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Effect of thermal and ultraviolet treatments on the stability of antioxidant compounds in single strength pineapple juice throughout refrigerated storage

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Abstract: Thermal treatment is commonly applied in juice manufacturing as a method to pasteurize juices. However the heat may deteriorate some of the essential compounds in the juice, especially heat-sensitive antioxidants. Therefore non-thermal treatment such as ultraviolet (UV) ray has been proposed as an alternative for pasteurization. The objective of this study was to compare the effect of thermal and UV treatments on the content of antioxidants (phenolic acids, flavonoids, carotenoids, ascorbic acids) and antioxidant capacity of single strength pineapple juice. The antioxidants stability of juices throughout 14 days of refrigerated storage was also studied. Ultraviolet treatment shows higher ascorbic acid content after treatment as compared to thermally treated single strength pineapple juice. Storage time affected the studied antioxidants, where UV treatment provided better stability to ascorbic acid content while thermal treatment provided better stability to flavonoids and carotenoids.

Keywords: Thermal, ultraviolet, pineapple juice, antioxidant, storage stability

Introduction

Pineapple (*Ananas comosus* L.) is one of the most appreciated tropical fruits around the world and is consumed as fresh fruits, juice and others. Pineapple was reported to contain polyphenolic compounds and also possess antioxidant activity (Hossain and Rahman, 2011). Studies show that compounds such as phenolics and flavonoids are responsible for providing antioxidant activity (Kahkonen *et al*., 1999; Alothman *et a*l., 2009a; Mhatre *et al*., 2009; Danino *et al*., 2009). Juice extracted from pineapple can be processed into many forms such as concentrated juice, mixed juice and single strength juice. Single strength pineapple juice is categorized as juice that possess 12.5% total soluble solids, pH of 3.6, 0.54% of titratable acidity and 5.6% of suspended insoluble solid (Hongvaleerat *et al.,* 2008).

Thermal and non-thermal processes are usually applied to ensure the safety of processed juice. Thermal treatment is the most common treatment used in the food industry to ensure safety of the products. The absence of heat in non-thermal technology i.e. UV irradiation produces better quality products compared to thermal treatment (Noranizan and Benchamaporn, 2007). Irradiation is a non-thermal preservation method which involves the exposure of food materials or products to radiation, for example ultraviolet rays. Ultraviolet light at 254 nm is lethal to most microorganisms (Bintsis *et al*., 2000) such as Pseudomonas spp. on fresh meat (Stermer *et al*., 1987) and Salmonella typhimurium on fish (Huang and Toledo, 1982), and it also can be applied to prolong shelf life of food such as orange juice (Tran and Farid, 2004). Ultraviolet was reported to result in 90.99% inactivation of total viable bacteria and total inactivation of yeast and mold in pineapple juice (Noranizan *et al*., 2011).

Given the great benefits provided by antioxidants, processed fruit juices that retained high amount of antioxidants from the fresh fruits are in demand. However, some of the antioxidants are destroyed by thermal treatment (Lessin *et al*., 1997; Lee and Coates, 2003; Cortés *et al*., 2006; Aguilar-Rosas *et al*., 2007; Gama and Sylos, 2007) and UV treatment (Pan *et al*., 2004; Koutchma, 2009). The losses of antioxidants may lead to colourless products, such as reported by Chen e*t al*. (1995) in carrot juice, and thus causes lower quality products. Hence, the objective of this study was to compare the effect of thermal and UV treatments on the antioxidant stability of single strength pineapple juice throughout 14 days of refrigerated storage.

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Materials and Methods

Pineapple juice production

Pineapple (Ananas comosus) fruits of Morris variety were purchased froma Pasar Borong Selangor, Malaysia. The pineapples were washed using tap water, shells were removed and the flesh was cut into small pieces using Food Slicer (EMURA ECA-201, Japan). The pineapple juice was extracted using a milling machine (Super mass colloider, Masuko Sangyo, Japan).

Juice clarification

Extracted pineapple juice was clarified by treatment with Pectinex Ultra SP-L enzyme (0.025%, v/v) at 30°C using water bath (circulating) for 30 mins. The clarified juice was then filtered using muslin cloth and the collected juice was subjected to either thermal or UV treatment immediately.

Juice treatments

Ultraviolet treatment was performed using CiderSure 3500-B (Macedon, New York) with average power of 2.3 mW/cm^2, exposure duration of 3.3013 seconds and dosage applied was 7.5 mJ/cm^2. A preliminary study has shown that pectinase was inactivated at this UV dose. Thermal pasteurization was performed by heating the pineapple juice at 97^{o}C for 5 mins, and this inactivated the pectinase enzyme (Sairi, 2005). Freshly clarified pineapple juice was used as the control. All treated and control samples were stored at 4±1^{o}C before analysis.

Antioxidants extraction

Extraction was performed according to Alothman *et al.* (2009a) with slight modifications on the centrifugation speed. Antioxidants of treated pineapple juice were extracted with ethanol (70%) for 3 hrs at ambient temperature using Snaker Bath (Hotech Instrument, Taiwan) with stirring. Then, it was centrifuged at 1500 x g using Kubota 5800 Refrigerated Centrifuge (Tokyo, Japan) for 15 mins at 26^{o}C. The supernatant was concentrated using rotary evaporator (Tokyo, Japan) at 50^{o}C until no solvent can be collected and evaporation was continued for another 5 mins to ensure total evaporation of solvent. The concentrated sample was store at 4±1^{o}C until analysis.

Determination of total phenolics content

Total phenolic content (TPC) analysis was performed using Folin-Ciocalteu method according to Alothman *et al*. (2009a). Wavelength used was 765nm and TPC of the sample was expressed as mg of gallic acid equivalent (GAE) / 100g sample on fresh weight basis.

Determination of total flavonoids content

Total flavonoids content (TFC) analysis was performed according to Alothman *et al*. (2009a). Wavelength used was 510 nm and TFC of the sample was expressed as catechin equivalent (CEQ)/ 100g sample on fresh weight basis.

Determination of ascorbic acid content

Ascorbic acid content (AAC) of juice were determined according to method of AOAC Method 967.21, 45.1.14(1) using 2, 6-dichloroindophenol (AOAC, 2000).

Determination of total carotenoids content

Total carotenoids content (TCC) analysis was performed according to Moore (2003). The wavelength used was 450 nm and the TCC was calculated according to the following formula and using extinction coefficient of 2500 (Lima *et al*., 2005):

$$\text{Total carotenoid content (mg/L)} = \frac{\text{Absorbance x extraction volume}}{\text{Sample weight x 100 x extinction coefficient}}$$

Determination of antioxidant capacity

The antioxidant capacity of the extracted samples were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH)according to method of Alothman *et al*. (2009a)withmodificationsontheDPPHconcentration. Methanolic DPPH solution (0.15 mM) was added to the concentrated sample and absorbance at 515 nm against a blank solution (methanol solution) was measured using spectrophotometer. Methanolic DPPH solution without concentrated sample was used as absorbance control. Antioxidant capacity of sample was expressed as the percentage of inhibition of DPPH (Azizah *et al*., 2009).

Storage stability of antioxidants

Control and treated samples (thermal and UV treated) were refrigerated at 4±1^{o}C for 14 days. Antioxidants content of these samples were analysed every 2 days. The storage duration of 14 days were chosen based on the shelf life of juices studied by Tran and Farid (2004), Baxter *et al.* (2005) and Majid *et al.* (2008).

Statistical analysis

The data obtained were analyzed and interpreted by analysis of variance (ANOVA) using MINITAB v.14 Statistical Package (Minitab Inc., State College, Pennsylvania). Values expressed were mean ±

standard deviations. Significance level was set at $p \leq 0.05$. All analysis was carried out in triplicates.

Results and Discussions

Effect of treatments on antioxidants

According to Table 1, the ascorbic acid content (AAC) between juices studied has significant differences ($p \leq 0.05$) with the sequence: thermally treated juice < UV treated juice < control. This shows that UV treatment is a better processing method to retain the heat-sensitive ascorbic acid than thermal treatment due to absence of heat in UV treatment. Significant ($p \leq 0.05$) reduction of AAC of thermally treated juices in this study was in agreement with several authors (Achinewhu and Hart, 1994; Iversen, 1999; Tiwari *et al*., 2009; Alothman *et al*., 2009b). Ultraviolet treatment has also been reported to reduce AAC in juices (Tran and Farid, 2004; Alothman *et al*., 2009b; Bhat *et al*., 2011).

As for total carotenoids content (TCC), control sample had significantly ($p \leq 0.05$) higher content than both thermal and UV treated juice. The lower TCC in thermal and UV treated juice can be explained by the thermo labile and light sensitive nature of carotenoids. The TCC of UV treated juice was slightly higher than thermally treated juice but the difference was not significant ($p > 0.05$). This might be due to the nature of double bonds in carotenoids which easily absorbed UV and then undergo the process of UV photolysis. Significant reduction of TCC in orange juice after thermal treatment was also reported by other authors (Lee and Coates, 2003; Gama and Sylos, 2007).

The total phenolic content (TPC) of both thermal and UV treated samples shows reduction but not significant as compared to control sample ($p > 0.05$). The TPC between thermal and UV treated samples have no significant differences ($p > 0.05$). In significant reduction of TPC has also been reported in thermally and UV treated pomegranate juice (Pala and Toklucu, 2011).

For flavonoids, the total flavonoids content (TFC) of thermally treated juice has no significant differences ($p > 0.05$) compared to control sample. However, slight increment in TFC was observed. Flavonoids increment has been reported where thermal treatment releases more flavonoids out of cell matrix, which might be the reason for increment of TFC of thermally treated juice in this study (Bruijn *et al*., 2008). The TFC of UV treated juice in this study recorded insignificant changes ($p > 0.05$). However in the literature, it was reported that TFC increased after UV treatment such as in starfruit juice (Bhat *et al*., 2011) and citrus fruit (Arcas *et al*., 2000). The increment of TFC is due to the increased biosynthesis of phenylalanine ammonia lyase (PAL). After irradiation, PAL content increases (Hadwiger and Schwochau, 1971; González-Aguilar *et al*., 2007; Charles *et al*., 2008; Pombo *et al*., 2011;) and this in turn increases the biosynthesis of flavonoids (González-Aguilar, 2007; Alothman *et al*., 2009b). The reason that different result obtained in this study as compared to those in the literature was not determined, but the possible reason might be that UV dosage applied in this study was lower than authors discussed above.

Table 1. Antioxidants content and activity of pineapple juices

	Thermally pasteurized juice	UV irradiated juice	Control (no treatment)
Ascorbic acid (mg/100g)	10.065 ± 0.798^{b}	12.673 ± 1.113^{c}	16.424 ± 0.971^{a}
Carotenoids (µg/L)	5.084 ± 0.994^{b}	5.810 ± 0.866^{b}	6.694 ± 0.334^{a}
Phenolic acids (mg GAE/100g)	41.810 ± 14.590^{a}	43.020 ± 4.380^{a}	47.130 ± 10.860^{a}
Flavonoids (mg CEQ/100g)	24.933 ± 6.890^{a}	20.021 ± 5.181^{a}	22.423 ± 7.787^{a}
Antioxidant capacity (%)	19.807 ± 6.523^{b}	29.793 ± 4.991^{ab}	35.443 ± 4.433^{a}

Mean values having different superscript low case letter within the row are significant different ($p < 0.05$)

The antioxidant capacity of UV treated juice was higher than thermally treated juice but with no significant difference ($p > 0.05$).There was only ascorbic acid in UV treated juice that was significantly higher ($p \leq 0.05$) than thermally treated juice.

Storage stability of antioxidants

In this study, all samples showed similar decreasing trend of TPC throughout storage but both thermal and UV treated samples had significantly ($p \leq 0.05$) higher reduction after 2 weeks storage compared to control sample (Figure 1). The TPC between thermal and UV treated juices had no significant ($p > 0.05$) difference after 2 weeks of storage. This shows that UV and thermal treatments reduced TPC after 2 weeks of storage and neither UV nor thermal treatment offered good stability to phenolic acids during storage. Reduction of TPC in thermally treated grapefruit during storage has been reported (Igual *et al*., 2011).

The TFC between all samples studied had significant ($p \leq 0.05$) differences after 2 weeks of storage. Both thermally and UV treated samples exhibited decreasing trend during storage with thermally treated juice retaining higher flavonoids (Figure 2).The greater reduction of TCC in UV-treated juice might be due to the oxidation enzyme which cannot be inactivated by UV treatment, while thermal treatment inactivates enzymes. The reduction of TFC after storage was reported by other authors (Awad and Jager, 2000; Caro *et al*., 2004). Flavonoids of pineapple juice in this study were unstable as it was

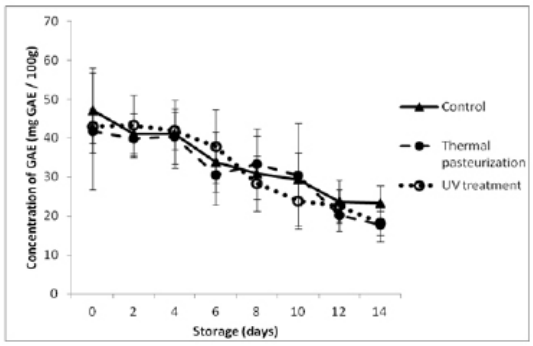

Figure 1. Storage stability of total phenolic content in treated and control samples throughout 14 days of refrigerated storage

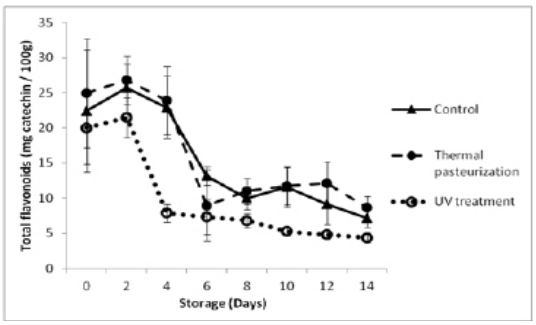

Figure 2. Storage stability of totalflavonoidscontent in treated and control samples throughout 14 days of refrigerated storage

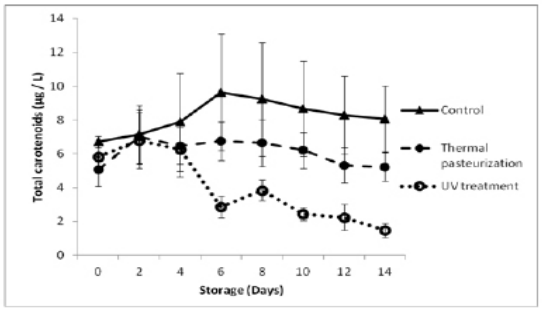

Figure 3. Storage stability of totalcarotenoids content in treated and control samples throughout 14 days of refrigerated storage

observed that TFC in all samples studied reduced drastically from day 2 to day 6.

The TCC between all samples studied had no significant ($p > 0.05$) difference after storage in the sequence of: UV treated juice < thermally treated juice < control sample. There was slight increment in the control sample with no significant ($p > 0.05$) difference during storage, which was also reported for kiwi fruit (Tavarini *et al*., 2008). The TCC of thermally treated juice was relatively stable and shows no significant ($p > 0.05$) difference after storage while UV treated juice shows reduction (Figure 3). The TFC and TCC of thermally treated juice were higher than UV treated juice after 2 weeks of storage. This shows that thermal treatment offered more stability to flavonoids and carotenoids of pineapple juice in this study.

The AAC of control sample was stable over time and had no significant ($p > 0.05$) reduction. This was in agreement with the stability of AAC in untreated orange juice during 12-day storage duration (Caro *et a*l., 2004). Throughout the whole storage period, the AAC of samples in this study followed the sequence: thermal treated juice < UV treated juice < control sample. This shows that UV treatment provides better stability to ascorbic acid than thermal treatment throughout 2 weeks of storage. Reduction of AAC has previously been reported in thermally pasteurized orange juice (Polydera *et al*., 2003; Plaza *et al*., 2006; Tiwari *et al*., 2009) and pineapple juice (Achinewhu and Hart, 1994) during storage.

Conclusions

Ultraviolet treatment shows higher ascorbic acid content after treatment as compared to thermally treated single strength pineapple juice. Storage time affected the studied antioxidants, where UV treatment provided better stability to ascorbic acid content while thermal treatment provided better stability to flavonoids and carotenoids. Further studies are recommended in order to know more about the effects of UV treatment and treatment parameter on the mechanism involving antioxidants stability.

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