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Effects of combining ultraviolet and mild heat treatments on enzymatic activities and total phenolic contents in pineapple juice

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ABSTRACT

Ultraviolet (UV) is able to inactivate most microorganisms in fruit juices with a low absorption coefficient but its effect is limited in inactivating undesired enzymes. The aim of this study was to overcome limitation of ultraviolet light (UV) by combining mild heat with UV. Pineapple juice was treated with mild heat (temperature: 50, 55 and 60 °C; holding time: 10, 20 and 30 min) and subsequently exposed to UV (5.61, 7.55 and 11.23 $mJ \cdot cm^{-2}$). The effects of these combined treatments on pectin methylesterase (PME), bromelain activities and total phenolic content (TPC) were determined. Both enzymatic activities were reduced by mild heat but not by UV treatment. Increasing holding time and UV dosage led to higher depletion of TPC. Treating pineapple juice with mild heat at 55 °C for 10 min and UV at 5.61 $mJ \cdot cm^{-2}$ decreased PME by 60.53% whilst retaining 61.57 ± 0.21% and 72.80 ± 0.33% of bromelain and TPC, respectively.

Industrial relevance: As opposed to traditional heat pasteurisation, ultraviolet (UV) treatment has the potential to produce pineapple juice with added value, such as high amount of health benefiting phenols and bromelain. Despite being known for being economically feasible, this technology is not widely adapted by the industry due to its inability to inactivate pectin methylesterase (PME). To overcome the limitation of UV, mild heat (MH) is introduced as hurdle technology. This study demonstrates that combining UV and MH could be able to effectively inactivate the PME in pineapple juice whilst preserving relatively high amount of bromelain and phenols.

1. Introduction

Pineapple juice is famous for its sweet and sour taste as well as beneficial health compounds, such as bromelain and phenols. Bromelain is the major proteolytic enzyme complex that is found prominently in pineapple (Hale, Greer, Trinh, & James, 2005). Hale et al. (2005) also reported that proteolytic activity is required for the therapeutic effect of bromelain. Bromelain exhibits therapeutic and pharmacological effects such as anti-inflammatory, tumour growth modulation and aid in digestion (Taussig & Batkin, 1988; Hale, 2004; Chobotova, Vernallis, Adibah, & Majid, 2010). On the other hand, phenols are known for their antioxidant properties. Epidemiological studies have shown that frequent consumption of natural antioxidants prevents cancers and lower cardiovascular disease.

Apart from the beneficial health compounds, pineapple juice is also well accepted for its distinctive mouthfeel and yellowish colour. Studies showed that the colour of pineapple juice is stable because there is no enzymatic browning; this is due to the low polyphenoloxidase activity in this juice (Das, Bhat, & Gowda, 1997; Chaisakdanugull, Theerakulkait, & Wrolstad, 2007; Perera, Gamage, Wakeling, Gamlath, & Versteeg, 2010; Supapvanich, Prathaan, & Tepsorn, 2012). Mouthfeel of juices can be influenced by the cloudiness of juice. Cloudiness of juices is attributed to the suspension of particles composed of a complex mixture of protein, pectin, lipids, hemicellulose, cellulose and other minor components (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2009). The cloud stability of juice can be affected by the presence of pectin methylesterase (PME). PME hydrolyses the ester bonds of pectin in juices, resulting in decreased cloud stability (Rouse & Atkins, 1952). PME is generally inactivated using conventional heat pasteurisation.

Although conventional heat pasteurisation is able to inactivate PME in pineapple juice, it compromises the heat sensitive phenolic compounds (Goh, Noranizan, Leong, Sew, & Sobhi, 2012) and bromelain (Bhattacharya & Bhattacharya, 2009) as well as the quality of the juice (Rattanathanalerk, Chiewchan, & Srichumpoung, 2005; Shamsudin, Noranizan, Yap, & Mansor, 2014; Shamsudin, Chia, Mohd Adzahan, & Wan Daud, 2013). Bhattacharya and Bhattacharya (2009) reported that devoid of bromelain leads to the absence of proteolytic activity in pineapple products. Similarly, the absence of phenols decreased the

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antioxidant capacity. Hence, researchers have looked into potential alternative treatments, such as ultraviolet (UV), to overcome the drawback of heat pasteurisation.

Ultraviolet (UV) treatment is one of the most promising and economical nonthermal preservation technologies (Mohd Adzahan & Benchamaporn, 2007; Mohd Adzahan et al., 2011a). Its efficacy on fruit juices depends on the absorption coefficient of the juice, which affects the UV penetration and thus microbial lethality. The absorption coefficient is influenced by the properties of juice, such as colour and cloudiness (Murakami, Jackson, Madsen, & Schickedanz, 2006).

UV has been reported to be effective in extending shelf life (Chia, Rosnah, Noranizan, & Wan Ramli, 2012) whilst preserving the quality (Caminiti et al., 2012; Falguera, Pagán, & Ibarz, 2011a) and phenolic compounds (Falguera, Pagán, & Ibarz, 2011a; Goh et al., 2012) of fruit juices. Its microbial lethality effects were also reported by many authors (Falguera, Pagán, Garza, Garvín, & Ibarz, 2011b; Guerrero-Beltrán & Barbosa-Cánovas, 2004; Mohd Adzahan, Sulaiman, Sew, & Karim, 2011b; Sew, Ghazali, Martín-Belloso, & Noranizan, 2012; Shama, 1999). The only drawback to this treatment is its inability to inactivate undesirable enzymes in fruit juice, especially PME. Although Falguera, Pagán, and Ibarz (2011a) and Mohd Adzahan, Lau, et al. (2011a) reported that UV totally inactivates PME in apple juice, this could only be achieved after 40 min of UV treatment. It is time consuming and not economically acceptable. Therefore, combined treatment has been suggested to overcome the shortcomings of UV treatment.

Combined treatments, such as UV with another treatment (either chemical or physical), could have synergistic effects on fruit juice (Gayán, Mañas, Álvarez, & Condón, 2013; Halim et al., 2012). One of the most commonly used treatments is the combination of UV with mild heat. Combined treatment with UV and mild heat was reported to achieve higher inactivation of PME (Gayán, Serrano, Monfort, Álvarez, & Condón, 2012) and microorganisms (Durak, Churey, & Worobo, 2012; Gayán et al., 2013; Marquenie et al., 2003; Tyrrell, 1976) than UV alone. Similar findings were reported in PME inactivation of juices treated with mild heat combined with other nonthermal treatments, such as thermosonication (Wu, Gamage, Vilkhu, Simons, & Mawson, 2008) and high intensity pulsed electric field (Vega-Mercado, Powers, Barbosa-Cánovas, & Swanson, 1995). This suggests that combining nonthermal treatments with mild heat could be a better alternative when nonthermal treatments alone are insufficient.

Despite the numerous published reports on the effect of nonthermal treatments combined with mild heat on fruit juices, none of them was on pineapple juice. There is a need for alternative combined treatments that can preserve phenols and bromelain in pineapple juice and simultaneously inactivate PME to ensure the juice quality. Furthermore, studies on UV treatment combined with mild heat are still insufficient. Thus, the present study was conducted to determine the effect of combined treatment (UV and mild heat) on bromelain and PME activities and the total phenolic contents of pineapple juice.

2. Materials and methods

2.1. Sample preparation

Pineapples (*Ananas comosus*) of the Morris variety at commercial maturity were bought from a local retailer (Pasar Borong Selangor, Malaysia). The fruits were washed, peeled and cut. Pineapple pieces were mashed with a milling machine (Super mass collider, Masuiko Sangyo, Japan). Pectinex® Ultra SP-L (Sigma Aldrich, Denmark) (0.025% v/v) was then added and allowed to react with pineapple mash at 30 °C for 30 min (Sreenath, Sudarshanakrishna, & Samthanam, 1994). Pectinex® Ultra SP-L was added to increase the juice yield and facilitate the subsequent filtration process. Pineapple juice was obtained after filtering the pineapple mash with a muslin cloth.

2.2. Juice treatment

Pineapple juice was treated with mild heat (MH) followed by ultraviolet (UV). The MH temperature (T) and holding time (t) ranged from 50 to 60 °C and 10 to 30 min, respectively. After mild heat treatment, the pineapple juice was exposed to a UV pasteuriser (FPE Inc, New York, USA) at different dosages (5.61, 7.55 and 11.23 $\text{mJ}\cdot\text{cm}^{-2}$) by modifying the flow rate (0.043, 0.064 and 0.086 $\text{L}\cdot\text{s}^{-1}$). This pasteuriser was equipped with two sensors that detected the UV intensity. The range limits of MH temperature, holding time and UV dosages were determined based on a study conducted by Sew et al. (2012). The authors reported that *Listeria innocua* in pineapple juice was totally inactivated (>7 log) when pineapple juice was treated at 55 °C for 10 min with UV dosage of 7.55 $\text{mJ}\cdot\text{cm}^{-2}$. The MH- and UV-treated pineapple juice (referred to as MH + UV) was then collected into clean, sterilised glass bottles and capped with sterilised cap bottles. The MH + UV-treated pineapple juices were stored at 4 ± 1 °C until analysis. Three samples were prepared for each treatment.

2.3. UV dosage measurement

2.3.1. Absorption coefficient of sample

The absorption coefficient of pineapple juice was calculated from the equation of Beer–Lambert's law (Murakami et al., 2006).

$$I_x = I_o \exp(-ax) \quad (1)$$

where α is the absorption coefficient of the solution, x is the distance between the UV source and the sensor (0.048 cm), I_x is the UV intensity measured by the sensor, and I_o is the UV intensity measured on the surface of the sample solution (4000 $\mu\text{J}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$).

2.3.2. Sample exposure time

The exposure time (t) of pineapple juice was calculated using the following equation, and expressed in seconds (s):

$$\text{Exposure time}, t = \frac{\text{volume of treatment chamber, L}}{\text{flow rate, L}\cdot\text{s}^{-1}} \quad (2)$$

where the volume of the treatment chamber is 0.213 L.

2.3.3. UV dosage

The dosage (D) for UV treatment of juice was calculated using the following equation:

$$\text{Dosage}, D\left(\text{mJ}\cdot\text{cm}^{-2}\right) = \frac{\text{Intensity}, I\left(\mu\text{J}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}\right) \times \text{Exposure time}, t(\text{s})}{1000}. \quad (3)$$

The UV dosages used in the present study were 5.61, 7.55 and 11.23 $\text{mJ}\cdot\text{cm}^{-2}$ (Table 1).

2.4. Enzyme assays

2.4.1. Proteolytic activity

The primary indicator of bromelain activity in pineapple products is proteolytic (PRO) activity (Bhattacharya & Bhattacharya, 2009; Hale

Table 1
Flow rate, exposure time, intensity and dosage of ultraviolet treatment on pineapple juice.

Flow rate, $\text{L}\cdot\text{s}^{-1}$	Exposure time, s	Intensity, $\mu\text{W}\cdot\text{cm}^{-2}$	Dosage, $\text{mJ}\cdot\text{cm}^{-2}$
0.043	4.94	2274	11.23
0.064	3.32	2274	7.55
0.086	2.47	2272	5.61