

## Chemical properties and biological properties of four varieties of pomelo (*Citrus grandis* (L) Osbeck) in the Mekong Delta of Vietnam

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### Abstract

Pomelo fruits with many different varieties are widely consumed in Vietnam that promote many beneficial health effects. This study provided the discrepancy in morphological structure, chemical properties, and biological properties in four pomelo varieties found in the Mekong Delta, Vietnam including Da Xanh, Nam Roi, Long Co Co, and Thanh Kieu. Da Xanh pomelo has pomelo segments of red-pink colour and high soluble solid content ( $11.88 \pm 0.26^\circ\text{Bx}$ ). Nam Roi pomelo is recognized by the yellowish colour of the pomelo segment and low soluble solid content ( $10.62 \pm 0.53^\circ\text{Bx}$ ) while Long Co Co and Thanh Kieu varieties present a pale pink colour. Pomelo segments were observed with high carbohydrate content, low protein and lipid content. Nam Roi pomelo showed the highest total polyphenolic content (TPC) as  $2.42 \pm 0.01$  mg GAE/g dry matter, total flavonoid content (TFC) of  $9.57 \pm 0.03$  mg QE/g dry matter, and vitamin C content of  $42.04 \pm 1.02$  mg/100 g compared to the lowest one (Da Xanh pomelo). Among these varieties, Da Xanh pomelo was favourable in the sensorial aspect of its visual appearance and sweetness while Nam Roi pomelo was observed to have the highest biological activities.

## 1. Introduction

Citrus fruits are considered a rich source of bioactive compounds including flavonoids, limonoids, and glycosides (Benavente-García and Castillo, 2008). These compounds can be simple phenolic molecules or complex molecules which are constituted by an aromatic ring with one or more hydroxyl groups (Ignat *et al.*, 2011). Flavonoids are mostly found in citrus fruits which are classified into flavanones, flavones, and flavanols (de Lourdes Mata Bilbao *et al.*, 2007). It has been evidenced that the flavonoid content in citrus fruits is dependent on the species, plantation, or environmental conditions (Zhang *et al.*, 2014). It has been found that citrus flavonoids were observed to significantly impact blood and microvascular endothelial cells (Manthey *et al.*, 2001). Citrus fruit has been observed to have many biological activities such as anti-tumour, anti-inflammatory, and anti-fungal (Abeyasinghe *et al.*, 2007). Ali *et al.* (2020) indicated that lemon juice appeared to have advanced protective and antioxidant properties on biochemical and haematological parameters of the treated mice in vivo. Flavonoids from orange juice were found to prevent DNA-oxidative damage in A549 cells

incubated with  $\text{H}_2\text{O}_2$ , the generation of reactive oxygen species, and membrane lipid peroxidation (Ferlazzo *et al.*, 2015). Besides, by-products of these citrus fruits such as the peel have been currently investigated in their biological activities (Abeyasinghe *et al.*, 2007; Dao *et al.*, 2019; Dao *et al.*, 2021).

Pomelo (*Citrus grandis* (L) Osbeck) is a vital citrus fruit originally cultivated in Southeast Asia and consumed all around the world (Cuenca *et al.*, 2018). In the FAO database, the plantation area and production of pomelo fruit in the world were  $3.7 \times 10^5$  ha and  $9.4 \times 10^6$  tons in 2018 in Vietnam contributed  $6.58 \times 10^5$  tons (FAO, 2018). Pomelo is a rich source of naringin, a bitter flavoured flavanone glycoside with previously reported antioxidant, anticancer, antiviral, and anti-inflammatory properties (Sudto *et al.*, 2009). Different pomelo varieties were reportedly observed to reveal different biological capacities (Toh *et al.*, 2013). The pink pomelo juice (variety PO 52) was reported to contain a higher number of volatile compounds but lower terpenoids than the white pomelo juice (variety PO 51) (Cheong *et al.*, 2012). The pomelo extract showed very high antioxidant capacity by scavenging 1,1-diphenyl-2-picrylhydrazyl

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(DPPH) radicals due to the high content of polyphenols, flavonoids, and ascorbic acid in the extract (Ali *et al.*, 2019).

To date, pomelo fruits are commonly subjected to juice processing, whereas their bioactive compounds with biological properties such as flavonoids could be employed as food preservatives. Besides, it has been significantly gaining the attention of researchers to avoid using chemical preservatives, and the exploration of preservative alternatives from natural resources has been recently investigated instead (Damián-Reyna *et al.*, 2017). In the Mekong Delta, Vietnam, four commonly found pomelo cultivars are Da Xanh, Nam Roi, Long Co Co, and Thanh Kieu, contributing to the most important section in exporting of pomelo production. Due to the potential of phenols and flavonoids contributing to beneficial health effects, their characterization in terms of chemical and biological properties of each variety of pomelo should be evaluated. Therefore, this study aimed to investigate the chemical properties and biological properties of four pomelo varieties in Mekong Delta Vietnam. The revealed result can draw the attention of researchers to further research on how to preserve their valuable properties and employ their properties for developing functionally biological products.

## 2. Materials and methods

### 2.1 Materials

The four varieties of pomelo were directly harvested from April to July. Da Xanh pomelo was obtained from Ben Tre province, Nam Roi pomelo was selected from Vinh Long province. Long Co Co variety was from Tien Giang province while Thanh Kieu pomelo was chosen from Can Tho city. All the collected samples were then washed with water to remove impurity particles and were prepared for further experiments. Folin-Cialcalteu (purity of 99.5%), 2,6-dichloro-indophenol sodium salt hydrate (DCPIP), gallic acid, quercetin, and vitamin C were all purchased from Merck KGaA, Darmstadt, Germany.

### 2.2 Morphological structure and visual appearance of four pomelo varieties

Each pomelo cultivar was measured its size, peeled off, and determined its constituent ratios including exocarp, mesocarp, endocarp, juice segment, and seed by weighing. The colour measurement was evaluated by a colourimeter (CR-400, Konica Minolta, Inc., Tokyo, Japan) according to three colour indicators of the CIEL\*a\*b\* system. The L\* index presents the lightness of the sample (L\* = 0 is black and L\* = 100 is white), the a\* index indicates the colour band between red and green, and the b\* index presents the colour band between yellow to blue (Yildiz and İzli, 2019)

### 2.3 Chemical compositions of four pomelo cultivars

The physicochemical properties of pomelo juice segments from four pomelo varieties were evaluated. The moisture content was determined following the AOAC method (AOAC, 1999). The protein determination was evaluated by the Kjeldahl method while lipid was determined following the Soxhlet method. The ash content was conducted by heating the sample at 550-600°C until unchangeable weight which was determined as the ash content. The total carbohydrate was calculated as followed:

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ lipid})$$

The total solid content (°Bx) was measured by using a refractometer. The total acid content was evaluated using a titration method with NaOH, and phenolphthalein as a colour indicator.

### 2.4 Total polyphenolic content measurement

The TPC was analysed following Folin-Ciocalteu (FC) assay with some modifications. Briefly, 0.1 mL of juice was mixed with 1.5 mL of 10% FC reagent with the later addition of four mL of 20% Na<sub>2</sub>CO<sub>3</sub> and was topped up to 10 mL by distilled water. The mixture was kept for 30 mins in the dark. The absorbance was recorded at the wavelength of 725 nm using a 722-Visible spectrophotometer (China Yangzhou Wandong Medical Co., Ltd, China). A standard curve for the calculation of TPC was plotted by using gallic acid (2-14 µg/mL). The TPC was expressed as mg gallic acid equivalent (GAE) per gram of dry matter (DM) (Toh *et al.*, 2013).

### 2.5 Total flavonoid content determination

The TFC of the sample was measured according to the aluminium chloride colourimetric assay. One mL of the extract was mixed with three mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M sodium acetate, and 5.8 mL of distilled water. The mixture was kept for 30 mins and the absorbance was recorded at 415 nm. A standard curve for the quantitative determination of TFC was plotted by using quercetin (20-160 µg/mL). The TFC was expressed as mg quercetin equivalent (QE) per gram of dry matter (Abudayeh *et al.*, 2019).

### 2.6 Vitamin C content determination

The vitamin C content was evaluated using the DCPIP titration method (Dinesh *et al.*, 2015). A total of 5 g of pomelo flesh were homogenized with 20 mL of 1% HCl and topped up to 100 mL by using a 1% oxalic acid solution. The mixture was allowed to stand for 10 mins and filtered. The filtered solution (10 mL) was titrated using 0.001N 2,6 dichlorophenol – indophenol

(DCPIP) until the presence of a pale pink stabilized in 30 seconds. The blank sample was a mixture of 8 mL of 1% oxalic acid and two mL of 1% HCl. The vitamin C content in mg/100 g was calculated as followed:

$$\text{Vitamin C content} = \frac{(V - v) \times E \times V_0 \times 100}{m \times V_s}$$

Where V is the titrated volume of the test sample, v is the titrated volume of the blank sample, E is the mg ascorbic acid equivalent to one mL of DCPIP solution, V<sub>0</sub> is the volume of initial test solution, V<sub>s</sub> is the volume of test solution titrated and m is the weight of the solid sample.

### 2.7 Statistical analysis

Each experiment was in three replications. The statistical software as Statgraphics Centurions 16.1 (Statpoint Technologies, Inc., Warrenton, VA, USA) was utilized to perform a number of statistical tests. Analysis of variance (ANOVA) and least significant difference (LSD) tests were conducted to compare means in an analysis of variance with a significance of 0.05.

## 3. Results and discussion

### 3.1 Morphological structure of four pomelo varieties

The morphological structure of each constituent in four pomelo varieties including Da Xanh, Nam Roi, Long Co Co, and Thanh Kieu is presented in Figure 1. All the pomelos have a round shape which is consistent with a previous study (Sawant and Panhekar, 2017). The general structure of four pomelo varieties is composed of a thick peel (exocarp, mesocarp, endocarp), 11-18 segments, central core, seeds, and peduncle. The exocarp generally has a greenish or yellowish colour with a hard structure while the mesocarp shows a white colour or pale pink with a soft structure. The colour of pomelo segments is dependent on the cultivars of pomelo which are pink, pale pink, or yellowish. The pomelo segments obtained from Thanh Kieu and Da Xanh variety were found to achieve the highest yield 58.64% and 56.89% (w/w), respectively. The visual appearance of Da Xanh pomelo segments was preferable due to the red-pink colour, indicated by the L\* value of 32.73±0.20 and a\* value of 9.76±0.24, shown in Table 1. Nam Roi pomelo is able to distinguish compared to the others with a yellowish colour, demonstrated by the brighter L\* value of 48.05±0.82. Besides, the number of seeds from the Da Xanh cultivar was the least, contributing to only 0.01% (w/w) while the seed section of Long Co Co pomelo took up 0.64%.

The exocarp of Da Xanh Pomelo appears a dark green colour, contributing to the highest portion of the exocarp in comparison with the others. The mesocarp

and endocarp of Nam Roi pomelo are found to be whiter than the others. In general, the characteristics of these varieties in the Mekong Delta were fairly similar to many varieties of pomelo in Thailand such as Tubtim Siam and Thong Dee with the red-pink or pale pink segments, or Kaotai, Kao Tangkwa, and Nungpueng with yellowish segments (Rosales and Suwonsichon, 2015).

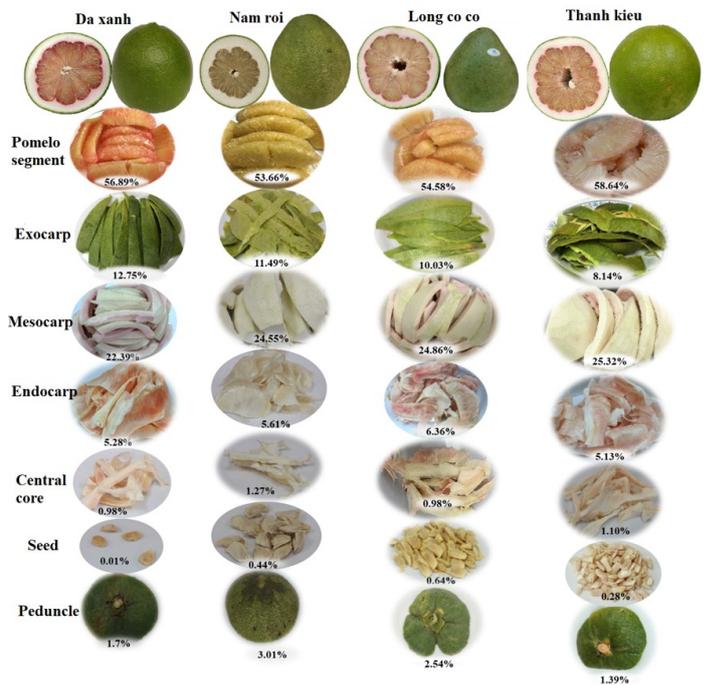


Figure 1. Morphological structure of four pomelo varieties  
Table 1. Colour parameters of pomelo segments of four pomelo varieties

Pomelo variety	L*	a*	b*
Da Xanh	32.73±0.20 <sup>a</sup>	9.76±0.24 <sup>c</sup>	15.69±0.25 <sup>a</sup>
Nam Roi	48.05±0.82 <sup>d</sup>	-0.23±0.03 <sup>a</sup>	19.04±0.27 <sup>b</sup>
Long Co Co	41.23±0.5 <sup>b</sup>	2.46±0.09 <sup>b</sup>	15.13±0.28 <sup>a</sup>
Thanh Kieu	46.52±0.52 <sup>c</sup>	2.57±0.05 <sup>b</sup>	15.63±0.42 <sup>a</sup>

Values are expressed as mean±standard deviation. Values with different superscripts within the same column are significantly different (p < 0.05).

### 3.2 Chemical properties of four pomelo varieties

Table 2 presents the chemical compositions of four pomelo cultivars including moisture, protein, lipid, ash, and total carbohydrate. The moisture content was within the range of 86.5-89%. The protein and lipid content of four pomelo cultivars were found to be low (0.002 % and 1.04-1.44%, respectively). It was evidenced that the citrus fruit contained a very low amount of protein (<1%) (Liu et al., 2012). The ash content was also observed to be low (0.33-0.43%) which was consistent with the study of Liu et al. (2012). The ash content was mostly composed of potassium (40%), sodium, and a small amount of calcium, magnesium, and phosphate (Liu et al., 2012; Abd Rahman et al., 2016). In terms of the total carbohydrate, Da Xanh variety contained the

Table 2. Nutritional ingredients of pomelo segments in four pomelo varieties

Pomelo variety	Nutritional ingredients				
	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)
Da Xanh	86.49±0.30 <sup>a</sup>	0.002±0.00 <sup>a</sup>	1.44±0.17 <sup>ab</sup>	0.43±0.01 <sup>c</sup>	11.64±0.17 <sup>d</sup>
Nam Roi	87.38±0.10 <sup>b</sup>	0.002±0.00 <sup>a</sup>	1.47±0.09 <sup>b</sup>	0.36±0.00 <sup>b</sup>	10.78±0.09 <sup>c</sup>
Long Co Co	88.78±0.39 <sup>c</sup>	0.002±0.00 <sup>a</sup>	1.17±0.10 <sup>a</sup>	0.33±0.02 <sup>ab</sup>	9.72±0.08 <sup>a</sup>
Thanh Kieu	88.44±0.16 <sup>c</sup>	0.002±0.00 <sup>a</sup>	1.04±0.10 <sup>a</sup>	0.34±0.01 <sup>a</sup>	10.08±0.10 <sup>b</sup>

Values are expressed as mean±standard deviation. Values with different superscripts within the same column are significantly different ( $p < 0.05$ ).

highest content of carbohydrate (11.64±0.17%) which was mostly soluble carbohydrate (Milind, 2008). The carbohydrate content is varied from 5-10% which is majorly composed of sucrose, fructose, and glucose, contributing to the sweetness of the pomelo (Liu *et al.*, 2012). Thus, in this study, the Da Xanh cultivar could be favourable due to its higher sweetness compared to the others.

The quality of pomelo segments is described in Table 3, expressed as the total solid content, total acid, and pH. Among four varieties, Da Xanh pomelo appeared to have the highest total solid content (11.88±0.26°Bx), indicating the highest sweetness compared to the others. The average solid content of the others was around 10°Bx. The total solid content of these varieties in the Mekong Delta was found to be higher than those in Thailand which varied from 6-9.45°Bx (Lado *et al.*, 2014; Pichaiyongvongdee *et al.*, 2014; Susanto *et al.*, 2018). The total acid content was considerably low due to the high content of total solids in the pomelo segments, leading to the high TSS/TA ratio. In terms of pH results, four pomelo varieties indicated the acidic pH (pH 4), showing no statistical difference with  $p > 0.05$ .

### 3.3 Biological properties of four pomelo varieties

The biological properties of four pomelo varieties could be evaluated via their TPC, TFC, and vitamin C content, illustrated in Table 4. There was a difference in TPC, TFC, vitamin C content among these varieties, which could be ascribed to the difference in species, plantation conditions, environmental conditions, etc. (Zhang *et al.*, 2014). Nam Roi pomelo was found to have the highest TPC of 2.42±0.01 mg GAE/g DM. Wu *et al.* (2011) showed that the TPC in white pomelo segments was higher than pink pomelo segments, elucidating the

higher TPC in Nam Roi pomelo compared to the others.

Similarly, the TFC in Nam Roi pomelo was found to be the highest one as compared to the others (9.57±0.03 mg QE/g DM). The TFC in four pomelo varieties in the Mekong Delta was higher than in some Chinese pomelo varieties which ranged from around 0.13-1.19 mg QE/g DM (Xu *et al.*, 2009; Toh *et al.*, 2013). The difference in the TPC and TFC among these cultivars was ascribed to the difference in species, plantation methods, or the influence of environmental conditions such as sunlight, soil, temperature, or rainfall on the biosynthesis activity of flavonoids in citrus fruit (Zhang *et al.*, 2014). Flavonoids are among the polyphenolic group; however, the TPC was less than the TFC in this study. This could be ascribed to the Folin-Ciocalteu method and aluminium chloride colourimetric assay used in this study (Matić *et al.*, 2017). The TPC and TFC are calculated according to the polyphenol standard that represents the whole polyphenolic content. Using different standards will reveal different results. Therefore, it cannot evaluate the TPC in the whole sample but only calculate the polyphenol or flavonoid content which reacts with certain polyphenol groups to the full extent. The measurement of TPC in wine using

Table 4. Total polyphenolic, flavonoid, and vitamin C content of four pomelo varieties

Pomelo variety	TPC (mg GAE/g DM)	TFC (mg QE/g DM)	Vitamin C content
Da Xanh	1.63±0.02 <sup>a</sup>	5.15±0.02 <sup>a</sup>	34.67±0.24 <sup>c</sup>
Nam Roi	2.42±0.01 <sup>d</sup>	9.57±0.03 <sup>d</sup>	42.04±1.02 <sup>d</sup>
Long Co Co	2.39±0.01 <sup>c</sup>	7.86±0.03 <sup>c</sup>	29.18±0.18 <sup>a</sup>
Thanh Kieu	1.95±0.02 <sup>b</sup>	6.59±0.01 <sup>b</sup>	30.33±0.09 <sup>b</sup>

Values are expressed as mean±standard deviation. Values with different superscripts within the same column are significantly different ( $p < 0.05$ ).

Table 3. Quality measurement of pomelo segments in four pomelo varieties

Pomelo variety	TSS (°Bx)	TA (%)	TSS/TA ratio	pH
Da Xanh	11.88±0.26 <sup>b</sup>	0.44±0.02 <sup>a</sup>	27.00±0.14 <sup>d</sup>	4.24±0.19 <sup>a</sup>
Nam Roi	10.62±0.53 <sup>a</sup>	0.52±0.17 <sup>a</sup>	20.42±0.35 <sup>b</sup>	3.85±0.10 <sup>a</sup>
Long Co Co	10.23±0.53 <sup>a</sup>	0.60±0.08 <sup>a</sup>	17.05±0.03 <sup>a</sup>	3.75±0.17 <sup>a</sup>
Thanh Kieu	10.43±1.02 <sup>a</sup>	0.44±0.02 <sup>a</sup>	23.70±0.50 <sup>c</sup>	4.10±0.35 <sup>a</sup>

Values are expressed as mean±standard deviation. Values with different superscripts within the same column are significantly different ( $p < 0.05$ ).

gallic acid is due to the very high amount of gallic acid in wine, therefore, gallic acid is an optimal choice to represent the TPC (Waterhouse, 2002). It is indicated that the best standard for the TPC or TFC method is based on the predominant polyphenol presented in the sample with a very high amount (Matić *et al.*, 2017). Therefore, the lower TPC could be due to the polyphenol standard in this study, gallic acid, which could not represent the whole polyphenols in the sample. In terms of vitamin C content, the highest vitamin C content was again recorded at Nam Roi pomelo (42.04±1.02 mg/100 g) which was consistent with the previous study by Toh *et al.* (2013). The vitamin C content in the white pomelo segment (Malaysian White Tambun) (41.21±1.43 mg/100 g) was normally higher than in the pink pomelo segment (Malaysia Pink Tambun) (33.77±1.43 mg/100 g) (Toh *et al.*, 2013).

#### 4. Conclusion

Among four varieties of pomelo fruit in this study, Da Xanh pomelo was observed to have a good visual appearance with the red-pink colour of pomelo segments, and high sweetness while the Nam Roi cultivar was distinguished with the yellowish colour and the low sweetness. In contrast, Nam Roi pomelo was found to have the highest biological activities by achieving the highest TPC, TFC, and vitamin C content. Nutritional ingredients in four varieties were considered to be similar with high carbohydrate content but low protein and lipid content. The revealed result will possibly give a basic understanding of the properties of these varieties in the Mekong Delta, Vietnam so that further research could employ their beneficial biological properties.

#### Conflict of interest

The authors declare no conflict of interest.

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