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STUDY ON FRUIT QUALITY OF SELECTED SEEDED PUMMELO CULTIVARS AND ITS RELATIONSHIP WITH ANTIOXIDANT ACTIVITY CONTENT DURING STORAGE PERIOD

Wahyu Fikrinda, Slamet Susanto *), Darda Efendi and Maya Melati

Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia
*) Corresponding author E-mail: slmtsanto@gmail.com

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ABSTRACT

Indonesia has number of accessions and cultivars of pummelo which are prospective to be developed. Pummelo contains higher antioxidants thus beneficial for health. This research aimed to get information of physical and chemical quality differences, antioxidant capacity, and explain the relationship between fruit quality and antioxidant capacity of selected seeded pummelo cultivars. Fruit was harvested in Banyuwangi and Magetan while fruit quality assesment was conducted in Laboratory of Agronomy and Horticulture Department, IPB. The results showed that physical qualities of fruit (weight loss, peel softness and peel color) and chemical qualities (total soluble solids and total titratable acidity) were changed during storage. Adas Nambangan and Banyuwangi cultivars have better physical and chemical qualities than other cultivars during storage until 10 weeks after harvest because of good visual appearance, the lowest decreased in weight loss and the good ratio of TSS:TTA. Seeded pummelo cultivars with dark red to reddish white fruit pulp had significant higher total phenolic, carotenoid, anthocyanin and antioxidant capacity than white fruit pulp. There were negative correlations between antioxidant capacity with colored pulp and total phenolic content. Banyuwangi had the highest antioxidant capacity in the pulp, followed by Bali Merah, Adas Nambangan, Pamelo Magetan, Srinyonya, Bali Putih cultivars.

Keywords: antioxidant capacity; *Citrus grandis* (L.) Osbeck; colored pulp; seeded fruit

INTRODUCTION

Citrus is an annual fruit plant that is widely cultivated because the tastes is preferred by various segments of society. Until now, citrus has grown commercially in over 50 countries around the world. Grapefruit and pummelo production reached about 5% to 6% total citrus production of the world (Ladaniya, 2008). Production of grapefruit and pummelo in the world increased from 4,829,076 tonnes in 2006 to 8,040,038 tonnes in 2012 (FAOSTAT, 2015), while pummelo production in Indonesia is 91,131 tonnes in 2010 increased to 106,344 tonnes in 2013 (BPS, 2015). Pummelo originated from Indonesian native citrus has prospective to be developed because of its large fruits, fresh taste and long shelf life until 4 months (Susanto, 2004). Exploration results between 2009-2010 to Sumedang, Pati, Kudus, Magetan, Pankajene (South Sulawesi) and Bireuen (Aceh) found 24 pummelo cultivars, 8 are seedless and the other are seeded cultivars. Until 2013, 17 pummelo cultivars have been released and only several cultivars are produced commercially namely Magetan, Nambangan, Raja, Ratu and Srinyonya and all of them are seeded cultivars (Susanto *et al*., 2013).

Pummelo are categorized as seeded, potentially seedless, and seedless based on the number of seeds per fruit. Several character comparisons between seeded and seedless cultivars were found using various methods such as morphological characters, biochemical, and quality assessments. The fruit quality can be determined from physical, chemical and functional compounds for health. Parameter of quality assessments that have been done are total soluble solid (TSS), total titratable acid (TTA), the pH of the juice, vitamin C and naringin (Susanto *et al.*, 2010). Other important

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assessments related with quality have not been conducted yet in Indonesia are antioxidant activities.

Several bioactive compounds in fruits having antioxidant properties are carotenoid, polyphenolic, anthocyanin and vitamin (Dillard and German, 2000). There are strong relationships between vitamin C and total phenolic content with antioxidant capacity in fruit juices that determined by the FRAP assay (Gardner *et al*., 2000). Pummelo has the highest antioxidant content and function as an anti-cancer agent. High antioxidant activity is also good for diet (Gorinstein *et al*., 2004). Red pummelo juice has higher total phenolic and carotenoid content than white pummelo juice thus become a good source of antioxidants and is more efficient at capturing variety forms of free radicals (Tsai *et al*., 2007).

Up to now, research on the antioxidant content in Indonesian seeded pummelo cultivars which are often found on market has not been reported yet. This research aimed to get information of physical and chemical quality differences, antioxidant capacity, and explain the relationship between fruit quality and antioxidant capacity of selected seeded pummelo cultivars.

MATERIALS AND METHODS

Research was conducted from December 2013 to June 2014. Fruit was harvested in Banyuwangi and Magetan, East Java, while fruit quality assesment was conducted in Postharvest Laboratory and Plant Analysis and Chromatography, Department of Agronomy and Horticulture, IPB, Bogor, West Java. Fruit of six pummelo cultivars consisted of dark red pulp (Pamelo Magetan), red pulp (Bali Merah), dark pink pulp (Adas Nambangan), pink pulp (Banyuwangi), reddish white pulp (Srinyonya), and white pulp (Bali Putih) with same level of maturity (24 until 26 week after flowering (WAF)) (Figure 1). This research was conducted using environmental design completely randomized design (CRD) with single factor, i.e. cultivar. The treatment consisted of 6 cultivars with 3 replications, and one experimental unit consisted of 2 pummelo fruits so there were 288 fruits. In each cultivar, there were fruits for non-destructive and destructive observation. Data analyzed by F test, if there was a significant effect of treatment, data then analyzed by Duncan Multiple Range Test (DMRT) at 5%. Pearson correlation test was conducted between anti-oxidant capacity (IC 50) with color pulp, vitamin C, total phenolic, flavonoid, anthocyanin and carotenoid content.

The research consisted of destructive and non-destructive observation which conducted every 2 weeks for 6 times observation during storage (at 0 to 10 week after harvest). Non-destructive parameters consisted of physical qualities such as weight loss (AOAC, 1995) and the changes in fruit peel color (scoring). Destructive parameters consisted of physical quality (fruit softness), chemical and phytochemical assess-ment. Chemical assessment consist of total soluble solids (TSS), total titratable acidity (TTA), TSS:TTA ratio, and vitamin C content (AOAC, 1995). Phytochemical assessment which con-ducted once at 0 week after harvest, consisted of:

1. Total phenolic. Extract pummelo juice of 100 µl was added with 2.5 ml of water and 100 µl Folin-Ciocalteu reagent, mixed by vortex, then allowed to stand for 8 minutes. Furthermore, the solution was added to 300 µl sodium carbonate of 25%, mixed by vortex, and incubated in a water bath at 45^o C for 30 minutes. The reaction mixture was measured at 765 nm using spectrophotometer. Standard curve was made using 0-80 mg l^{-1} galic acid equivalent (GAE) (y = 0.0024x - 0.0269, R^2 = 0.966) (Waterhouse, 2002).
2. Flavonoid. Extract pummelo juice of 0.1 ml was added with 1.9 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water then mixed by vortex. After incubated at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm using spectrophotometer. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Standard curve was made using the 0-400 mg l^{-1} quercetin in methanol (y = 0.0014x - 0.0148, R^2 = 0998). The result was expressed as milligrams equivalents of gallic acid per ml of fruit juice (mg GAE ml^{-1} juice) (Chang *et al*., 2002).
3. Anthocyanin and carotenoid. Pummelo juice of 1 ml was added with 4 ml acetris then centrifuged at a speed of 14,000 rpm for 10

seconds. The absorbance were measured using UV/VIS at 663 nm, 647 nm, 537 nm and 470 nm (Sims and Gamon, 2002).

4. Antioxidant capacity. Modified from Brand-Williams *et al*. (1995) and Payet *et al*. (2005) methods. Briefly, 5 ml of 0.1 mM 2,2-Diphenyl-1-picrilhydrazyl (DPPH) ethanol solution was add to several concentrations of pummelo juice, then mixed with 5 ml of 0.1 mM DPPH solution in ethanol by vortex. The solution stored at 27^{o} C for 20 minutes in a dark room condition. The extraction solvent was used as control. The absorbance of the reaction mixture was measured at 517 nm using spectrophotometer. Antioxidants capacity expressed as inhibition percent. From inhibition percent obtained, linear regression equation and 50% radical scavenging (IC50) were determined.

Figure 1. (a) Dark Red (Pamelo Magetan), (b) Red (Bali Merah), (c) Dark Pink (Adas Nambangan), (d) Pink (Banyuwangi), (e) Raddish White (Srinyonya), (f) White (Bali Putih)

RESULTS AND DISCUSSION

Fruit Quality Changes During Storage

Physical and chemical qualities of pummelo changed during storage. Physical changes occured were changing of color peel from green to yellowish as well as changes in the peel surface from smooth to wrinkled, while weight loss and peel softness were increased. Chemical changes during storage were increasing content of the TSS and decreasing content of TTA and vitamin C.

Seeded pummelo cultivars, namely Adas Nambangan, Bali Putih, Pamelo Magetan, Banyuwangi and Srinyonya were stored at room temperature (± 27^{o} C) for 10 week after harvest (WAH) while Bali Merah cultivar could be stored only for 8 WAH. Adas Nambangan and Bali Putih cultivars could be stored with better appearance than other cultivars with yellowish green and slightly wrinkled peel. Bali Merah cultivar had a shorter shelf life because of faster degradation in physical and chemical properties than other cultivars. This was determined from occurrence of fruit tissue damage at 8-10 WAH and then fruit decayed. Pamelo Magetan and Srinyonya cultivars had poor visual appearance with dark yellow, wrinkled fruit peel and desiccation (hardening of the peel). The appearance of the fruit will determine whether the fruit to be marketable. Fruit with good appearance and long shelf life will be accepted more easily by consumers.

Fruit weight loss increased during storage 2-10 WAH. During storage, Bali Merah, Adas Nambangan and Banyuwangi cultivars had the lowest in weight loss of 31.56%, 34.13% and 38.47% respectively, while Srinyonya and Pamelo Magetan had the highest in weight loss of 51.31% and 46.70% (Figure 2). High of weight loss in Srinyonya and Pamelo Magetan cultivars were due to rougher texture peel and bigger skin pores so that the transpiration was higher and resulted in peel shrinkage. High weight loss on the fruit caused the fruit to lose its freshness, shriveled and become less attractive. Fruit weight loss occured mainly due to the transpiration while effect of respiration was lower because pummelo include non-climacteric fruit.

Peel softness in seeded pummelo fruit during storage were 8.67 until 17.99 mm 50 g^{-1} 5 s^{-1} at 0 WAH and increased to 19.26 until 30.23 mm 50 g^{-1} 5 s^{-1} at 10 WAH (Figure 3). Fruit peel softness of Bali Putih and Srinyonya cultivars were the lowest of 4.78 mm 50 g^{-1} 5 s^{-1} and 6.56 mm 50 g^{-1} 5 s^{-1} while Bali Merah, Banyuwangi and Adas Nambangan cultivars had the highest of 13.03, 12.24, 11.80 mm 50 g^{-1} 5 s^{-1}. Low peel softness in Bali Puith and Srinyonya cultivar were because fruit shrinked more quickly and lead to desiccation peel. High peel softness in Bali Merah cultivar was due to faster changes in physical and chemical quality so that the degradation of pectin in the peel occurred much sooner. Peel softness occurred due to changes in water-insoluble protopectin that turned into water-soluble pectin which caused decrease in fruit firmness and the fruit peel become soft (Seymour *et al*., 1993).

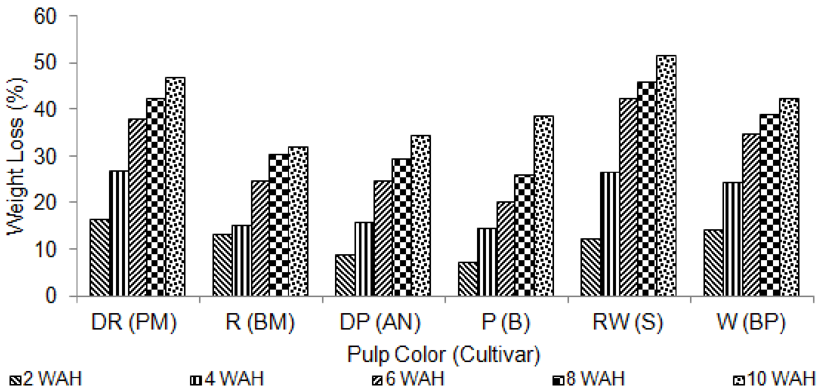

Figure 2. Fruit weight loss in various seeded pummelo cultivars

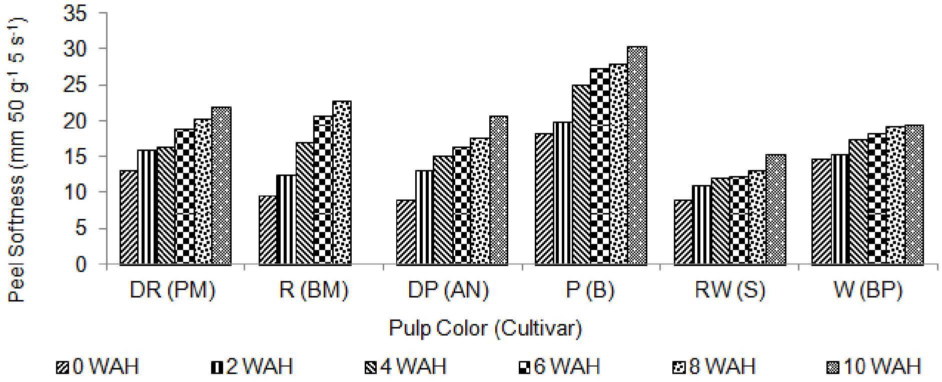

Figure 3. Peel softness in various seeded pummelo cultivars

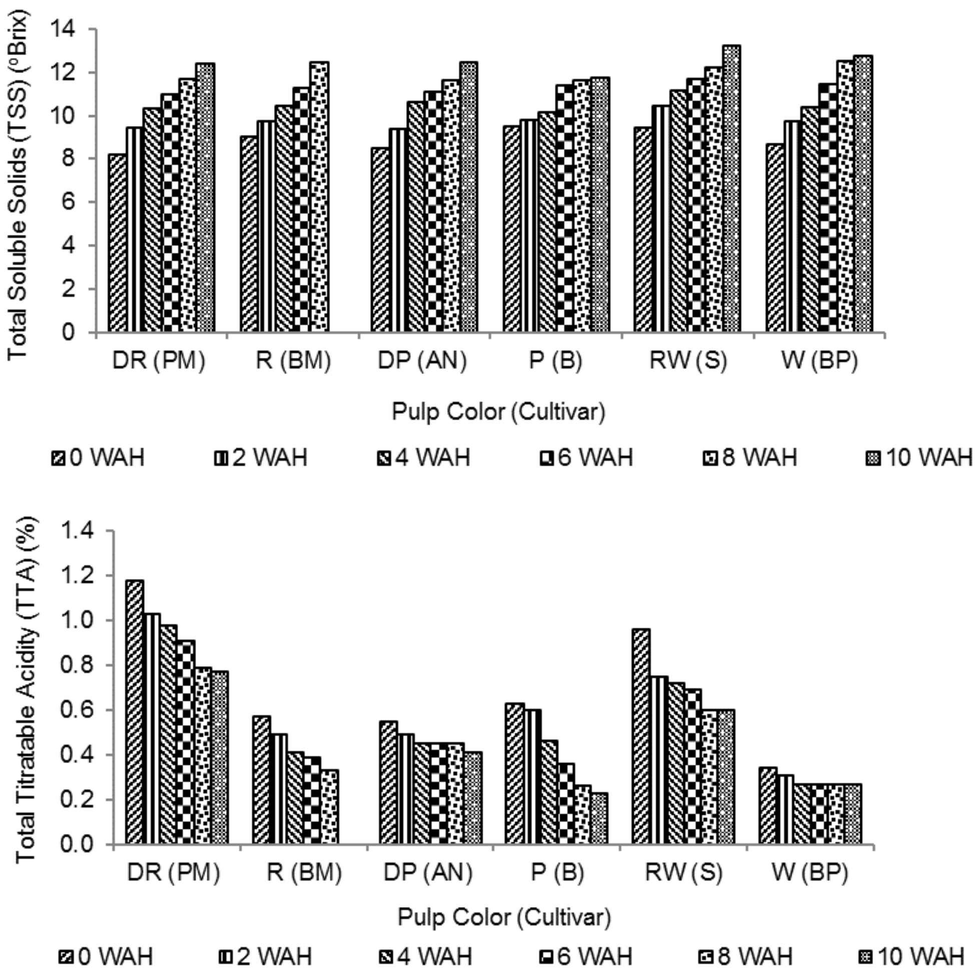

Figure 4. The Content of TSS and TTA in Various Seeeded Pummelo Cultivars

During storage, total soluble solids (TSS) in seeded pummelo fruit significantly increased whereas total titratable acid (TTA) decreased in 0 until 10 WAH (Figure 4). The TSS content of this six pummelo cultivars ranged at 8.20 until 9.53^{o} Brix (0 WAH) and increased during storage at 12.40 until 13.20^{o} Brix (10 WAH). The increase of TSS of Pamelo Magetan cultivar was the highest but not significantly different with Bali Merah, Adas Nambangan, Srinyonya and Bali Putih while Banyuwangi cultivar was the lowest. The TTA content in six pummelo cultivars ranged from 0.34 until 1.18% at 0 WAH and decreased during storage to 0.21 until 0.77% at 10 WAH (Figure 4).

The TSS:TTA ratio is one of the parameters on fruit maturity index that is related to citrus. Taste of the citrus fruit was determined by its TSS:TTA ratio. The TSS:TTA ratio between 8-10 is generally accepted as minimum maturity, while the ratio of 10-16 is criteria of good quality acceptance (Ladaniya, 2008). Pumelo Magetan cultivar had the smallest TSS:TTA ratio by 6.9 so it was not include in the minimum maturity criteria of 8-10 and causing poor fruit taste. Bali Merah, Adas Nambangan and Banyuwangi cultivars were included in the criteria of good quality acceptance. The interesting thing was Srinyonya cultivar that contained high TTA and TSS with TSS:TTA ratio of 9.851 that almost fitted into the criteria of good quality acceptance and tasted best.

Phytochemical Assessment Result

Phytochemical assessments in seeded pummelos have significantly different among cultivars in anthocyanin, carotenoid, flavonoid and total phenolic. Anthocyanin and carotenoid pigments assessments conducted to determine the role of bioactive compounds that give color to the pulp of pummelo. Anthocyanin content in six seeded pummelo cultivars was 0.011-0.030 μmol ml^{-1} (Table 1). Banyuwangi cultivar has the highest anthocyanin content and not significantly different with Pamelo Magetan, Srinyonya, Adas Nambangan> Bali Merah > Bali Putih. Carotenoid content in six seeded pummelo cultivars was 0.006-0.020 μmol ml^{-1}. Banyuwangi cultivar has the highest carotenoid content and not significantly different with Adas Nambangan, Srinyonya and Pamelo Magetan > Bali Merah and Bali Putih.

Chlorophyll content in ripe fruit is gradually reduced. Chlorophyll breakdown can be used for the synthesis of carotenoids and flavonoids such as anthocyanins (Pantastico, 1975). Carotenoids are source of yellow, orange and red colors in various crops. Anthocyanins are part of the flavonoid compounds and source of red and purple colors in the fruit. In plants, carotenoids have essential antioxidant functions that are inactivation of singlet oxygen, an oxidant which is formed during the process of photosynthesis. Carotenoid that responsible for oranges color on sweet oranges and tangerine are α-carotene, β-carotene, zeta-antheraxanthin (yellowish), violaxantin (yellowish), β-citraurin (reddish orange), and β- kriptoxantin (orange); red or pink color on grapefruit is lycopenen and red color on sweet orange is anthocyanin (Kimball, 1999).

The content of flavonoids in six seeded pummelo cultivars ranged between 0.047 until 0.274 mg QE ml^{-1} (Table 1). The highest flavonoid was found in Banyuwangi cultivar > Adas Nambangan > Pamelo Magetan, Bali Putih, Bali Merah and Srinyonya. In pummelo, naringin is the dominant flavonoid compounds that found in flavedo and juice (Zhang *et al*., 2011). Some of factors that affect the levels of flavonoids include agricultural practices applied, environmental factors, the level of maturity of materials, processing, storage and ripening (Muchtadi, 2012).

Total phenolicic content of the six seeded pummelo cultivars were 1.24 to 2.28 mg GAE ml^{-1}, Banyuwangi cultivar had the highest total phenolic content followed with Adas Nambangan > Pamelo Magetan > Bali Merah and Srinyonya > Bali Putih. These results are equal with Tsai *et al*. (2007) that the content of phenolic content of red fruit juice is higher than those of the white fruit juice.

Table 1. Phytochemical content in various seed pummelo cultivars

Pulp Color (Cultivar)	Vit C (mg 100 g^{-1} FP)	Total Phenolics (mg ml^{-1})	Flavonoid (mg ml^{-1})	Anthocyanin (µmol ml^{-1})	Carotenoid (µmol ml^{-1})	IC 50 (%)
Dark Red (Pamelo Magetan)	44.42 c	1.63 c	0.095 bc	0.029 ab	0.011 ab	30.1
Red (Bali Merah)	59.58 a	1.48 d	0.080 bc	0.025 c	0.008 b	25.8
Dark Pink (Adas Nambangan)	41.89 c	1.77 b	0.117 b	0.028 b	0.014 ab	28.8
Pink (Banyuwangi)	59.47 a	2.28 a	0.274 a	0.030 a	0.020 a	22.6
Raddish White (Srinyonya)	50.12 b	1.42 d	0.047 c	0.029 ab	0.013 ab	33.6
White (Bali Putih)	51.28 b	1.24 e	0.092 bc	0.011 d	0.006 b	35.2

Remarks: Number followed by the same letter in the same columns are not significantly different based on DMRT at level α=5%

Table 2. Correlation test between phytochemical content with the antioxidant capacity

	Color pulp	Vit C	Total Phenolics	Flavonoid	Anthocyanin	Carotenoid	IC 50
Color Pulp	1						
Vit C	0.02	1					
Total Phenolics	0.63	0.23	1				
Flavonoid	0.42	0.39	0.92**	1			
Anthocyanin	0.70	-0.01	0.54	0.18	1		
Carotenoid	0.26	0.55	0.87*	0.96**	0.20	1	
IC 50	-0.80*	-0.52	-0.83*	-0.74	-0.61	-0.70	1

Remarks: *: significant at α = 5%, **significant at α = 1%

The content of vitamin C in six seeded pummelo cultivars ranged between 41.89-59.58 mg 100g^{-1} Fresh Pulp (FP) (Table 1). Bali Merah cultivar had the highest vitamin C content of 59.580 mg 100g^{-1} FP that not significantly different with Banyuwangi > Srinyonya and Bali Putih > Pamelo Magetan and Adas Nambangan. Differences in vitamin C is influenced by differences in varieties, cultivation practices, level of maturity, fresh fruit handling, packaging and storage conditions (Nagy *et al*., 1977). Vitamin C is also much influenced by temperature and storage time (Rapisarda *et al*., 2008). On the sweet orange, vitamin C is largely derived from ascorbic acid (90%) and less than 10% of the dehydroascorbic acid (DHA) (Ladaniya, 2008).

Antioxidant capacity was indicated by IC50. The IC50 obtained from the regression equation formed from several juice concentration and not tested statistically. IC50 value is related inversely to its antioxidant capacity, this value indicates the concentration of antioxidants needed to reduce the concentration of free radicals by 50% (Molyneux, 2004). The low IC50 value indicated a higher antioxidant capacity compound (Molyneux 2004; Kelebek *et al.*, 2008). Antioxidant capacity of six seeded pummelo cultivars ranging between 35.2-22.6%. Antioxidant capacity was highest in Banyuwangi cultivar (22.6%) followed by Bali Merah > Adas Nambangan > Pamelo Magetan > Srinyonya > Bali Putih (35.2%) (Table 4). This indicates that the antioxidants capacity of pummelo with dark red pulp (Pamelo Magetan) to reddish white (Srinyonya) are higher than that of pummelo with white pulp (Bali Putih). Antioxidant capacity of seeded pummelos are higher than pummelo var. Robab and Khaloi and sweet orange var. Valencia and Sohniangriang which have IC50 of 44.43-286.51% (Kumari and Handique, 2013). Surinrut *et al*. (2005) reported that pummelo has moderate antioxidant capacity (IC50 of 11.18-32.80 mg ml^{-1}) with some fruits such as mangosteen, sweet oranges, grapes and papaya included. Fruits with high antioxidant capacity including mango, guava and lychee fruit while fruit with low antioxidant capacity including orange juice, jackfruit and apple.

Correlation Test Result

The results of correlation analysis showed an association between antioxidant capacity with pulp color and total phenolics (Table 2). Antioxidant capacity (IC50) was negatively correlated with pummelo fruit pulp color (r = -0.80) and total phenolics (r = -0.83). These results indicated that the pulp color of pummelo affected the value of IC50. Pummelo fruit with red pulp had a high antioxidant capacity than pummelo fruit with white pulp. Besides, the higher content of total phenolics the lower value of IC50 (indicating a high antioxidant capacity). These results are consistent with research of Gardner *et al*., (2000) and Xu *et al*., (2008) that the total phenolics in fruit juices have a strong relationship with the antioxidant capacity.

Correlation test was also conducted between phenolics content with the flavonoid. This test was done because flavonoids are part of the phenolics compounds. There was a relationship between phenolics and flavonoid compounds (r = 0.92), which explained that most of the compounds that make up the phenolics compounds were flavonoids. Most types of flavonoids that contained in pummelo allegedly was naringin. Naringin is a type of flavonoid that has highest content in pummelo juice (Pichaiyongvongdee and Haruenkit, 2009; Zhang *et al*., 2011). Naringin is compound that cause bitter taste in pummelo fruit (Ladaniya, 2008).

CONCLUSION

Physical qualities of fruit (weight loss, peel softness and peel color) and chemical qualities (total soluble solids (TSS) and total titratable acidity (TTA)) of seeded pummelo cultivars changed during storage. Adas Nambangan and Banyuwangi cultivars have physical qualities and chemical qualities better than other cultivars during storage until 10 week after harvest (WAH) because of good visual appearance, the lowest decreased in weight loss and the ratio PTT:ATT were good. Seeded pummelo cultivars with dark red, red, dark pink, pink and reddish white fruit pulp had significantly higher total phenolic, carotenoid, anthocyanin and antioxidant capacity than cultivars with white fruit pulp. There were negative correlations between antioxidant capacity (IC50) with colored pulp (r = -0.80) and total phenolic content (r = -0.83). Banyuwangi cultivar has the highest antioxidant capacity in the pulp, followed by Bali Merah > Adas Nambangan > Pamelo Magetan > Srinyonya > Bali Putih cultivars.

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