Effects of Vacuum Concentration on Color, Polyphenol and Flavonoid Contents and Antioxidant Activity of Pomelo Citrus maxima (Burm. f.) Merr. Juice

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Abstract. Pomelo (Citrus maxima (Burm. f.) Merr.), juice is known as an enriched source of antioxidants and nutrients. It is therefore necessary to develop a preservation method for freshly prepared juice. This study aims to determine the effect of the vacuum concentration process on the change in color, bioactive compounds and antioxidant activity of pomelo juice. High pressure showed negligible effects on color. However, longer heating time seemed to cause browning in juice. In comparison with the fresh sample, total color difference (TCD) value of the treated sample was 6.73 ± 0.58 after 150 min of heating at 85°C. The total polyphenol content (TPC) values in the sample also increased with longer heating time or increased pressure. The total flavonoid content (TFC) seemed to be non-responsive to changes in heating time and pressure but was closely related to total soluble solid. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) of pomelo juice are affected by heat and, to a lesser extent, by pressure. The changes caused by the application of vacuum dehydration in the juice texture resulted in a higher water loss and higher sugar content (52.92 ± 0.79 °Brix).

1. Introduction
Plants provide an abundant source of natural antioxidants which exhibit a number of health benefits [1]–[3]. There has been an increased attention in nutritional value of natural products such as anthocyanins and essential oils [4]–[10], typical with some similar to herbs [11]–[13], and most applied in antibacterial and cytotoxic studies [14], [15].

The citrus family is mentioned for a variety of volatile compounds and flavonoids [16]. Pomelo (Citrus maxima), also known as grapefruit, is a citrus fruit tree in the Rutaceae family and originates in Southeast Asia (mostly in Thailand and Malaysia) [17]. It has been found that the composition of
grapefruit is dominated by the sucrose content (40.08-59.68% of the total sugar and the fructose to glucose ratio was almost 1:1) [18]. In addition, citric acid is the main organic acid and accounts for 39.10-63.55% of the total acid composition, followed by quinic acid. These two contents determine the taste of the fruit. Regarding volatile composition in grapefruit, monoterpens and sesquiterpenes are two abundant terpenes, in which D-limonene and caryophyllene were especially prevalent compounds. Other aromatic compounds in grapefruits include Caryophyllene, α-humulene, humulen-(v1), blinalool and tert-butyl 2-methylpropanoate. It has been found that the combination of zeaxanthin, b-cryptoxanthin and lycopene determines the color of the flesh while β-carotene is the main carotene in grapefruit [18]. The bioflavonoids found in grapefruit and citrus family also contributes in detection and prevention of proliferation of breast cancer cells by collecting excess estrogen from the body [19]–[21].

In production of grape juice, high-pressure pumping technology is often applied to minimize the loss of nutrients, antioxidant activity, and color variation. A previous study which performed deoxidization before heating grapefruit juice indicated that the obtained product had volatile compounds destroyed, necessitating further mitigation measures [22]. Despite that, studies on the effect of condensation conditions on the content of phenolics and flavonoids, as well as the antioxidant properties of grapefruit juice remain lacking. Therefore, this study was conducted to determine the influence of heating temperature, pressure conditions (50-55-60-65cmHg), stirring mode and concentration time on color and total phenolic content, flavonoids, antioxidant activity of pomelo juice. The results are expected to aid in valorization of pomelo fruits in particular and of citrus fruits in general.

2. Material and method

2.1. Prepare of pomelo juice

Pomelo were collected in a local farm in Ben Tre province, Vietnam. The materials were then cleaned, had peels removed and squeezed. Then, 500ml of crude juice was filtered and centrifuged at 6000 rpm within 15 minutes. 400ml of the obtained solution was placed in a pear-shaped glass jar and concentrated by system Heidolph rotary vacuum system (Model Hei-VAP Value P/N 560-01100-00, Glassware G1 Diagonal, Germany) with a temperature suitable for evaporation of the solution (85 °C).

![Figure 1. (A) Pomelo fruits (Citrus maxima (Burm.merr.)), (B) Pomelo Pulp](image)

2.2. Total polyphenol content (TPC)

To determine total polyphenol content, Folin-Ciocalteu colorimetric methods were adopted with gallic acid being used as a standard [23]. First, 0.1 mL of the extracts was transferred into a dark tube, followed by addition of 0.5ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 0.4 ml of sodium carbonate solution (7.5% w/v). The tube was stored in the dark and then measured using a photometer at an absorption of 765nm. The results were described as mg of ascorbic acid equivalent per gram of dry matter (mg AA/g dry matter).
2.3. Total flavonoid content (TFC)
The total flavonoid content was determined following aluminum chloride colorimetric method [24]. First, 0.5 mL of the extract was pipetted into a test tube containing 0.1 mL AlCl₃ (10% w/v), followed by the addition of 0.1 mL of 1M CH₃COOK and 4.3 mL of distilled water. The mixture was then vigorously shaken, followed by incubation in 30 minutes in the dark and spectrophotometric measurement at 415 nm. Quercetin was used as a standard. The results were shown in mg a standard equivalent per 1 gram of dried matter (mgQE/g). The standard curve was y=0.0073x-0.0179 (R²=0.9999) with the standard equation.

2.4. Free-radical scavenging Activity by DPPH
The antioxidant activity of juice was evaluated using DPPH assay following Braca et al [25]. The method is based on the basis that the scavenging of free radicals, caused by antioxidant compounds, could discolor the purple color in DPPH solution. First, 1.5mL of DPPH (OD₅17 nm = 1.1 ± 0.02) was added into 0.5 mL solution sample. The mixture was allowed to stand at room temperature in the dark for 30 min and then optically measured by UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. The result was expressed by mg of Trolox equivalent per gram of dry matter (mg QE/g dry matter).

2.5. ABTS Scavenging Activity
The free-radical scavenging was determined by ABTS assay [26]. First, the working solutions were prepared by adding 10ml of 2.6mL K₃S₂O₈ into 10 mL of 7.4 mM ABTS solution. The mixture was then allowed to stand in 24 hours. Following that, 1ml of the mixture was diluted with 60ml of ethanol (OD₇34nm = 1.1 ± 0.02). Then, 0.5ml of the extract was added in 1.5ml of the working solution. The mixture was kept in the dark for 30 minutes, followed by optical measurement for absorbance at 734nm. The scavenging ability of pomelo juice was calculated using the standard equation: y=20.46x-1.3855 with R²= 0.9985

2.6. Determination of Lightness
The CIELAB color space was used as the reference [27]. Three values including L* (from black to white), a* and b* were used to calculate total color difference (TCD) as follows:

\[ TCD = \sqrt{(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2} \]  

(1)

2.7. Statistical Analyses
Experiments are performed in triplicate. SPSS 15.0 (SPSS Inc., Chicago, USA) software was used to perform statistical analysis including variance analysis (ANOVA) and determination of standard deviations. Results are expressed as mean value ± standard deviations and differences were considered significant at p < 0.05.

3. Results and discussions

3.1. The effect of concentration time on bioactive activities and compounds and color of pomelo juice
The vacuum concentration time at 85°C significantly affected the color and soluble solids of juice, as demonstrated in Table 1. The L* value decreased from 34.71 ± 0.53 to the 30.65 ± 0.45 when rising the duration from zero to 150 min. On the contrary, a* and b* value seemed to be proportional to the duration. Interestingly, at 120 min, the TCD difference between the original and post-treated samples was large (6.80 ± 0.90). However, it was not significantly different from that of the 150 min sample (6.73 ± 0.58). In general, the pressed juice was light pink in color and had a significant change to dark brown after heating. The reported color change could be attributed by the Maillard reaction that is formed by the thermal treatment of amino acids and reducing sugar in juice [5], [28]. On the other hand, the evaporation process also increased the soluble solids content in the solution resulting in a non-enzymatic browning during concentration process [29].
The analytical characterization was mentioned in Figure 2 where different indicators were showed at four concentration durations (60-90-120-150 minutes) at 50cmHg and 85°C. Polyphenols showed no significant difference between 90 minutes (1.44 ± 0.04mg AA/g) and 120 minutes (1.46 ± 0.05mg/g) and peaked at 150 min. Similarly, the flavonoid content is high (fresh: 4.44 ± 0.04mg/g) and is proportional to the heating time. It is possible that the flavonoid group is affected by the growing conditions. It was also shown that increased soluble solid concentration leads to an increase in free-radical scavenging activity, measured by DPPH (from 0.58 ± 0.01 mgAA/g up 0.496mg after heating at 120 min) and ABTS. However, during this phase, the denaturation of compounds also occurs by polymer chain reactions, resulting in reduced polyphenol and flavonoid content [30]. In a comparison with commercial red grape juice and some other concentrated fruit juices, pomelo concentrate juice in this study is 10 times higher in terms of TPC content. However, the free-radicals scavenging activity measured by DDPH and ABTS of this pomelo juice is lower [31]. We selected the time parameters of 120 minutes based on free-radical scavenging activity. On the other hand, the color of the product at this stage is greatly affected and the solids content is acceptable.

**Table 1. Effects of pressure vacuum’s time on pomelo juice’s color**

| Sample | L*       | a*       | b*       | TCD      | Soluble solids (°Brix) |
|--------|----------|----------|----------|----------|------------------------|
| Fresh  | 34.71 ± 0.53a | -0.77 ± 0.02a | 5.31 ± 0.08a | 0a       | 12.01 ± 0.01a          |
| 60     | 37.08 ± 0.23b | -0.63 ± 0.02b | 6.84 ± 0.07b | 2.84 ± 0.63b | 18.75 ± 0.25b          |
| 90     | 37.11 ± 1.32b | 0.36 ± 0.01c | 8.64 ± 0.21cd | 4.39 ± 1.01c | 29.90 ± 0.10c          |
| 120    | 30.04 ± 1.64c | 3.01 ± 0.10d | 8.29 ± 0.75c | 6.80 ± 0.90d | 47.55 ± 0.45d          |
| 150    | 30.65 ± 0.45c | 3.08 ± 0.10d | 8.93 ± 0.14e | 6.73 ± 0.58d | 51.30 ± 0.20e          |

a–e means the difference between columns

**Figure 2. Change in bioactive content over concentrated time**
3.2. The effect of concentration pressure on bioactive activities and compounds and color of pomelo juice

The change of pressure level caused light effect on the color and soluble solids in pomelo juice as shown in Table 2. However, pressure exerted on the concentration process darkened the solution, evidenced by the decrease in \(L^*\) value after 2 hours. The increase in the value of \(a^*\) in pomelo juice indicates a color shift towards the red hue. Similarly, \(b^*\) moving up to the yellow hue results in a large difference in TCD. However, when changing the pressure from 50 to 60 cmHg, no significant difference in the color of the reprocessed sample was observed. The \(ab^*\) value may partially due to disintegration of chromoplast in the pressed liquid into pigments [32]. Besides, as the concentration of dissolved solids increases, the density of particles distribution in the solution also increases, which intensifies the hue color [33].

The TPC, TFC and antioxidation activity measured by DPPH, ABTS of the juice were shown in Figure 3. Under pressure of 50cmHg and at 85°C, the TFC coefficient peaked at 5.50 ± 0.14 mgQE/g. Rising the pressure past 50cmHg did not seem to induce any further changes in TFC. TPC, DPPH, and ABTS, all seemed to be affected by pressure. The concentration of DPPH increased from 1.08 ± 0.01 to 1.21 ± 0.03mg AA/g when rising the pressure from 55 to 60 cmHg and was stable afterwards. ABTS of the sample produced at the highest pressure (1.22 ± 0.01mg / g) was about 2.04 times higher than the original sample. This is closely related to Soluble solids content in Table 2 and can be explained by the increased denatured content and rapid dehydration, leading to the increase in ABTS concentration during each analysis period. As previously mentioned, high pressure treatment accelerates chemicals and biochemical reactions in cells, which could be either desirable or undesirable [34]. Moreover, high pressure treatment provides the ability to inactivate degradable enzymes that can account for higher yield and antioxidant activity than other methods [35].

Table 2. Effects of pressure vacuum (cmHg) on pomelo juice’s color

| Sample | \(L^*\) | \(a^*\) | \(b^*\) | TCD | Soluble solids (°Brix) |
|--------|--------|--------|--------|-----|------------------------|
| Fresh  | 34.71 ± 0.53\(^a\) | -0.77 ± 0.02\(^a\) | 5.31 ± 0.08\(^a\) | 0\(^a\) | 12.01 ± 0.01\(^a\) |
| 50     | 31.93 ± 0.67\(^b\) | 1.99 ± 0.11\(^b\) | 6.91 ± 0.20\(^b\) | 4.24 ± 0.24\(^b\) | 46.14 ± 0.03\(^b\) |
| 55     | 31.12 ± 1.00\(^b\) | 1.65 ± 0.43\(^b\) | 7.90 ± 0.22\(^bc\) | 5.19 ± 0.71\(^bc\) | 46.85 ± 0.33\(^b\) |
| 60     | 32.71 ± 1.50\(^bc\) | 2.70 ± 0.51\(^c\) | 7.87 ± 1.15\(^bc\) | 5.08 ± 0.89\(^bc\) | 52.92 ± 0.79\(^c\) |
| 65     | 31.04 ± 1.64\(^b\) | 3.04 ± 0.07\(^c\) | 7.98 ± 0.32\(^d\) | 6.01 ± 0.96\(^d\) | 53.73 ± 0.62\(^c\) |

\(^a-d\) means the difference between columns
4. Conclusions
Pomelo juice is a potentially good source of nutrients, antioxidants and essential mineral elements suitable for human consumption. Therefore, it is recommended to adopt a proper preservation method for the fresh pomelo juice. The present study aimed to determine the influence of heating temperature, pressure conditions, stirring mode and concentration time on color and total phenolic content, flavonoids, antioxidant activity of pomelo juice. In this study, the concentrated vacuum method was adopted to produce pomelo juice with minimal anti-nutrients and adequate minerals. The results obtained in this study show that concentration time significantly affects color, total soluble solids, and biologically active compounds. Heating the fresh juice sample at 85 °C for 120 minutes (60 cmHg) induced a total color difference of 5.08 ± 0.89. This figure corresponds with solid content of 52.92°Brix. Under these conditions, TPC, DPPH and ABTS content of the treated sample also increased by 1.33, 2.08, 1.96 in comparison with those of the fresh sample, respectively. Further studies should consider additional process parameters and other plant materials to discovery new use of the vacuum concentration technique.

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