spoilage are generally yeasts/molds and bacteria such as *Lactobacilus*, *Leuconostoc*, thermophilic *Bacillus* (*Bacillus subtilis* and/or *Bacillus pumilus* spore formers) and *Alicyclobacillus acidoterrestris* which is a thermophilic spore forming bacteria targeting the juice industry (Walls and Chuyate, 2000; Yamazaki *et al*., 1997; Kimball, 1991, 1996). Thermal processing is applied in order to prevent spoilage and to extend products shelf life but the juice color alteration and loss of volatile compounds is inevitable, thus alternative techniques should be investigated so safety of the product can be obtained with a quality competing with fresh juice. One goal of this study was to evaluate the effect of UV-C on standard aerobic plate count microorganisms. The organisms in treated and untreated samples were analyzed based on plate count methods.

Antioxidant vitamins play a key role in human health and are nutritionally important. Vitamin C maintains vasculature structure by regulating collagen synthesis, prostacyclin production, and maintenance of nitric oxide levels (Gaby, 2010). Ascorbic acid is used as an additive in juice industry in order to prevent color change and Millard's reaction in the product and also as a chelating agent.

PME enzyme deesterifies pectin content of the juice which would lead to pectate (LMP) formation. Pectate's reaction with calcium ion leads to gelation and cloud loss phenomenon (Zhang *et al*., 2011). To prevent serious quality defect resulting from cloud loss, 99.99% of designated enzyme should be deactivated. Enzyme deactivation is achieved by heating the product for 60 s at 90^{o}C (Eagerman and Rouse, 1976). In this article the effect of UV-C on enzyme deactivation is also discussed.

Materials and Methods

Oranges were purchased from local store and juice was obtained mechanically (Moulinex VITAPRESS 600, Spain), then filtered by 1 mm^2 sieve made of stainless steel to obtain 25 l orange juice. The juice was refrigerated at 4^{o}C for 8 h then set at room temperature for 8 h before UV treatment.

Concentrated orange juice was diluted with a ratio of 1:1. 2 l of the juice was left in room temperature for 5 days to elevate the microorganism count in the specimen. Then it was mixed with 20 l of reconstituted orange juice.

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Ultra Violet treatment

The UV-C device consisted of a 50 cm long glass rod, setup vertically with a 45 cm UV lamp (30W, 254 nm, and low pressure). 6 W of germicidal UV emitted from the lamp. It was fixed in the center of the tube and was shielded by quartz so the juice would not be in direct contact with the lamp. Air was pumped inside the vessel in order to decrees the temperature of the UV lamp so the efficiency of UV lamp would not drop because of temperature elevation. The juice was pumped through the instrument using a small Peristaltic pump 6-600 rpm. Flow speed was regulated by a controller. The flow (0.6 l/min) was adjusted in a manner that liquid film thickness was between 0.21 and 0.48 mm. The substance was considered transparent to UV light if it formed a liquid film with thickness less than 1.6 mm (Sarkin, 1977).

Micro organism analysis

One milliliter of treated and untreated sample dilutions was pipetted to petri dishes. Pour plate technique was applied on PCA to enumerate total aerobic count, then plates were incubated at 35°C for 48 h. Total amounts of molds and yeasts were enumerated by applying pour plate technique on potato dextrose agar plates and these plates were then incubated at 26°C for five days. All tests were performed twice and the total count experiments were done each 3 days for two weeks.

Vitamin C concentration analysis by the HPLC method

UV treated samples were centrifuged for 20 min at 4°C to remove solid particles. The supernatant phase was mixed with 4 ml metaphosphoric acid and well filtered by PTFE membrane. The samples were transferred to auto sampler prior to HPLC injection. For more accurate results samples were prepared in triplicates. The analysis was performed by Surveyor Thermo Electron system comprising a vacuum degasser, HPLC pump Surveyor Plus LCPMPP pump, Surveyor Plus ASP auto sampler and diode array detector with 5 cm flow cell. Integration, data storage and processing were performed by Chrom Quest 4.2 software.

Aqueous ammonium dihydrogen orthophosphate was considered as the mobile phase. It was adjusted to a Ph of 2.8 by phosphoric acid at a flow rate of 0.7 ml/min. The temperature was set to 20°C. Samples were injected to instrument in 5 micro liter volumes and were monitored at 254 nm for up to 20 min. In order to calibrate the instrument standard ascorbic acid were prepared in 3% meta-phosphoric acid solution and the calibration curve and standard tests were run in triplicates.

Vitamin C concentration analysis by titration method

Samples were centrifuged at 4°C for 20 min. The obtained supernatant was mixed with 25 ml of 20%meta-phosphoric acid as a stabilizing agent (FAO, 1986) then it was diluted to a volume of 100 ml. A mixture of the diluted specimen and 2.5 ml acetone was titrated with 2, 6-dichloindophenol till a pink color remains for 15 sec. 10 ml of standard ascorbic was prepared by adding 50 mg of pure ascorbic acid in 60 ml of 20% metaphosphoricacid. Dilution was done to a volume of 250 ml and titrated with 2, 6-dichloindophenol until a pink color persisted for 15 s.

Pectin methylesterase (PME) assay

PME activity of samples treated by different doses of UV or heated at 70°C for 2 seconds were determined by the method proposed by Kimball (1991). This method was based on carboxyl group titration. 0.2 ml of the sample was mixed with 20 ml of 1% pectin containing 0.1M NaCl and was then set in a 23°C incubator. 0.025 ml of 0.1N NaOH was added to the solution and pH was adjusted to 7.5 by adding 0.1N NaOH. Pectin methylesterase units were calculated by Eq. (1):

$$A = \frac{C_{NaOH} \times V_{NaOH}}{V_{sample} \times t} = \frac{1}{400 \times V_{sample} \times t} \quad (1)$$

A, C_{NaOH} , V_{NaOH} and V_{sample} are PME activity, NaOH concentration (0.1 mol/l), volume of NaOH (0.025 ml) and applied sample volume (0.20 ml), respectively. Time needed for pH to return to 7.5 after addition of NaOH (min) is demonsted as t. PME residual level was calculated by Eq. (2):

$$PME\ residual\ level = \frac{A_{sample}}{A_{control}} \times 100\% \quad (2)$$

The rate of acid formation at pH 7.5 was calculated in PME unit by Eq. (3):

$$\frac{PMEU}{ml} Brix = \frac{(\text{ml NaOH})(\text{NaOH normality})}{(\text{reaction time})\,(\text{ml sample})\text{Brix}} \quad (3)$$

Obtained data was analyzed by Microsoft excel 2007.

Results

Impact of UV ray on standard aerobic plate count and yeast and molds in reconstituted and fresh orange juice

Figure 1 shows the dose response of bacteria and mold to UVC treatment. As demonstrated the response was linear with of R^2 0.96 and 0.91 for bacteria and mold respectively. Statistical analysis results revealed that there is significant difference in APC population in different irradiated doses (p=0.002). Regarding yeast and mold a p value of 0.001 was calculated that revealed meaningful difference between these populations in different UV doses. This experiment was done in ambient temperature so the constant (curve slope) is only valid in this condition.

Eqs 4 and 5 show the destruction kinetics for APC and yeast/mold respectively:

$$N = N_0 e^{-0.027 X} \quad (4)$$

$$N = N_0 e^{-0.022 X} \quad (5)$$

Where N, X and N_0 are viable microorganisms, ultraviolet (mJ/cm^2) and initial number of microorganisms in the juice prior to treatment. Decimal reduction constant is the amount of required UV dose in order to inactivate 90% of microorganisms (Towasend, 1938). D_{10} values for APC and Yeast/mold were reported 82 and 105, respectively. It was found that yeast and molds are more resistive to UV-C treatment than bacteria and 96.8% &95.3% of organisms were inactivated at 125 mJ/cm^2 dose. Shelf life of fresh orange juice was prolonged for 7 days and it could be extended for more by applying higher doses of UV (Table 1).

Table 1. Organism count of treated and untreated samples at storage

	APC		Yeast and Mold	
Storage day	Untreated juice	Treated juice	Untreated juice	Treated juice
0	7500	2900	7800	3200
2	5800	1700	8900	2500
7	11200	6300	28000	6700
14	42000	22000	90000	23000

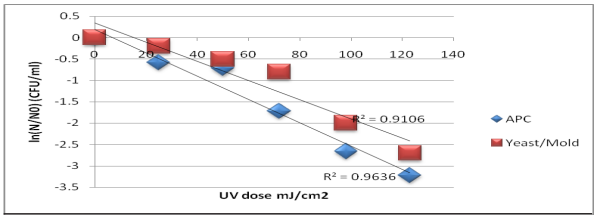

Figure 1. Dose response of bacteria and mold to UVC treatment

Effect of UV on Vitamin C

As shown in the Figure 2 the amount of vitamin C in reconstituted juice steeply decrees by increasing UV dose. The first order kinetic model which explains the plot is stated bellow:

$$C = C_0 e^{-0.002 X}$$

C, C_0 and X are vitamin C content (mg/100 ml) after UV treatment, vitamin C content before treatment and UV dose respectively. The analysis was done by HPLC and titration method. No major difference was seen and UV destruction is similar to vitamin C loss in thermal processing which was 18% and vitamin C destruction due to air oxidation was reported 1% (Lopez *et al*., 1967).

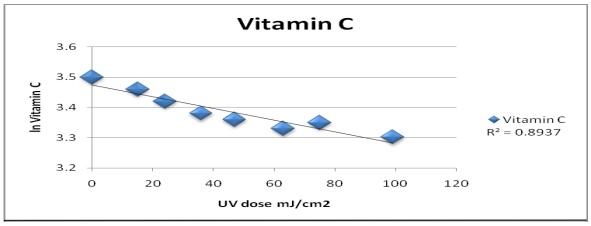

Figure 2. Residual Vitamin C in different UVC dosages

Effect of UV on PME activity

UV treatment (73.8 mWs/cm^2) in comparison to thermal treatment (70°C 2 sec) didn't reduce the enzyme content significantly. Only 8% was inactivated which is too little compared to the 75% deactivation by conventional heating treatment, however UV treated juice maintains its freshness and color.

UV dose and energy needed for treatment

UV dose applied for microorganism eradication is calculated by the following equations (Eq. 6, 7):

$$U = It/A \quad (6)$$

$$A = \pi DL \quad (7)$$

Where I, t , A ,D and L are UV intensity of lamp (6 W), time of irradiation, exposure surface area, inner diameter of vessel (4.5 cm) and length of vessel (45 cm), respectively:

$$6 \times \frac{1.24}{635.85} = 0.0117 \frac{J}{cm^2}$$

$$A = 3.14 \times 4.5 \times 45 = 635.85$$

UV irradiation time was calculated as about 1.24 sec by measuring the resident time of added tracers. The experiment was repeated 40 times to achieve higher accuracy rates. The liquid volume (V) was circulated 12 times (n) in order to elevate UV dose from 13 to 144 mJ/cm^2. Amount of energy consumed by thermal treatment and UV processing was calculated by following equations (Eq. 8, 9):

$$Q_{thermal} = mc\Delta\theta \quad 1000 \times \frac{4.2(90-20)}{1000} = 294\frac{MJ}{m^3} or\ 81.67\frac{kWh}{m^3} \quad (8)$$

$$Q_{UV} = \frac{n\,I}{\dot{V}} \quad 12 \times 6 \times \frac{10^{-3}}{0.036} = 2.0\ kW\frac{h}{m^3} \quad (9)$$

Discussion and Conclusions

Our study demonstrated that bacteria are more susceptible to UV treatment. This could be explained by 3 reasons. First, bacteria are smaller than yeasts/molds; this causes easier UV passage (Montgometry, 1985). Second bacteria have different cell wall constructions, and finally existence of higher levels of pyrimidine in bacteria DNA increases the chance of cross linkage of neighboring thymin and cytosine (Miller *et al*., 1999). As demonstrated in Figure 3 sensitivity to UVC is as follows: bacteria >yeast/mold >fungi. Another probable reason for this could be difference in pigmentation or the existence or capacity of repair systems in yeast/mold and fungi. A decrease in APC was seen in the first two days in fresh juice which was due to chilling injury. Air oxidation can be responsible for vitamin C degradation but since the samples were stored for maximum 2 hours before UV treatment this could be compromised. The energy consumed by the UV-C device varies from 2-10 KW h/m^3, which depends on the type of lamp used and device efficacy.

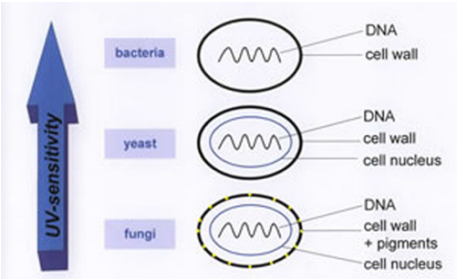

Figure 3. Comparison of UVC sensitivity in micro organisms

In this study it was demonstrated that UV treatment is an effective way to extend fresh orange juice shelf life from 2 to 7 days. The utilized energy was tremendously less than conventional processes and the color of irradiated product were stable but it cannot completely be applied as a substitute to thermal processes, since there was no considerable difference in vitamin C stability and the thermal method was more efficient in inactivating enzymes.

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