

Furan development In Dean vortex UVC treated Pummelo (*Citrus grandis* L. Osbeck) fruit juice

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Abstract

Furan has been classified as a possible carcinogen and substantiated evidences have also shown that conventional thermal treatment could stimulate furan development in food products. Ultraviolet light (UVC) is currently known as an alternative method widely used in pasteurizing juice. However, the effect of UVC in inducing furan development has not been studied specifically on UVC-treated pummelo juice. Thus, the aim of this study was to investigate the development of furan in UVC-treated pummelo juice and its relationships to the juice sugar contents. Our results showed that furan development within the juice post-UVC treatment was dose-dependent and inversely proportional to the amount of sugar and ascorbic acid ($p < 0.05$).

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Introduction

The approval of ultraviolet (UVC) light as an alternative treatment to thermal pasteurization of fresh juice products by the United States' Food and Drug Administration (USDA, 2000) has led to a growing interest and research in UVC technology. It is a non-thermal technology suitable for fruit juice pasteurization with a positive consumer image and it is attractive to the food industry due to its low cost and energy efficiency (Koutchma, 2009). UVC light is part of an electromagnetic spectrum between 200 to 280 nm. Approximately 85% of the output from low-pressure mercury arc lamps is monochromatic at a wavelength of 254 nm (Water Environment Federation, 1996). Its antimicrobial mechanism is based on the absorption of UVC light by microbial DNA, which causes the formation of cyclobutane pyrimidine dimers to interrupt the transcription, translation and replication of DNA and eventually rendering cells death (Harm, 1980).

Furan is a heterocyclic aromatic compound containing one oxygen atom. Furan is a colorless, flammable, highly volatile liquid with a boiling point close to room temperature. It is soluble in common organic solvents, including alcohol, ether, and acetone, and is slightly soluble in water (Jakubke and

Jeschkeit, 1994). It is a suspected carcinogenic and classified in Group 2B as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (WHO, 1995). Furan does not have a practical application as a final product. However, it plays a role in the production of (co)polymers and furan has been identified in a number of food that have been heat treated (Wegener and Lopez-Sanchez, 2010) and recently, in UVC-treated fruit juice (Fan and Geveke, 2007). In 2004, a FDA survey had found that furan was present in a large number of thermally processed foods, such as baby foods, canned vegetables, fruits, meats, fish, pasta sauces, nutrition drinks, fruit preservers and coffee, with levels of furan at ~100 parts per billion (ppb) (USFDA, 2004).

In addition to that, furan was found to form from carbohydrates, ascorbic acid, fatty acids and a mixture of all three (Locas and Yaylayan, 2004; Fan 2005a). In another study done by Fan (2005b), it was found that irradiation was proven to induce furan formation in fruit juices and its constituents. It was also found that furan formation was triggered by ascorbic acid and carbohydrates. These compounds may serve as a precursor for furan. Locas and Yaylayan (2004) suggested that ascorbic acid had the highest potential to produce furan upon thermal treatment, followed by sugar/amino acids mixture. Becalski and Seamen

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(2005) in their study has identified two pathways of thermally induced furan formation in model systems: the oxidation of polyunsaturated fatty acids and the decomposition of ascorbic acid derivatives. Therefore, the objective of this study was to examine the development of furan post-UVC treatment on pummelo fruit juice and its correlations to juice quality, in a bid to have a natural, fresh-like and safe fruit juice.

Materials and Methods

Preparation of clarified juice

Frozen fresh juice (-22°C for a maximum of 6-weeks) was thawed at room temperature for three hours to prepare for clarified treatments. The optimized range of the variables for enzymatic treatment conditions (Enzyme concentration: 0.02%, incubation time: 100 minutes and incubation temperature: 50°C) was based on the preliminary RSM experiments conducted earlier (unpublished works). Enzyme used in this study was Pectinex Smash XXL (Novozymes A/S, Denmark). The temperature of the enzymatic treatment was adjusted to the desired level using a constant temperature water bath. The pH of pummelo juice was kept at its natural pH value of 4.0 and was excluded from the RSM experimental design as the pH was considered optimal for exopectinase (Kashyap *et al.*, 2001). The enzymatic treatment was performed according to the method of Rai *et al.* (2004) with slight modification on enzyme inactivation. Heat treatment to inactivate enzyme was excluded and replaced with ultrasonication method (Ultrasonic time: 10 minutes, ultrasonic power: 400 Watt). This method was introduced to avoid unwanted changes to the flavour and juice characteristics from the conventional thermal treatment to inactivate the pectinase (90°C for 15 s). The treated juices were then centrifuged at 3000 g for 10 minutes and the supernatant was collected. Subsequently, the juice was filtered through a Whatman no. 1 filter paper using vacuum suction at 25 mm Hg. The filtrate was then collected for further analysis.

Ultraviolet apparatus and treatments

The experimental setup consisted of Dean Vortex reactor, juice feeding tank, overflow tank and two UVC sensors. A 914-mm arc length low pressure mercury lamps (Philips, UK) were used in the UVC reactor. The high output UVC lamp has an energy density of 573 W/m² and a durability of 9000 hours. A coil made of perfluoroalkoxy (PFA), with a length of 19.27 m, an inner diameter of 1.65 mm and a pitch of 3.18 mm, was used to create a helically

wound tube around the UVC lamp. The feeding and overflow tank were made out of food-grade stainless steel with an inner diameter of 250 mm and 850 mm height. The reactor was mounted in vertical position with fluid flowing from the bottom to the top in order to fill the tube fully and to avoid bubbles. The fluence rate is highly dependent on the volume of the juice (10 – 100 m³), flowrate (50 – 70 mL/min), retention time distribution (35 – 50 s), turbidity and flow field.

The performance of UVC reactor was evaluated at three frequencies (30, 35 and 40 Hz). Prior to each experimental run, the UVC reactor and all accessories were cleaned with hot water and chemically sterilized with 2% sodium hydroxide solution (NaOH) to eliminate any microbial contamination. Upon start-up, the reactor was left to warm up until the temperature of UVC lamp reached 40°C. This was done to ensure the UVC radiation from the lamp is at a steady state for efficient usage. The samples were pumped through the UVC reactor at set frequency using a peristaltic pump until all of the juice flowed out. The temperature of UVC lamp and samples were recorded together with the time for the whole volume of juice to be pumped out. Samples were then collected from the inlet and outlet for microbial analysis. All measurements were done in triplicate.

Furan analysis

Furan content in samples was determined using a method adapted from Fan (2005a) where modification to the method was done without spiking the juice with furan. Ultraviolet treated samples were kept in a -20°C deep freezer before being analysed for furan. Samples in 15 mL glass vials were incubated at 35°C for 25 minutes on a stir plate (Supelco) before a solid-phase microextraction (SPME) fiber (75 µm Carboxen-PDMS) was inserted into the headspace of a vial. After 20 minutes of extraction time, the SPME fiber was inserted into the gas chromatography (GC) injection port at 240°C and held for 5 minutes to desorb volatile compounds. Volatile compounds were separated by a Hewlett-Packard 5890/5971 GC-MSD (Agilent Technologies, Palo Alto, CA, USA) equipped with a 3.5 M GasPro capillary column (0.32 mm inner diameter) connected to a DB-5 column (30 m x 0.32 mm inner diameter, 0.1 µm film thickness; J & W Scientific, Folsom, CA, USA) using a Universal Prestight Connector (Restek Chromatography Products, Bellefonte, CA, USA). The GS-GasPro column, which is ideal for separating compounds that are gases at ambient temperature, was connected to a DB-5 column to establish an appropriate flow rate as required by the mass spectrometer. The temperature program of the GC oven was set to

50°C for 2 minutes, increased to 130°C at 10°C/minute, then to 250°C at 15°C/minute and held for 2 minutes at the final temperature. Helium was the carrier gas at a flow rate of 39 cm/s. The transfer line was held at 250°C during the entire run. Furan was identified by comparison of the spectra of the sample compounds with those of standards and by comparing retention times of sample compounds with those of the standards. The m/z (mass/charge) 39/68 was used for the confirmation of furan and m/z 68 was used as the quantifier. Furan was quantified using a standard curve established with the individual matrix (pummelo fruit juice) and corrected using the internal standard. The standard curve for furan with coefficient of determination, $R^2 = 99\%$.

Sugar content and ascorbic acid analysis

Sugars composition was analyzed using a method by Mohd Asraf *et al.* (2013). The juice samples were centrifuged at 3000 rpm for 2 minutes using a bench top centrifuge (EBA-20 Hettich Zentrifugen, Germany). An aliquot of fruit juice solution was filtered through 0.45 µm nylon membrane filter and injected (20 µL) into HPLC equipped with Genesis Carbohydrate column (250 mm x 4.6 mm, 4 µm) and refractive index (RI) detector (2414, Waters Milford Inc. USA) using mobile phase of acetonitrile:deionized water (85:15) at a flow rate of 1.0 mL/minute. Respective coefficient of determination for sucrose, glucose and fructose, $R^2 = 97 - 99\%$.

Ascorbic acid content was determined using the 2, 6-dichlorophenol-indophenol titration method described in Association of Official Analytical Chemists (AOAC, 1996). The ascorbic acid in 20 mL of fresh sample was extracted with 3% metaphosphoric acid (w/v). The extract volume was made up to 100 mL and then it was mixed and filtered. Ten millilitres of diluted and extracted ascorbic acid were titrated against standard 2, 6-dichlorophenol-indophenol dye which was already standardized against L-ascorbic acid standard solution with coefficient of determination, $R^2 = 99\%$. The ascorbic acid concentration was calculated by comparison with the standard and expressed as mg/L.

Analytical measurements

The total soluble solids were determined using a digital refractometer (D Series, Graigar Technology, China). Turbidity was measured using a turbidimeter (TN-100, Eutech, Singapore) by shining an incident light beam through a 10 mL sample juice with a scattering angle of 90°. Clarity was determined by measuring the absorbance at 660 nm using a UV-Vis spectrophotometer (UV-mini 1240, Shimadzu,

Japan). Distilled water was used as reference. All measurements were done in triplicate.

Statistical analysis

The data obtained in the study were analyzed using Minitab Release 14 (Minitab Inc., PA, USA). Analysis of variance was performed by ANOVA procedure and significant differences ($p < 0.05$) between factors were determined using general linear regression. Pearson's correlations were performed to determine the relationships between all factors. All analyses were done in triplicate.

Results and Discussion

From Figure 1, it was observed that furan formation increased with increment of UVC fluence ($p < 0.05$). The initial level of furan within the non-treated clarified pummelo juice was found to be 0.01 ± 0.003 ppb/mL. Meanwhile, the final level of furan was found at 28 mJ/cm² of UVC fluence to be 2.38 ± 0.22 ppb/mL (Table 1). The furan formation can be seen to have increased linearly ($R^2 = 0.95$) at a rate of 0.58 ppb per mJ/cm². The results further show that more furan was formed at higher fluences in the UVC-treated clarified pummelo juice. The results in this study is comparable to a study by Mueller *et al.* (2013) where the authors had found furan formation in a range of 2.3 to 3.7 ppb after naturally cloudy apple juice had been treated with UVC fluence of 39 to 195 mJ/cm².

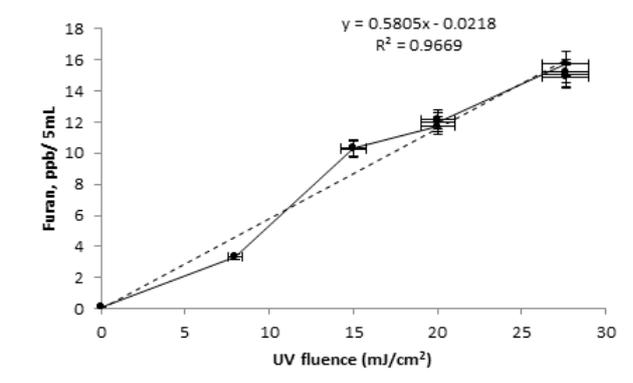


Figure 1. Formation of furan from the juice sample as a function of UVC fluence

The finding of this study, however was found to be unexpectedly higher than the results reported by Fan and Geveke (2007), where it was shown that 8.8 J/cm² (equivalent to 8800 mJ/cm²) would produce 14 ppb/mL of furan in commercial fresh apple cider, at a rate of 11 ppb per J/cm². The authors also claimed that significant amount of furan could be accumulated if the fruit juice was over-treated (Fan and Geveke,

Table 1 Physicochemical analysis of UVC-treated clarified pummelo juice as a function of UVC fluence

UVC fluence	Furan (ppb/mL)	pH	TSS‡ (°Brix)	Fructose (mg/mL)	Sucrose (mg/mL)	Glucose (mg/mL)	Total sugar (mg/mL)	Ascorbic acid (mg/L)	Clarity (abs)
Control	0.01 ± 0.00 ^a	4.02 ± 0.03 ^a	10.4 ± 0.01 ^a	15.77 ± 0.40 ^a	70.61 ± 0.34 ^a	14.86 ± 0.38 ^a	101.3 ± 0.26 ^a	516.7 ± 0.58 ^a	0.15 ± 0.01 ^a
15 mJ/cm ²	0.66 ± 0.00 ^b	3.95 ± 0.01 ^a	9.69 ± 0.11 ^b	14.60 ± 0.39 ^b	60.46 ± 0.30 ^b	12.82 ± 0.11 ^b	87.88 ± 0.77 ^b	296.0 ± 0.26 ^b	0.19 ± 0.00 ^b
18 mJ/cm ²	1.83 ± 0.21 ^c	3.95 ± 0.00 ^a	9.12 ± 0.01 ^c	11.56 ± 0.36 ^c	53.64 ± 1.49 ^c	12.64 ± 0.11 ^c	77.84 ± 1.85 ^c	274.0 ± 0.10 ^c	0.14 ± 0.00 ^a
28 mJ/cm ²	2.38 ± 0.22 ^d	3.97 ± 0.00 ^a	8.95 ± 0.02 ^d	10.73 ± 0.39 ^d	34.91 ± 0.30 ^d	11.35 ± 0.30 ^d	56.99 ± 0.71 ^d	266.3 ± 0.31 ^d	0.12 ± 0.00 ^c

*The means within the column followed by different letters were significantly different at p < 0.05

‡ Total Soluble Solids

Table 2 Correlation analysis on furan development in clarified juice

	UVC Treatment	Fructose	Sucrose	Glucose	Total sugar	Ascorbic acid	Clarity	TSS‡	pH
Fructose	-0.893* 0.000**								
Sucrose	-0.967 0.000	0.909 0.000							
Glucose	-0.982 0.000	0.848 0.000	0.929 0.000						
Total sugar	-0.974 0.000	0.930 0.000	0.998 0.000	0.938 0.000					
Ascorbic acid	-0.874 0.001	0.751 0.005	0.729 0.007	0.901 0.000	0.757 0.004				
Clarity	-0.523 0.081	0.758 0.004	0.689 0.013	0.418 0.176	0.688 0.013	0.163 0.612			
TSS‡	-0.946 0.000	0.962 0.000	0.904 0.000	0.932 0.000	0.928 0.000	0.889 0.000	0.581 0.047		
pH	-0.651 0.022	0.513 0.088	0.466 0.127	0.740 0.006	0.501 0.097	0.899 0.000	-0.135 0.676	0.707 0.010	
Furan	0.935 0.000	-0.985 0.000	-0.945 0.000	-0.896 0.000	-0.961 0.000	-0.782 0.003	-0.733 0.007	-0.973 0.000	-0.549 0.065

* Pearson's correlation value

**P-value at 0.05

‡ Total Soluble Solids

**Nine total samples were analysed for each of the physicochemical characteristics.

2007). However, contrasting to the finding of Fan and Geveke (2007), the furan formation in this study seemed to be diminutive in comparison to a study done by Bule *et al.* (2010) where the amount of furan found in apple juice treated with UVC fluence rate of 3.3×10^{-3} mW/cm² (equivalent to 5.94 mJ/cm²) was 1648 ppb/mL. Both literatures have claimed that furan formation is highly associated with the physicochemical properties of fruit juice, where fructose was found to be the main cause.

From the preliminary study, major components of pummelo fruit juice are sugars (fructose, sucrose and glucose) followed by ascorbic acid. Thus, with high amount of sugars and ascorbic acid, it is expected that the amount of furan will be high post-UVC treatment in addition to the effect of UVC fluence. Table 1 shows the post-UVC effect on the

physicochemical properties of clarified pummelo juice. With the exception of pH, all other properties were shown to have a significant 5% effect from the control sample. Furan was observed to have a drastic increase, moreover after 28 mJ/cm² of UVC fluence. UVC wavelength at 254 nm has been reported to have higher energy and may induce more furan formation (Fan and Geveke, 2007) in comparison to a wavelength of 260 – 270 nm.

Table 2 further summarizes the correlation analysis of furan development and its dependency on the physicochemical properties of UVC-treated clarified pummelo juice. Fan and Geveke (2007) had also found that solution with lower pH could have an effect on furan formation. In addition to that, they also found that sucrose, glucose and ascorbic acid had little effect towards furan formation. Contrastingly,

in the present study, furan formation post-UVC treatment was observed to have high correlations with significant p-values ($p < 0.05$) towards sugar content (fructose, sucrose and glucose) and total soluble solids of the tested sample. Soluble solids content, which measures mostly sugar, was seen to have a linear relationship with the total sugar content ($r = 0.93$, $p < 0.05$) and subsequently had a direct correlation towards furan formation ($r = 0.96$, $p < 0.05$).

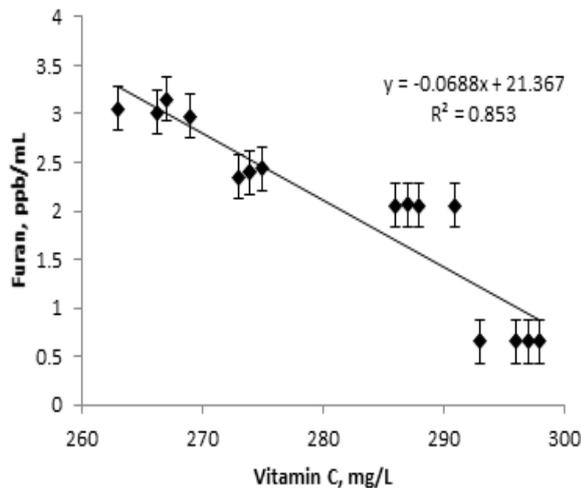


Figure 2. Furan formations as a function of ascorbic acid content in samples

These findings are comparable to the study of gamma-irradiation (Fan, 2005a) and thermally treated fruit juice (apple and orange juices) (Fan, 2005b), where both studies had found that ascorbic acid, sucrose, fructose and glucose were responsible in promoting furan formation. A claim by Fan and Geveke (2007) that suggested UVC had a different mechanism in inducing furan formation compared to gamma-irradiation and thermal, seemed to be in the opposite with the findings of this study. Weak relationships between furan formation and clarity ($r = 0.733$, $p < 0.05$) together with pH ($r = 0.55$, $p > 0.05$) showed that these properties may not influence the furan formation within the UVC-treated samples. These findings further decline the results of Owczarek-Fendor *et al.* (2010) where the authors observed significant furan formation with increasing pH level. However, it was interesting to note that pH increment affecting the furan formation was found in a starch-based model, whereas pummelo juice does not contain starch. Thus, the findings of present study could have a substantial reason to not follow the trend of the previous reports. Furan formation was found to be inversely proportional to the content of ascorbic acid. Pearson's correlation, $r = 0.782$ and significant p-value of 0.003 ($p < 0.05$) showed that furan formation is influenced by the amount of

ascorbic acid within the UVC-treated juice (Figure 2). Similarly, Owczarek-Fendor *et al.* (2010) also reported of decrement in furan formation when ascorbic acid is increased within the tested samples.

In addition to that, Owczarek-Fendor *et al.* (2010) also added that a clear influence of temperature on furan formation was obtained, especially with the presence of ascorbic acid. They further added that the furan formation was attained at a rate of 1 ppb for every 28°C increment. In comparison, the results of this study are significantly ($p < 0.05$) high, at a rate of 1 ppb to 20°C in which the maximum temperature recorded was 48°C (UVC fluence of 30 mJ/cm²) (Figure 3). Nevertheless, according to the USFDA survey (USFDA, 2004), the amount of furan found in UVC-treated clarified pummelo juice (0.66 to 2.4 ppb/mL) was much less than the ones reported in thermally processed juices (2.5 – 8.4 ppb/mL). Therefore, UVC disinfection can be an excellent alternative pasteurization method producing healthier and safer juice for public consumption.

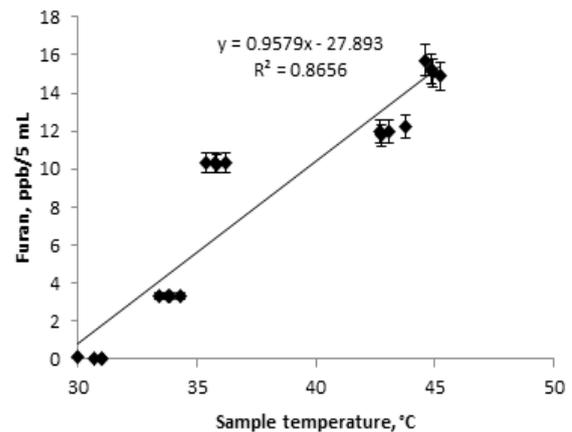


Figure 3. Furan formations in relation to sample's temperature

Conclusion

The present study evaluated the effects of UVC pasteurization on the furan development in pummelo fruit juice. Furan development was seen to be closely dose-dependent and inversely proportional to the amount of fructose, sucrose, glucose and ascorbic acid ($p < 0.05$) contradicting with the results of previous studies. Nevertheless, the amount of furan found in UVC-treated clarified pummelo juice (between 0.66 to 2.4 ppb/mL) was much less than the ones reported by the USFDA survey (2004) of various food products thermally treated. Thus, clarified pummelo juice with absorption coefficient, α of 17 cm⁻¹ can be safely treated with a minimum of 20 to 30 mJ/cm² of UVC irradiance using dean vortex ultraviolet light system.

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