Combined effects of pulsed electric field and ultrasound on bioactive compounds and microbial quality of grapefruit juice

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Abstract
The combined effect of ultrasound (US) and pulsed electric field (PEF) was investigated on microbial load and bioactive compounds of grapefruit juice. Grapefruit juice was PEF treated (flow rate: 80 ml/min, pulse frequency: 1 kHz, 20 kV cm−1 electric field strength, temperature: 40 ± 8°C, time: 600 s) followed by US treatment in an ultrasonic bath cleaner radiating 600 W at frequency of 28 KHz and 20 ± 8°C for 30 min. PEF and US treatment resulted in a significant reduction in microbial load as compared to the control group. Using combined (PEF + US) treatment, carotenoids, lycopene, anthocyanin contents, and total antioxidant activity were increased from 0.84 µg/ml, 0.32 µg/ml, 1.37 mg/L, and 177.48 ascorbic acid equivalent mg/g (control) to 1.26 µg/ml, 0.92 µg/ml, 1.68 mg/L, and 262.32 ascorbic acid equivalent mg/g, respectively. The findings demonstrated that PEF + US could be successfully used for preserving bioactive compounds in grapefruit juice while improving the microbial quality for a better shelf-life.

1 INTRODUCTION

Among citrus juices, grapefruit juice is one of the most popular and nutrient enriched beverages that contains significant amounts of citric acid and phenolic compounds, which play a vital role in lowering the onset of many diseases (Aadil, Zeng, Han, & Sun, 2013). Keeping in view the short shelf-life and consumers’ preferences toward fresh and minimally processed juices, there is dire need to develop various novel processing techniques (Tournas, Heeres, & Burgess, 2006, Tran & Farid 2004). Additionally, consumers’ concerns for healthy food choices, food industry is paying more attention on those innovative techniques that can keep the juices fresh for longer time with minimal nutrient depletion. Nonthermal processing technologies are much applicable alternative for thermal processing of food. The most commonly employed techniques include US, high pressure processing (HPP), PEF, ultraviolet, and irradiation, etc. The key benefit associated with the use of these techniques is improved nutritive value of food products due to the less thermal degradation of heat sensitive nutrients. Additionally, nonthermal food processing (Liu, Zeng, Sun, & Aadil, 2015; Saeeduddin et al., 2015, 2016; Zhang et al., 2015) techniques also provide microbial safety to the food without thermal degradation (Grahl & Märkl, 1996). A wide range of studies has confirmed the potential of nonthermal processing methods to enhance the quality and shelf stability of various food products (Aadil, Zeng, Sun, et al., 2015; Aadil, Zeng, Wang, et al., 2015). In this regard, combination of PEF and sonication is a novel nonthermal method, which has shown its capability to augment the safety and quality of fruit juices with minimal nutrient losses (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006; Yeom, Streakeaker, Zhang, & Min, 2000). Further, the other potential benefits of this method are reduced processing time, less energy inputs, high throughput, and environment friendly (Tiwari, O’Donnell, Patras, Brunton, & Cullen, 2009).
In recent years, the expectancy of combinative effect of these nonthermal techniques in quality enhancement and reduction in the quality deterioration of fruit juices have been extensively studied, which has proved their potential to attenuate the microbial load in juices besides increasing the shelf life of the product (Noci et al., 2008; Ross, Griffiths, Mittal, & Deeth, 2003). Furthermore, ultrasound processing of fruit juices has also shown up to 5 log reductions in the microbial population (Salleh-Mack & Roberts, 2007; Valero et al., 2007) without imposing adverse effects on the quality of juice. Likewise, several studies have also confirmed significant microbial reduction, enzyme inactivation, and quality improvement of the fruit juices without affecting the physicochemical attributes in a negative way by using nonthermal techniques (Qin et al., 1995). However, very little scientific data on the combination of PEF & US are available. Accordingly, the main objective of the present work is to investigate the effect of PEF + US on various quality attributes of grapefruit juice. This is potentially the first study reporting on the combined effect of PEF and US on microbial growth, lycopene contents, anthocyanins, total carotenoids, and bioactive compounds in grapefruit juice.

2 MATERIALS AND METHODS

2.1 Chemicals required

Ascorbic acid was obtained from Sigma-Aldrich (St. Louis, MO). Quercetin, sulfural acid gallic acid, DPPH, ammonium molybdate, catechin hydrate, and Folin–Ciocalteu reagent were taken from the Aladdin Industries (Shanghai, China). Potato dextrose agar media (PDA), peptone, and molten plate count agar were purchased from Guangdong Huankai Sci. & Tech. (Guangzhou, China). BHT n-hexane/acetone, aluminum trichloride, sodium acetate anhydrous, sodium carbonate, potassium chloride buffer, methanol, sodium sulfate, sodium nitrite, ethanol, sodium phosphate, and sodium acetate buffer were procured from the Sinopharm Chemicals Industry Reagent (Shanghai, China). All chemicals and reagents which are used in this study are of analytical grade.

2.2 Preparation of grapefruit juice sample, PEF, and US treatments

Fresh grapefruits (Citrus maxima, family Rutaceae) were bought from a fruit market (Guangzhou, China). Grapefruits were washed clearly and crushed by using juice extractor (JM 352, Midea Group Limited, Guangzhou, China) to produce fresh grapefruit juice. Then, fresh grapefruit juice was thoroughly mixed and classified as control, US, PEF & PEF + US. PEF treatment was done in a continuous PEF system as method mentioned previously by Aadil, Zeng, Wang, et al. (2015), while US treatment was performed in an ultrasonic bath cleaner as method cited prior by Aadil, Zeng, Wang, et al. (2015). PEF + US was processed by PEF treatment followed by US treatment.

2.3 Total carotenoids, lycopene, and total anthocyanins contents

The TC contents were calculated by the method reported by Liao et al. (2007) with some changes as earlier reported by Aadil, Zeng, Wang, et al. (2015). Lycopene was evaluated by using the method reported by Oliu, Serrano, Fortuny, and Belloso (2009) with some minor amendments earlier reported by Aadil, Zeng, Wang, et al. (2015). Total anthocyanins were determined by the pH-differential method described by Lee, Durst, and Wrolstad (2005) with some minor amendments earlier reported by Aadil, Zeng, Wang, et al. (2015).

2.4 Microbiological analysis

Microbiological analysis was performed according to the method mentioned in FDA’s Bacteriological Analytical Manual (FDA, 2001). Total plate counts (TPC) using nutrient agar medium were counted by pour plate method, while yeast, and mold (Y&M) counts were performed on PDA medium. The results were depicted as log colony-forming units (CFU/ml) of grapefruit juice.

2.5 Total antioxidant capacity and DPPH radical scavenging activity

Antioxidant capacity of the grapefruit juice was measured by the method described by Prieto, Pineda, and Aguilar (1999) with some minor amendments earlier reported by Aadil et al. (2013). DPPH free radical scavenging activity of the grapefruit juice was measured using a method as described by Yi, Yu, Liang, and Zeng (2008) with some minor amendments earlier reported by Aadil et al. (2013).

2.6 Total phenolics, total flavonols, and total flavonoids

Total phenolics of grapefruit juice were determined as by spectrophotometric method using Folin–Ciocalteu reagent by the method proposed by Slinkard and Singleton (1977) with some minor amendments earlier reported by Aadil et al. (2013). Total flavonols of grapefruit juice samples were measured using the method of Kumaran and Karunakaran (2007) with some minor amendments earlier reported by Aadil et al. (2013).

Total flavonoids were determined by using 5% NaNO2, 10% AlCl3, and 1 mol L\(^{-1}\) NaOH and measured by the spectrophotometer at 415 nm using quercetin as standard (Kim et al., 2012). The results were expressed as mg of quercetin equivalents (QE) per 100 g of fresh weight.

2.7 Statistical analysis

All the experiments were conducted using three replicates. Completely randomized design followed by one-way ANOVA at significance level of \(p < .05\), and significant differences between mean values were determined by Tukey HSD test using Statistix 9.0 software (Analytical
3 | RESULTS AND DISCUSSION

3.1 | Effects of PEF and US on microbial count

As shown in Figure 1, a significant decrease was observed in the activity of TPC and Y&M during US, PEF, and combined. As control values of TPC and Y&M (4.03 and 3.63 log CFU/ml), the survival of TPC and Y&M decreased in all treatments, that is, US (3.51 and 3.11 log CFU/ml), PEF (2.57 and 2.24 log CFU/ml), and PEF + US treatment (2.12 and 1.91 log CFU/ml), respectively. Additionally, the highest inactivation was noted in the case of PEF + US that shows the higher reduction in efficiency of microbial cells in grapefruit juice. In this study, trend of microorganism reduction was counted in agreement as reported earlier in kasturi lime (Bhat, Kamaruddin, Min-Tze, & Karim, 2011) and pear juice (Saeeduddin et al., 2015). This reduction in the activities of TPC and Y&M count might be due to the combination of chemical and physical operations occurred during the cavitation process. It has been reported that the cavitation process causes the production of free radicals and increased localized heating, which could be a reason for the killing of microorganisms (Jabbar, Abid, Hu, Wu, et al., 2014). Similarly, the findings of current investigation were in confirmation with the results of Marsellès-Fontanet, Puig-Pujol, Olmos, Minguez-Sanz, and Martín-Belloso (2013) and Barbosa-Canovas, Pothakamury, Gongora-Nieto, and Swanson (1999) who revealed that PEF treatment reduced the activity of Y&M due to electroporation and electrofusion. Moreover, combined effect of examined treatments exhibited the highest reduction in microbial activity than a single treatment of US, PEF, and control samples. Recently, synergistic effect of HPP + US on microbial inactivation was observed by Jabbar, Abid, Hu, Muhammad Hashim, et al. (2014) who reported significant microbial reduction in carrot juice after combined treatment with HPP + US.

3.2 | Effects of PEF and US on lycopene

In Figure 2, a significant increase in lycopene content was noted during US (0.73 ± 0.03 µg/ml), PEF (0.62 ± 0.04 µg/ml), and PEF + US (0.92 ± 0.06 µg/ml) than control (0.32 ± 0.05 µg/ml). An increase in the concentration of lycopene might be due to sonication that is in confirmation of the result as reported in sonicated carrot juice (Jabbar, Abid, Hu, Muhammad Hashim, et al., 2014). In ultrasound treatment, disruption of chromoplast membrane and collapse of cell-wall occurs due to the cavitation that results in release of more lycopene contents (Jabbar, Abid, Hu, Wu, et al., 2014). Odriozola-Serrano, Soliva-Fortuny, and Martín-Belloso (2008) stated that lycopene was raised in high intensity pulsed electric fields (HIPEF) treated strawberry juice when pulse width and frequency were increased, which showed that HIPEF could stimulate change of total carotenoids into lycopene. Overall, PEF + US treatment appeared to be more adequate in retention of lycopene whereas US and PEF were more effective than control.

3.3 | Effect of PEF and US on total anthocyanins

Results regarding anthocyanins revealed significant increase in anthocyanins contents during US, PEF, and PEF + US as compared to control (Figure 2). Anthocyanin contents were increased significantly in US (1.47 ± 0.05 mg/L), PEF (1.58 ± 0.03 mg/L), and PEF + US (1.68 ± 0.09 mg/L) as compared to control (1.37 ± 0.03 mg/L). Previously, a considerable change in anthocyanin contents of sonicated strawberry was observed by Sala, Burgos, Condon, Lopez, and Raso (1995) and mainly due to the cavitation process that regulates various chemical or biological reactions including increase in the diffusion rates and disintegration of affected particles (Tiwari et al., 2009). Significant increase in anthocyanins content was reported after a pretreatment of PEF in grape juice (Knorr, 2003) and PEF treated strawberry juice by using low pulse widths and high frequencies (Odriozola-Serrano et al., 2008), Anthocyanins retention is dependent on pulse frequency width, polarity, and treatment time during the PEF treatment (Odriozola-Serrano et al., 2008). It was observed that anthocyanin contents in PEF + US
treated samples showed the highest values as compared to the control group and juices treated with individual applications of US and PEF. The significant increase due to PEF + US treatment might be due to prominent chemical effects of PEF and US.

3.4 | Effect of PEF and US on total carotenoids

Results regarding TC contents in grapefruit juice are given in Figure 2, a significant increase in carotenoids during US, PEF, and PEF + US was observed as compared to control. Total carotenoid contents were increased from $0.84 \pm 0.05 \, \mu g/ml$ (control) to $1.03 \pm 0.06 \, \mu g/ml$, $1.03 \pm 0.04 \, \mu g/ml$, and $1.26 \pm 0.03 \, \mu g/ml$ in US, PEF, and PEF + US, respectively. The increase in the TC could be due to ruptured cell walls during cavitation process resulting in release of free carotenoids (Abid et al., 2014). A substantial increase in carotenoids was noticed in HPP treated orange and HIPEF treated orange-carrot juice (Plaza et al., 2011; Torregrosa, Cortés, Esteve, & Frígola, 2005). In our results, PEF + US treatment showed higher values for carotenoids than individually treated US and PEF juices and similar trend was observed using ultrasound and HPP treatment of carrot juice (Jabbar, Abid, Hu, Muhammad Hashim, et al., 2014) which supports our findings.

3.5 | Total antioxidant capacity and DPPH free radical scavenging activity

A significant increase in TAC and DPPH activity was observed during US, PEF, and PEF + US as compared to control (Figures 3 and 4). TAC was increased significantly from $177.48 \pm 0.05$ ascorbic acid equivalent mg/g (control) to $224.90 \pm 0.05$, $226.73 \pm 0.04$, and $262.32 \pm 0.04$ ascorbic acid equivalent mg/g in US, PEF, and PEF + US treated samples respectively. DPPH activity was also increased from $32.80 \pm 0.04$ (control) to $39.65 \pm 0.04$, $38.74 \pm 0.05$, and $28.78 \pm 0.05$ DPPH Inhibition Percentage (%) in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after US treatment. Earlier, significant elevation in TAC and DPPH activity were noticed in sonicated grapefruit juice by Aadil et al. (2013) while increase in TAC in sonicated pear juice was also observed by Saeeduddin et al. (2016) and HIPEF strawberry juice by Odriozola-Serrano et al. (2008). The increased antioxidative activity could be due to an enhanced release of matrix-bound phenolic compounds after PEF treatment (Schilling et al., 2007). The highest level of TAC and DPPH was observed in PEF + US might be due to the synergistic effect of both treatments in grapefruit juice. Our results are in harmony with the earlier findings of Abid et al. (2014) who reported improved TAC and DPPH activity in US and HPP treated apple juice might be due to inactivation of oxidative enzymes and release of phenolics from cell structure.

3.6 | Effect of PEF and US on total flavonoids, total flavonols, and total phenolics

As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment. As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment. As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment. As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment. As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment. As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment. As data displayed in Table 1, significant increase was achieved in TF and total flavonols during US, PEF, and PEF + US as compared to control. TF contents were increased from $2.19 \pm 0.02$ quercetin equivalent mg/g (control) to $2.95 \pm 0.03$, $2.93 \pm 0.04$, and $3.30 \pm 0.03$ quercetin equivalent mg/g in US, PEF, and PEF + US treated grapefruit juices respectively. This significant increase could possibly be due to the improved level of phenolic compounds that produce cavitations after PEF treatment.
equivalent μg/g) in PEF + US treated grapefruit juice. Previous studies have shown significant increase in TF and total flavonols in UV treated starfruit juice (Bhat, Ameran, Voon, Karim, & Tze, 2011) and sonicated pear juice (Saeeduddin et al., 2016) & increase in flavanone in HIPEF treated strawberry juice (Odriozola-Serrano et al., 2008).

As shown in Table 1, significant increase was noted in the TP during US, PEF, and PEF + US treatments of grapefruit juice as compared to control. As compared to control (640.10 ± 0.05 catechin equivalent μg/g), TP contents were increased to 700.37 ± 0.05, 701.10 ± 0.05, and 730.19 ± 0.05 catechin equivalent μg/g in US, PEF, and PEF + US, respectively. During sonication, the increase could be due to release of the phenolics from ruptured cell membranes mainly owing to the cavitation process (Aadil et al., 2013). Fruits having higher phenolic contents usually exhibit the strongest total antioxidant capacities (Patras, Brunton, DA Pieve, Butler, & Downey, 2009). A similar elevation in TP contents was observed in sonicated kasturi lime juice (Bhat, Kamaruddin, et al., 2011) and PEF treated Tempranillo grape juice (López, Puértolas, Condón, Álvarez, & Raso, 2008). The increase in TP, TF, and total flavonols have health beneficial effects, because these compounds possess potent antioxidants for ultimate consumers (Balasundram, Sundram, & Samman, 2006). The highest levels of TP, TF, and total flavonols in PEF + US treatment may be due to the complementary effects of both techniques on grapefruit juice. Our results are in agreement with earlier investigations conducted using combination of HPP + US treatment in apple juice (Abid et al., 2014) and sonicated carrot juice for shelf-life extension (Jabbar, Abid, Hu, Muhammad Hashim, et al., 2014). Likewise, results obtained from the present investigation are also inlined with the outcomes of Lieu (2010) who reported a significant increase in TP contents of grape juice due to the effect of ultrasound and enzymes. Our results suggested that combination of two nonthermal technologies could be the best option to obtain the optimal results related to bioactive compounds.

4 | CONCLUSIONS

Combination of different nonthermal technologies is a good alternative technique in the food industries. This study depicts the effects of the PEF + US treatment on the quality and microbial safety of grapefruit juice. PEF + US treatment resulted in improvement of DPPH activity, TAC, flavonols, TF, TP, and reduction in microbial load. Moreover, this combination also improved lycopene, anthocyanin, and carotenoid contents of grapefruit juice. In general, PEF + US treatment has greater advantages to maintain the quality and can be used at commercial level to produce the safe, healthy, and high quality grapefruit juice to increase the market value.

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