Research Article

Quality Evaluation of Grapefruit Juice by Thermal and High Pressure Processing Treatment

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Abstract | The effects of control, thermal and high pressure processing (HPP) techniques on some quality parameters of grapefruit juice were investigated based on pH, acidity, °Brix, viscosity, cloud value, hunter color values, bioactive compounds, microorganisms and enzyme analysis. The grapefruit juice was treated with processing variables of HPP treatment (0, 150, 200 and 250 MPa), temperature (40, 50 and 60 °C), for processing time of 3 minutes and thermal treatment was done in a water bath at 70 °C for 90 seconds. In HPP treatment (250 MPa/60 °C/3 min), significant reduction was observed in microorganisms, PME and PPO whereas improvement was observed in cloud value, total carotenoids, total anthocyanins, DPPH activity, total antioxidant capacity (TAC), total flavonoids (TF) and total flavonols as equated to thermal and control, while °Brix, pH and acidity remain unchanged in all HPP treatments with control and thermal. After this, we suggested that HPP treatment (250 MPa/60 °C/3 min) has a potential against thermal to progress the value of grapefruit juice and could be applicable at industrial scales.

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Introduction

Among citrus fruit, grapefruit juice is a good source of bioactive compounds, which are valuable due to their healthiness assistances can perform an imperative share in prevention of abundant chronic diseases owing to the accessibility of bioactive compounds (Kaur and Kapoor, 2001). Nowadays, consumers are paying more attention towards health to obtain quality products from fruits and vegetables. They are expecting healthy food products which can give a good and natural taste (Gómez et al., 2011).

Thermal (conventional) methods stay efficient to outspread quality and shelf life of the juice products by controlling the activity of enzymes and microorganisms. Such thermal technologies have effect on flavor, color and other sensory losses in foods (Ludikhuyze et al., 2003). Novel technologies such as non-thermal techniques have been discovered as a good alternative for heat treatment (Butz et al., 2003). These modern techniques, such as HPP, pulsed high intensity light, ultraviolet irradiation, oscillating magnetic fields, sonication and pulsed electric field (PEF) were studied recently extending juice quality and minimizing the nutritional losses (Cserhalmi et al., 2006; Sánchez-Moreno et al., 2005; Aadil et al., 2015 a,b,c; Varela-Santos et al., 2012; Liu et al., 2014; Zhang et

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al., 2015).

Among these, HPP have a significant importance because it affects the microorganisms and enzymatic activity. It can also maintain the food product quality, i.e. taste, vitamins, flavor, freshness, color and sensorial characteristics (Dede et al., 2007). Also, HPP have been applied on different fruits juices along with orange juice (Sánchez-Moreno et al., 2005; Plaza et al., 2006); carrot juice (Dede et al., 2007); pomegranate juice (Varela-Santos et al., 2012).

Keeping in view the importance of microorganism analysis and bioactive compounds in grapefruit juice, HPP could be helpful for consumers and juice industry. During this study, the effects of thermal and HPP were investigated in comparison with control on color, cloud value, viscosity, ascorbic acid, microorganism analysis, PME, PPO, DPPH activity, TAC, TF, carotenoids, anthocyanins and flavonols of grapefruit juice.

Materials and Methods

Chemicals

Ascorbic acid was bought from Accu Standard Inc. (New Heaven, CT 06513, USA). Catechin hydrate, sodium hydroxide, quercetin was purchased from Aladdin Industrial Company Limited (Shanghai, China). Count agar and potato dextrose agar media (PDA) was obtained from Huankai Science and Technology Limited, (Guangzhou, China). Ethanol, catechol, diabasic potassium phosphate (K_2HPO_4), methanol, monobasic potassium phosphate (KH_2PO_4), sodium nitrite, KCI, tartaric acid, sodium acetate anhydrous, aluminium chloride and sodium acetate anhydrous were got from Sinopharm Chemical Company and Limited (Shanghai, China). All chemicals and reagents used in the study were of analytical (AR) grade.

Preparation of grapefruit juice

Fresh grapefruits (*Citrus maxima,* family Rutaceae) were bought from a local super 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. After getting juice, it was filtered through muslin cloth (sterilized double layer) to remove the impurities before subjecting to further analysis.

Thermal and HPP treatments

The freshly squeezed grapefruit juice was subjected to thermal treatment of grapefruit juice samples were done in a water bath at 70 °C for 90 seconds. After each treatment, grapefruit juice was cooled, quickly in ice water bath for further analysis. Secondly, a high-pressure treatment with continuous high-pressure equipment (Baotou Science Development and High Technology Co. Ltd., Inner Magnolia, China). Approximately 50 mL grapefruit juice samples were filled in pressure resistant polyethylene bags and pressurized at 150 MPa, 200 MPa and 250 MPa, at 40°C, 50°C and 60°C for 3 minutes. The temperature was controlled by a thermostat (Changzhou Ao-Hua instrument Co. Ltd., Changzhou, China) coupled to sample chamber. All measurements were performed in triplicate.

°Brix, pH, acidity, viscosity, color analysis and cloud value

Acidity was determined by the standard method mentioned in AOAC (1999), °Brix with Abbe refractometer, pH with a digital pH meter, colors by colorimeter and viscosity by rotary viscometer.

About 5mL sample was centrifuged (988 g, 10 min, 20 °C), and cloud value supernatant was determined at 660 nm using a spectrophotometer (UV 17800, Shimadzu Suzhou Industrial Mfg. Co. Ltd., Jiangsu, China) with distilled water serving as a blank (Versteeg et al., 1980).

Determination of total anthocyanins and carotenoids

The contents of total carotenoids were examined by the method of Liao et al. (2007) as reported by Aadil et al. (2015a) with some changes. Total anthocyanins were determined by the method of Lee et al. (2005) as stated by Aadil et al. (2015b).

Determination of microbiological analysis

The microbiological analysis of grapefruit juice samples was evaluated by the FDA's standard method of Bacteriological Analytical Manual (FDA, 2001). Total plate counts were counted by pour plate method using nutrient agar media whereas yeasts and molds (Y&M) were counted by using Potato Dextrose Agar media.

Determination of PME and PPO

PME residual activity was examined by the method of de Assis et al. (2007) reported by Aadil et al.

(2015b) while PPO residual activity was measured by following the procedure explained by Augustin et al. (1985) reported by Aadil et al. (2015b).

Determination of ascorbic acid, DPPH radical scavenging activity, total antioxidant capacity, total flavonoids and total flavonols

Ascorbic acid, total antioxidant capacity (TAC), DPPH free radical scavenging activity, total flavonoids (TF), total flavonols and phenolics (TPC) were measured by using the modified method of Aadil et al. (2013).

Statistical analysis

Data obtained were presented as mean value ± standard deviation (SD). Completely randomized design (CRD) was conducted with one-way ANOVA at a significance level of $P < 0.05$, significant differences between mean values were determined by LSD (least significant differences) pair-wise comparison test. Statistical analyses were determined by using Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA).

Results and Discussion

Effects of thermal and HPP treatment on pH, °Brix, acidity, cloud value and viscosity

Effects of HPP on pH, °Brix and acidity of grapefruit juice were compared with thermal and control treatment (Table 1). °Brix, pH and titratable acidity of the grapefruit juice related to thermal and HPP treatment was statistically similar to control. Similarly, Liu et al. (2013) did not detect any change in °Brix, acidity and pH of HPP treated watermelon juice.

Effects of HPP on the cloud value and viscosity were compared with thermal and control in grapefruit juice (Table 1). The cloud value related to thermal and HPP treatments was significantly higher than control treatment. Thermal treatment has a lower value than all HPP treated values and maximum value of cloud was determined in the HPP treatment (250 MPa/60 °C/3 min). During HPP, an increase might be owing to the breakage of superior molecules into minor ones owed to high pressure applied during HPP treatment, which might develop the amount of adjourned particles, depresses the space between particles by the expansion of surface area which could the possible reason cloud improvement in juice. Previously, Aadil et al. (2013) observed an increase in ultrasound grapefruit juice as compared to control.

Viscosity is an imperative quality property of fruit juices which play a significant aspect in consumer acceptability. Table 1 shows that the viscosity of the grapefruit juice related to thermal and HHP treatments were significantly decreased than control. The viscosity of the control grapefruit juice was 3.51, while thermal treatment was reduced to 2.04 and HPP treatment reduced from 2.32 to 2.16. Viscosity loss in juices could be due to the degradation of pectin enzymes such as polygalacturonase (PG) and PME. It had demonstrated that pectin is the main factor and could be depraved through hydroxyl radical mediated scission of the polymer (Cao et al., 2012).

Effects of thermal and HPP on the color

Color plays a significant contribution in the satisfaction of consumers, also enormous economic losses can also be accumulated by the companies due to the loss of attractive product color. Effects of HPP on total color values of grapefruit juice were compared to thermal and control (Table 2). The color values of grapefruit juice related to HPP and thermal treatments were significantly decreased as compared to control treatment. Thermally treatment shown the lowest lightness (L*), redness (a*) and yellowness (b*) while the highest values for L*, a* and b* were detected in control. A significant decrease in all the color values was detected in cloudy apple juice treated with high pressure CO_2 (Xu et al., 2011), apricot nectars treated with HHP (Huang et al., 2013) as compared to control. These changes in color values might be temperature reliant on and collective pressure at ambient temperature caused a decrease of color values. Although, HPP treatments brought deviations that cannot be easily observed with naked eyes in color values. So, it is proposed that HPP treatment could be applied for juice processing industry.

Effects of thermal and HPP treatment on total carotenoids and total anthocyanins

Effects of HPP on carotenoids and anthocyanins were compared with thermal and control in grapefruit juice (Table 2). The carotenoids of the grapefruit juice related to thermal and HPP treatments were significantly improved as control. In this study, thermally treated sample showed the lowest value of carotenoids as compared to all HPP treatments, but the maximum improvement of total carotenoids was observed in HPP treatment (250 MPa/60 °C/3 min).

Table 1: *Effect of thermal and HPP on pH, TA, TSS (°Brix), cloud value and viscosity of grapefruit juice*

Samples	pH	TA (%)	TSS (°Brix)	Cloud value	Viscosity (cP)
Control	4.79±0.01a	0.11±0.01a	9.83±0.06a	0.659±0.010g	3.51±0.08a
HPP (150 MPa/40 ⁰C/3 min)	4.80±0.01a	0.12±0.01a	9.80±0.10a	1.225±0.06e	2.32±0.09b
HPP (200 MPa/40 ⁰C/3 min)	4.80±0.01a	0.11±0.01a	9.83±0.12a	1.229±0.011e	2.31±0.12b
HPP (250 MPa/40 ⁰C/3 min)	4.79±0.01a	0.11±0.01a	9.85±0.05a	1.232±0.08e	2.30±0.10b
HPP (150 MPa/50 ⁰C/3 min)	4.79±0.01a	0.12±0.01a	9.80±0.10a	1.241±0.07d	2.29±0.09b
HPP (200 MPa/50 ⁰C/3 min)	4.80±0.01a	0.12±0.01a	9.82±0.13a	1.245±0.06d	2.26±0.16b
HPP (250 MPa/50 ⁰C/3 min)	4.80±0.01a	0.11±0.01a	9.78±0.18a	1.247±0.10d	2.31±0.06b
HPP (150 MPa/60 ⁰C/3 min)	4.79±0.01a	0.11±0.01a	9.75±0.15a	1.268±0.07c	2.21±0.08c
HPP (200 MPa/60 ⁰C/3 min)	4.80±0.01a	0.12±0.01a	9.80±0.10a	1.284±0.09b	2.23±0.12c
HPP (250 MPa/60 ⁰C/3 min)	4.80±0.01a	0.11±0.01a	9.80±0.05a	1.333±0.08a	2.16±0.09d
Thermal	4.80±0.01a	0.12±0.01a	9.73±0.15a	0.991±0.09f	2.04±0.08e

Values with different letters in the same column (a-k) are significantly different (P < 0.05) from each other. HPP: high pressure processing, TA: titratable acidity

Table 2: *Effect of thermal and HPP on color attributes, total carotenoids total anthocyanins of grapefruit juice*

Samples	Color attributes			Total carotenoids (µg/mL)	Total anthocyanins (mg/100 mL)
	*L**	*a**	*b**		
Control	6.65±0.08a	3.80±0.09a	4.95±0.08a	0.95±0.06h	81.09±0.07d
HPP (150 MPa/40 ⁰C/3 min)	6.52±0.08b	3.65±0.05b	4.79±0.07b	1.73±0.06f	89.88±0.32c
HPP (200 MPa/40 ⁰C/3 min)	6.50±0.08b	3.63±0.02b	4.77±0.04b	1.74±0.09f	91.71±0.76c
HPP (250 MPa/40 ⁰C/3 min)	6.47±0.09b	3.63±0.09b	4.75±0.04b	1.76±0.06f	92.90±0.98c
HPP (150 MPa/50 ⁰C/3 min)	6.46±0.06b	3.60±0.06b	4.74±0.06b	1.82±0.08e	90.30±0.47c
HPP (200 MPa/50 ⁰C/3 min)	6.49±0.04b	3.58±0.08b	4.73±0.04b	1.84±0.04e	93.69±0.48c
HPP (250 MPa/50 ⁰C/3 min)	6.45±0.03b	3.58±0.07b	4.70±0.06b	1.90±0.07d	92.10±0.63c
HPP (150 MPa/60 ⁰C/3 min)	6.48±0.04b	3.60±0.07b	4.71±0.05b	1.99±0.04c	101.55±0.89b
HPP (200 MPa/60 ⁰C/3 min)	6.47±0.07b	3.56±0.07b	4.72±0.07b	2.09±0.09b	103.26±0.38b
HPP (250 MPa/60 ⁰C/3 min)	6.44±0.06b	3.54±0.07b	4.69±0.03b	2.25±0.09a	110.43±0.59a
Thermal	6.33±0.06c	3.51±0.08c	4.67±0.09c	1.18±0.04g	65.63±0.08e

Values with different letters in the same column (a-k) are significantly different (P < 0.05) from each other; HPP: high pressure processing

While, HPP treatments had higher total anthocyanins content compared to thermal and control. Among HPP treatments, the highest amount was noticed in processed juice at 250 MPa/60 ˚C/3 min. While thermal treatment showed the lowest value as compared to control and HPP treatments. Every treatment ought to change effect on total anthocyanins activity. Total anthocyanins of grapefruit juice subjected to HPP treatment, increased when pressure and temperature increased while control treatment shown the lowest value. In foods, HPP have some unfavourable effects on pigments, flavouring agents and vitamins because covalent bonds were not interrupted by used pressure level. Previously, some researchers have done some research on effects of HPP on carotenoids and anthocyanins in different vegetables and fruits (Butz et al., 2003). Less detailed reports available in HPP treated anthocyanins and carotenoids while few have attained increase in carotenoid with HPP and pasteurized juice (Plaza et al., 2011). Also, similar trend of increase in anthocyanin was observed in strawberry and blackberry purées treated with HPP and thermal treatment (Patras et al., 2009). Anthocyanin degradation in grapefruit juice could also be due to the ascorbic acid oxidation products such as hydrogen peroxide.

Effects of thermal and HPP on microorganism analysis

Effects of HPP on microorganism analysis of grapefruit juice were compared with thermal and control (Figure 1). In this study, total plate counts, aerobic bacteria, yeasts and molds (Y&M) showed a significant reduction in all HPP and thermal treatment as compared to control. The maximum reduction of microorganisms was observed in HPP treatment (250 MPa/60 °C/3 min). In HPP, higher pressure and temperature lead toward more inactivation of microorganism. HPP was used to decline the activity of microorganisms and enhance the orange juice shelf-life (Basak et al., 2002), orange, apricot apple and sour cherry juices (Bayindirli et al., 2006). Similarly, microorganism inactivation was observed in thermal treated juice. However, thermal treatment was used to inactivate microorganisms, caused some undesirable changes in juice quality.

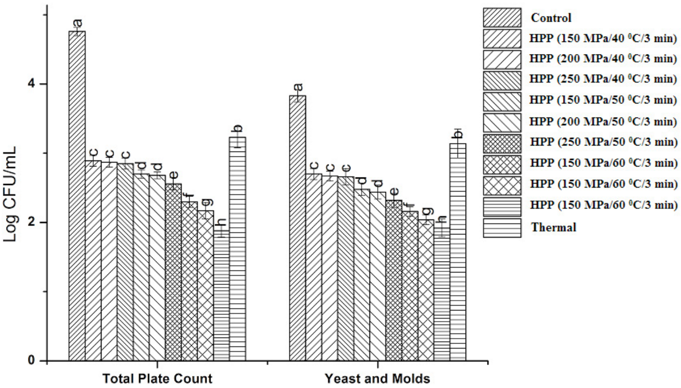

Figure 1: *Effect of thermal and HPP on total plate count and yeasts and molds of grapefruit juice HPP: High pressure processing*

Effects of thermal and HPP on the PME and PPO residual activity

PPO activity is a generic term for a group of enzymes responsible for oxidation of phenolics compounds and development of browning reaction in fruits and vegetables. The inactivation of PPO is much desirable due to the related change in the organoleptic properties (Rocha and Morais 2001). Effects of HPP on PME and PPO residual activity of grapefruit juice were compared with thermal and control (Figure 2). PME and PPO activity decreased with increase in pressure and temperature. It indicates that HPP is effective to control enzyme activity. Control treatment showed highest value than thermal, but maximum inactivation of enzymes was observed in HPP treatment (250 MPa/60 °C/3 min). Similar trend of significant decrease in PME and PPO residual activity was observed with HPP treated apple juice (Abid et al., 2014). The declines in enzymes activities increase with the level of pressure which express the compassion to HHP treatment. PPO activity steadily lessened with collective increase in level of pressure/treatment times in strawberry pulps (Cao et al., 2011). PPO activity exhibited dissimilar sensitivities to HHP and these modifications were in need of pressure, temperature, time and substrate (Mújica-Paz et al., 2011).

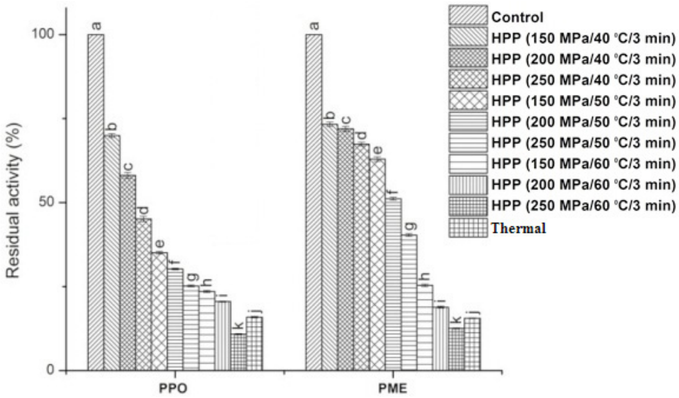

Figure 2: *Effect of thermal and HPP on PPO and PME residual activity percentage of grapefruit juice. HPP: High pressure processing, PPO: Polyphenoloxidose, PME: Pectin methyl esterase*

Effects of thermal and HPP on ascorbic acid

Effects of HPP on ascorbic acid of grapefruit juice were compared with thermal and control (Table 3). Ascorbic acid contents of control sample were 28.65 (mg/100 ml) in grapefruit juice. However, ascorbic acid contents were reduced from 25.58 to 19.32 (mg/100 ml) in HPP and 17.28 (mg/100 ml) in thermal processing. These findings are in line with HPP treated apple juice (Abid et al., 2014). Moreover, control showed the highest ascorbic acid content than HPP treatment and thermal treatments (Sánchez-Moreno et al., 2006). Even though, thermal treatments showed the lowest value among all treatments, temperature and the time used for pasteurization treatment might be responsible for the depletion in ascorbic acid.

Effects of thermal and HPP on DPPH radical scavenging activity, TAC, TF and total flavonols

Effects of HPP on DPPH radical scavenging activity and total antioxidant capacity, total flavonoids and total flavonols of grapefruit juice were compared with thermal and control (Table 3). Our results about DPPH activity and TAC showed that significant increase was in HPP treatment as compared to control while thermal treatment showed the lowest value than HPP and control. The maximum improvement was investigated in HPP treatment (250 MPa/60 °C/3 min). Recently, significant increase was observed DPPH radical scavenging activity and TAC, TF and total flavonols in HPP treatment as control (Abid et al., 2014). Flavonoids have got a significant attention because

Table 3: *Effect of thermal and HPP on total antioxidant capacity, percentage inhibition (DPPH radical), total flavonoids, total flavonols and ascorbic acid of grapefruit juice*

Samples	Antioxidant capacity (Ascorbic acid equivalent µg/g)	Percentage inhibition (DPPH radical)	Total flavonoids (Catechin equivalent µg/g)	Total flavonols (Quercetin equivalent µg/g)	Ascorbic acid (mg/100 ml)
Control	282.60±0.06j	33.54±0.06j	535.52±0.09j	2.53±0.11j	28.65±0.06a
HPP (150 MPa/40 ⁰C/3 min)	330.57±0.10i	36.59±0.08i	629.22±1.37i	2.33±0.09i	25.58±0.8b
HPP (200 MPa/40 ⁰C/3 min)	339.22±0.12h	37.26±0.07h	641.38±1.65h	2.38±0.07h	24.10±0.07c
HPP (250 MPa/40 ⁰C/3 min)	345.93±0.05g	37.96±0.04g	652.18±1.77g	2.47±0.07g	23.17±0.04d
HPP (150 MPa/50 ⁰C/3 min)	349.59±0.10f	39.83±0.11f	663.79±1.69f	2.55±0.06f	22.40±0.11e
HPP (200 MPa/50 ⁰C/3 min)	353.33±0.03e	41.78±0.04e	675.14±1.79e	2.64±0.06e	21.97±0.04f
HPP (250 MPa/50 ⁰C/3 min)	365.22±0.08d	42.99±0.09d	688.35±1.36d	2.73±0.10d	21.09±0.09g
HPP (150 MPa/60 ⁰C/3 min)	369.93±0.15c	44.57±0.06c	699.11±1.99c	2.87±0.08c	20.52±0.06h
HPP (200 MPa/60 ⁰C/3 min)	373.58±0.09b	45.34±0.07b	709.57±1.29b	2.94±0.07b	19.97±0.07i
HPP (250 MPa/60 ⁰C/3 min)	380.30±0.06a	46.23±0.05a	733.20±1.56a	3.00±0.07a	19.32±0.05j
Thermal	274.13±0.04k	30.05±0.07k	515.64±0.07k	2.20±0.10k	17.28±0.07k

Values with different letters in the same column (a-k) are significantly different (P < 0.05) from each other; HPP: high pressure processing

of their high pervasiveness among foods and very important and beneficial polyphenolic contents. Epidemiological studies establish a relationship of individual flavonoids, which lowers the risks of many diseases (Knekt et al., 1996). We observed that among HPP treatments, TF and total flavonols contents increased significantly and maximum improvement was investigated in HPP treatment (250 MPa/60 ˚C/3 min). The rise in TF and total flavonols owing HHP treatment in grapefruit juice could be ascribed to develop compound's extraction by distraction of some cells (Kim et al., 2012). Another reason might be due to the improver effects of both methods which exert pressure cause cell interruption. Previously, a rise in total flavonols and TF was attained in HPP treated apple juice than control (Abid et al., 2014). In fruit juices, these compounds are core important suppliers which exist in different plants which have numerous biotic and antioxidant actions (Jayaprakasha et al., 2008).

Conclusions

In this study, non-significant change in pH, TA, TSS (ᵒBrix) but a maximum increase in cloud value, total carotenoids, total anthocyanins, DPPH activity, TAC, TF and total flavonols while reduction in microbiological analysis, PPO and PME were observed in HPP treatment (250 MPa/60 ˚C/3 min) as compared to control and thermal. The results suggested that use of HPP treatment (250 MPa/60 ˚C/3 min) may produce a good quality grapefruit juice with microbial safety as a consumer point of view and it may be successfully implemented at industrial scale.

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Authors Contribution

RMA and XZ planned and designed the study. RMA wrote teh manuscript and AN did SPSS analysis. SJ, AN, AAM, MKIK, AR and Abdllah helped in writing the manuscript.

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