

## Nutrient content of three clones of red fruit (*Pandanus conoideus*) during the maturity development

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### Article history

Received: 23 November 2014

Received in revised form:

28 August 2015

Accepted: 9 September 2015

### Keywords

Red fruit (*Pandanus conoideus*)

Ripening stages

Carotenoids

Tocopherol

Nutrient composition

### Abstract

The purpose of this study was to determine the best harvest time of three clones red fruit (*Pandanus conoideus*) based on their nutrient contents. Fruit flesh of three red fruit clones (namely Monsor, Edewewits and Memeri) were analyzed the nutritional content, during the development of the maturity i.e. unripe, half ripe, ripe and overripe. The results show that the maturity stages had a significant effect on the nutrient contents of three clones of red fruit. Nutritional components in the red fruit on are fat (50.8-55.58%), carbohydrate (36.78-46.3%), vitamin C (24-45 mg per 100 g), phosphorus (654-792 ppm), calcium (4919-5176 ppm), total carotenoids (976-1592 ppm) and total tocopherols (1256-2016 ppm). The changed of nutrient composition of fruits vary in each clone during ripening. Using the principal component analysis (PCA), commonly three clones of red fruit in unripe and half ripe stages could be characterized by high content of ash, calcium, phosphor, and carbohydrate, while red fruit in maturity level of ripe and overripe were characterized by high content of fat, total carotenoids and total tocopherol content. Based on these results, the red fruit is perfect to harvest on ripe or overripe phase so that they have abundant nutrient content.

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### Introduction

Red fruit (*Pandanus conoideus*) is one of *Pandanus* plants, which is characterized by its cylindrical shape, small pieces of stone fruit, single cavity, grouped in a dense, pericarp oily, red, with a small and relatively thin endocarp (Stone, 1997). Red fruit has been traditionally used by the Papuan as a food, natural dyes and medicine for various diseases. It was also reported that red fruit and red-fruit oil contains natural antioxidant component such as  $\alpha$ -carotenoids,  $\beta$ -carotenoids,  $\beta$ -cryptoxanthin,  $\alpha$ -tocopherol and unsaturated fatty acids, particularly oleic, linoleic, linolenic and palmitoleic (Murtiningrum *et al.*, 2005; Surono *et al.*, 2008) and minerals such as Fe, Ca and P (Murtiningrum *et al.*, 2012).

Various studies have shown that extract oil of red fruits safe for human consumption (Nishigaki *et al.*, 2011) and also has a high antioxidant activity (Rohman *et al.* 2010), good effect on health benefits related to the in vivo studies, as the tumor inhibitor and cancer cells killers (Mun'im *et al.*, 2006; Surono *et al.*, 2008), anti-diabetic (Winarto *et al.*, 2009), anti-inflammatory and it can enhance immune cell (Khiong *et al.*, 2009). The development of research

on the efficacy of red-fruit oil opens its chances as a source of natural antioxidants to be developed into functional food products.

Since red fruit oil was a natural product, it has a various chemical composition, which not only influenced by cultivar, cultivation, climatic conditions, but also the level of fruit maturity at harvest (Lee and Kader, 2000). In addition, growth phase on the fruit harvest greatly affect the possibility of fruit damage during storage. If raw fruit picked unripe, the development of taste and nutrition will be disrupted. Conversely, if the fruit was picked at the stage through overripe also the nutrient content of food would quickly disappear. There were levels of development between the two extreme stages above that were associated with susceptibility to damage the fruit (Krochta and Feinberg, 1989).

The development of the fruit during the maturation greatly affect on the nutrient composition and the active components (Lee and Kader, 2000), so it can be used to determine the optimal timing of harvest. Oil content increases with the increase of the maturity level, but the content of phenols and stability was decline (Shibasaki, 2005). Similarly, the fat content of almonds (Piscopo *et al.*, 2010), and

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peanuts (Hertiningsih, 2003) were reported that it increase during maturation and varies in each clones. Santoso *et al.* (2011) reported that during the process of maturation, red fruit seeds embedded in the pith will be more stretchable and easily separated, with the soft texture of the meat, thus making it easy bruising as a result of physical injury and susceptible to chemical damage (hydrolysis and oxidation). Furthermore, Sambanthamurthi *et al.* (1991) explained that tissue injury during harvesting process of palm fruit can initiated by activity of endogenous lipases to hydrolysis fatty acid and produced free fatty acid (FFA) of palm oil. The FFA content is one of the important quality criteria of vegetable oils; which associated with undesirable rancid flavor (hydrolytic rancidity) and it is very easily oxidized (Bhosle and Subramanian, 2005).

Red fruit plants like other *Pandanus* plants are dioecious. They are unisexual, bearing male and female flowers in separate trees. In addition, *Pandanus conoideus* is believed to be parthenogenic (Hyndman, 1984). The formation of the red fruit will be formed after pollination of the flowers, which always come out at the top of the stem. Santoso *et al.*, (2011) stated that in general there were four stages of development since red fruit formation, that were unripe, half ripe, ripe, and overripe. In addition, the harvest time of each red fruit clone was variety, i.e. Monsor, Memeri and Edewewits each about 4, 6 and 8 months since the formation of the fruit. The differences in harvest time among clones of red fruit could be affect the nutritional content of fruit as well as the quality of oil of red fruit (Santoso *et al.*, 2011).

Some researcher also reported that total carotenoid content of red fruit oil was variated depend on clones (Murtiningrum *et al.*, 2012), post harvest handling (Sarungallo *et al.*, 2013) and extraction method (Andarwulan *et al.*, 2006; Sarungallo *et al.*, 2014); meanwhile no information about the change of nutrient content in red fruit during the maturity development. The purpose of this study was to determine the best harvest time of three clones red fruit based on their nutrient contents. This information is important to prove that the harvest time of each clone red fruit was different and could be influence the quality of oil.

## Materials and Methods

### Materials

The main materials in this study were three red fruit clones, namely Monsor, Edewewits and Memeri obtained from the Experimental Farm, University of Papua, Manokwari, West Papua Province, Indonesia.

Table 1. The development of maturity stages of red fruit (*Pandanus conoideus*)\*

Maturity stages		Characteristics of the red fruit changes
Unripe	Sub phase 1	<ul style="list-style-type: none"> <li>• Green fruit</li> <li>• Fruit grains have not formed</li> <li>• Fruit position perpendicular</li> <li>• Tightly wrapped in the leaf sheath</li> </ul>
	Sub phase 2	<ul style="list-style-type: none"> <li>• Pink fruit</li> <li>• Fruit grains have formed incomplete</li> <li>• Fruit position perpendicular</li> <li>• Slightly open leaf sheath</li> </ul>
	Sub phase 3	<ul style="list-style-type: none"> <li>• Dark red fruit</li> <li>• Grains have been formed entirely of fruit but not yet filled</li> <li>• Fruit slightly crouched position</li> <li>• Slightly open leaf sheath</li> </ul>
Half ripe		<ul style="list-style-type: none"> <li>• Dark red fruit</li> <li>• Empty or full spherical grains and hold tightly on the core Position of fruit on the tree with a slope of 160°</li> </ul>
Ripe		<ul style="list-style-type: none"> <li>• A little leaf sheath opening</li> <li>• Deep red colored fruit blackish red</li> <li>• Grain fruit already contains complete and sticking close to the pith</li> <li>• Position of fruit on the tree with a slope of 180°</li> <li>• Leaf sheath open and dry around <math>\pm 50\%</math></li> </ul>
Overripe	Sub phase 1	Grains fell at the end of the pith $\pm 3-5$ cm
	Sub phase 2	Grains fell at the end of the pith $\pm 5-15$ cm
	Sub phase 3	Grains fell at the end of the pith more than half
	Sub phase 4	All fruit grains fall 100%

\*Santoso *et al.* (2011)

Obsevation on the 8<sup>th</sup> years tree of three clones of red fruits using 2 fruits from difference tree. Red fruits were harvested twice at July and August 2011 on four ripening stages i.e. unripe, half ripe, ripe, and overripe. Determination of four ripening stages of red fruit based on the changes of fruit color, dried of leaf level, position of fruit on the tree, as well as shape of grains (drupa) and whole fruit (cepallum) were shown in Table 1 and Figure 1. The grains of red fruit were separated from the pith as soon after harvested and stored in freezer (-20°C) until analyzed. Before analyzed, the flesh of red fruit was separated from the seed using knife. The chemicals for analysis of nutrient content and active components of red fruit as of pro analysis quality.

### Analysis of nutrient content

Analysis of nutrient content of pulp of red fruit related to moisture content, ash, crude fat (soxhlet extraction), protein (micro kjeldahl), and vitamin C (titrimetry) (AOAC, 2005), while carbohydrates was determined using by difference method (FAO, 2003). Calcium content was determined by atomic absorption spectrophotometry (Perkin Elmer Analyst 100) (AOAC, 2005) and phosphor content was determined by the molybdenum blue method using stannous chloride as the reducing agent; the absorbance was measured by UV-VIS spectrophotometer (Shimadzu, Japan) at wavelength 400 nm (AOAC, 2005).



Figure 1. The development of maturity stages of red fruit (Santoso *et al.* 2011)

#### *Analysis of total carotenoids and total tocopherols*

Total carotenoids content of red fruit pulp was determined based on the method of Porim (2005). Two milligrams of each sample was dissolved in 10 ml hexane. The absorbance of the sample solution was measured by using spectrophotometer at wavelength of 446 nm. Total tocopherols was determined refers to the method of Wong *et al.* (1988). The weight of oil sample was 25 mg and added 5 ml of toluene. Oil solution diluted by adding 3.5 ml of 2,2-bipyridine (0.07% w/v in ethanol 95%) and 0.5 ml of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution (0.2% w/v in ethanol 95%) then 95% ethanol added to 10 ml. The solution then allowed to stand for 1 minute in a dark room and then measured absorbance with UV-VIS spectrophotometer (Shimadzu UV-2450, Kyoto, Japan) at wavelength of 520 nm. The apparent total tocopherols content was calculated on the standard curve.

#### *Statistical analysis*

Data were shown as tables of mean values and standard deviation. The discussion was based on one-way analysis of variance (ANOVA) followed by Duncan Multiple Region Test (DMRT) with the level of significance at  $P < 0.05$  for every clones. All statistical analyses were performed using the Statistical Product and Service Solutions 8.0 (SPSS 8.0) program. Average data of triplicate samples were used in the analysis. The XLSTAT Version 2015.4.01.20270 was used for principle component analysis (PCA) of red fruit nutrient contents data of four maturity stages.

## **Results**

#### *Proximate analysis of red fruit pulps*

The proximate analysis of the red fruits during maturation stages were various among clones (Table 2). The ash content, that represents the amount of

mineral content of the red fruits, ranged from 2.9 to 11.9%, is continually decreasing in the four stages of development. The study showed that the ash content of these three clones significantly different ( $P < 0.05$ ). The decline of the ash content along with decreasing of the water content of the fruit, therefore the content of mineral metabolism contributes to the process of growth and development of the fruits in plants.

The range of carbohydrate content of three clones of red fruit at different maturity levels vary from 36.8 to 83.6% (Table 2), which has a tendency to decrease. Memeri and Monsor clones reach the lowest levels on ripe stage for all maturity levels, but increase again at overripe stage, while the lowest content of carbohydrate of Edewewits clone was achieved on the overripe stage.

Protein content of 3 clones red fruit that is significantly ( $P < 0.05$ ) influenced by ripening levels was various from 1.86 to 4.3% (Table 2), which had a tendency to reduce during maturity stage of growth. While the fat content of the 3 red fruit clones during fruit cell growth varies from 2.16 to 55.7%. The lowest level of fat content in Monsor clone found on the unripe stage. The highest level of fat content, however, happens on the ripe stage (55.22%), while the maximum levels of fat content in Edewewits clone was reached on overripe phase (53.5%). For Memeri clone, the maximal level of fat content achieved on the ripe stage (55.58%), but it tends to decrease on overripe stage as well.

#### *Vitamin and mineral content of red fruit pulps*

Table 3 showed that the vitamin C of three red fruit clones has range from 21.5 to 57.5 mg per 100 g, which tends to increase during fruit growth. Maximum level of vitamin C of Monsor clones reached on the ripe stage (44.5%), while the Memeri and Edewewits clones have a maximum level of 25% and 57.5%, respectively, on a half ripe phase and decrease thereafter. Raffo *et al.* (2004) reported that the content of vitamin C decreased with fruit ripening. However, Zhang *et al.* (2006) showed that the acid contents increase at the beginning and middle stages of fruit growth and then decrease after the development of fruit color.

The level of total carotenoids of three red fruit clones during maturation development level range from 0.4 to 1592 ppm (Table 3), which had a tendency to increase during maturity stage of growth. The highest level of total carotenoids of Monsor, Edewewits and Memeri clones were achieved on the overripe stage. Total tocopherols content of three red fruit clones during maturation range from 12 to 2016 ppm, which generally increase during maturity

Table 2. Proximate analysis results of three clones of red fruit in the four stages of maturation

Clones	Maturity stages	Moisture (% wb), mean <sup>*</sup> ± SD	Ash (% db), mean <sup>*</sup> ± SD	carbohydrates (% db), mean <sup>*</sup> ± SD	Protein (% db), mean <sup>*</sup> ± SD	Fat (% db), mean <sup>*</sup> ± SD
Monsor	Unripe	39.6±0.1 <sup>c</sup>	11.9±0.1 <sup>a</sup>	75.8±0.2 <sup>b</sup>	4.3±0.04 <sup>a</sup>	7.9±0.002 <sup>c</sup>
	Half ripe	46.9±0.05 <sup>b</sup>	8.7±0.12 <sup>b</sup>	77.71±0.24 <sup>a</sup>	2.57±0.00 <sup>b</sup>	11.0±0.11 <sup>b</sup>
	Ripe	53.7±0.2 <sup>a</sup>	4.72±0.08 <sup>c</sup>	37.49±0.09 <sup>d</sup>	2.55±0.10 <sup>b</sup>	55.22±0.07 <sup>a</sup>
	Overripe	37.2±0.2 <sup>d</sup>	3.03±0.01 <sup>d</sup>	39.44±0.7 <sup>c</sup>	2.56±0.03 <sup>b</sup>	54.95±0.7 <sup>a</sup>
Edewewits	Unripe	44.8±0.1 <sup>a</sup>	9.82±0.02 <sup>a</sup>	84.1±0.05 <sup>a</sup>	3.05±0.01 <sup>a</sup>	3.01±0.12 <sup>d</sup>
	Half ripe	41.3±0.3 <sup>b</sup>	3.55±0.03 <sup>b</sup>	42.7±0.33 <sup>b</sup>	2.91±0.03 <sup>a</sup>	48.0±0.03 <sup>c</sup>
	Ripe	33.6±0.2 <sup>c</sup>	2.98±0.00 <sup>c</sup>	46.3±0.17 <sup>c</sup>	2.66±0.06 <sup>b</sup>	50.8±0.39 <sup>b</sup>
	Overripe	33.1±0.1 <sup>c</sup>	2.9±0.03 <sup>c</sup>	41.0±0.06 <sup>d</sup>	2.5±0.11 <sup>b</sup>	53.5±0.003 <sup>a</sup>
Memeri	Unripe	43.7±0.2 <sup>b</sup>	10.6±0.03 <sup>a</sup>	83.6±0.18 <sup>a</sup>	3.62±0.15 <sup>a</sup>	2.16±0.00 <sup>d</sup>
	Half ripe	40.6±0.1 <sup>c</sup>	9.53±0.02 <sup>b</sup>	81.81±0.1 <sup>a</sup>	3.42±0.01 <sup>a</sup>	5.23±0.09 <sup>c</sup>
	Ripe	34.6±0.2 <sup>d</sup>	5.03±0.18 <sup>c</sup>	36.78±1.3 <sup>c</sup>	2.59±0.01 <sup>b</sup>	55.58±1.1 <sup>a</sup>
	Overripe	49.8±0.1 <sup>a</sup>	2.7±0.04 <sup>d</sup>	42.02±0.3 <sup>b</sup>	1.86±0.03 <sup>c</sup>	53.40±0.2 <sup>b</sup>

\*n = 3

wb = wet base

db = dry base

Value followed by different letter within a column indicate a significantly difference (p &lt; 0.05)

Table 3. Vitamin C, total carotenoids, total tocopherol, phosphor and calcium from three varieties of red fruit in the four stages of maturation

Clones	Maturity stages	Vitamin C, mg per 100 g, db (mean <sup>*</sup> ± SD)	Total Carotenoids, ppm, db (mean <sup>*</sup> ± SD)	Total Tocopherol, ppm, db (mean <sup>*</sup> ± SD)	Phosphor ppm, db (mean <sup>*</sup> ± SD)	Calcium ppm, db (mean <sup>*</sup> ± SD)
Monsor	Unripe	38±8.8 <sup>a</sup>	25±0.5 <sup>d</sup>	311±1.9 <sup>c</sup>	1747±149 <sup>a</sup>	8734±375 <sup>a</sup>
	Half ripe	44±9.6 <sup>a</sup>	225±14.7 <sup>c</sup>	269±19.7 <sup>d</sup>	1554±36 <sup>a</sup>	8517±111 <sup>a</sup>
	Ripe	45±0.71 <sup>a</sup>	552±50 <sup>b</sup>	544±20.2 <sup>b</sup>	792±2.07 <sup>b</sup>	5020±452 <sup>b</sup>
	Overripe	44±0.0 <sup>a</sup>	976±3.9 <sup>a</sup>	1256±0.6 <sup>a</sup>	661±70.9 <sup>b</sup>	4350±151 <sup>b</sup>
Edewewits	Unripe	22±2.1 <sup>b</sup>	0.6±0.17 <sup>c</sup>	28±0.5 <sup>d</sup>	1289±43 <sup>a</sup>	7094±257 <sup>a</sup>
	Half ripe	24±1.8 <sup>b</sup>	1098±523 <sup>b</sup>	1355±12.2 <sup>b</sup>	697±12 <sup>b</sup>	4498±90 <sup>c</sup>
	Ripe	24±1.1 <sup>b</sup>	1073±52 <sup>b</sup>	1294±38 <sup>c</sup>	654±6.8 <sup>b</sup>	4919±53 <sup>b</sup>
	Overripe	25±1.4 <sup>a</sup>	1592±89 <sup>a</sup>	2016±3.9 <sup>a</sup>	435±5.45 <sup>c</sup>	4146±64 <sup>c</sup>
Memeri	Unripe	26±0.71 <sup>c</sup>	0.4±0.03 <sup>c</sup>	12±0.6 <sup>d</sup>	1784±78.9 <sup>a</sup>	8460±109 <sup>a</sup>
	Half ripe	26±3.5 <sup>c</sup>	8.4±0.03 <sup>c</sup>	211±4.3 <sup>c</sup>	1718±26.9 <sup>a</sup>	7647±306 <sup>b</sup>
	Ripe	36±1.1 <sup>b</sup>	678±8.6 <sup>b</sup>	883±1.6 <sup>b</sup>	759±11.1 <sup>b</sup>	5176±166 <sup>c</sup>
	Overripe	58±4.95 <sup>a</sup>	1095±18.8 <sup>a</sup>	1921±1.7 <sup>a</sup>	700±26.3 <sup>b</sup>	4634±48 <sup>d</sup>

\*n = 3

db = dry base

Value followed by different letter within a column indicate a significantly difference (p &lt; 0.05)

stage of growth (Table 3). The maximum amount of tocopherols content of all clones reached on the ripe stage.

The content of Phosphor (P) of the three red fruit clones at different stages of maturity was quite high, which has ranged from 435 to 1784 ppm (Table 3). The P content tends to decrease during the maturation and decline on the unripe stage (1289-1784 ppm), while the lowest content was on the overripe stage (435-750 ppm). While the calcium (Ca) content of three red fruit clones on the four maturity levels was about 4146 to 8460 ppm, which tends to decrease during the ripening process, which followed by the decrease of ash and phosphorus content. The highest Ca content of Monsor clone was on the unripe phase,

and fall to its lowest level on the overripe phase. For Edewewits and Memeri clones, Ca levels gradually decline on overripe stage, except for Edewewit clone on the half ripe stage.

#### Principle component analysis (PCA) of the three red fruit clones nutrient content

Every red fruit clones have differences composition of the nutrition content as shown in Table 2 and Table 3. The PCA was applied to investigate the relationship between 3 clones of red fruit by their nutrients components (moisture, ash, calcium and phosphor, protein, carbohydrates, fat, total carotenoids, total tocopherol and vitamin C) content during maturation.

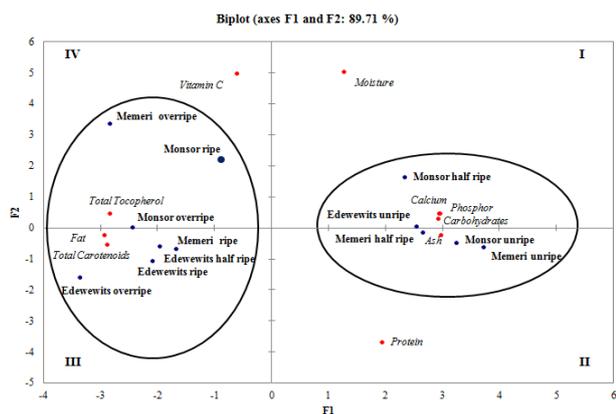


Figure 2. Distribution of three clones red fruit based Principle Component (PC) 1 (F1) and PC 2 (F2) with 8 variables of the nutrients compounds of the red fruit at four maturity stages

The PCA results in Figure 2 shows that 8 variables comprising the composition of nutrients components of red fruit from 3 clones in four maturity level has a total of 89,71% variance. The 3 clones of red fruit in four maturity stages (unripe, half ripe, ripe and overripe) and spread in four quadrants biplot can be classified into 2 groups based on the proximity of their nutrition component. In quadrant 1 and 2, the three clones of red fruit in maturity level of unripe and half ripe were characterized by high content of calcium, phosphor, carbohydrate, ash content, except Edewewits clones at half ripe stage. In quadrant III and IV, the three clones of red fruit in maturity level of ripe and overripe were characterized by high content of fat, total carotenoids and total tocopherol, except Edewewits clones at half ripe stage include in this group.

## Discussions

Based on data content ash (Table 2) as well as phosphor and calcium (Table 3) of red fruits tend to decrease during maturation. This result supported by data of PCA, which is show in Figure 2. Content of ash, calcium, and phosphor were higher at unripe and half ripe than ripe and overripe of all clones, except Edewewits was high in half ripe stage.

The decrease of phosphor content during maturation is very advantageous because the presence of phosphorus in red fruit is one of the triggers for high levels of gum in the extraction of oil. In this case, phosphorus compound often to remove through the process of oil refining by degumming process (Subramanian and Nakajima, 1997). According to Marschner (1995), during fruit development, the calcium content of the cell walls increased to the fully grown immature stage, but this was followed by a

drop in the content and change in the binding form of calcium in the tissue just before ripening (softening of the tissue). In this study, the calcium content of these red fruit pulps (Table 3) on the ripe stage are lower than the previous study of Murtiningrum *et al.* (2012) reported that calcium content of 16 red fruits clones from five regions in Papua was about 5000 to 11000 ppm. Piscopo *et al.* (2010) also reveals that the level of calcium in almonds was affected both by cultivar and harvest time. From this experiment, the level of calcium content of red fruit was higher than almond, which was around 899.7 to 1765 ppm (Piscopo *et al.*, 2010). Thereby the red fruit is potentially as a source of calcium.

The carbohydrate content of red fruit during maturation was fluctuate (Table 2) depends on the fat accumulation. Hertiningsih (2003) reported that the content of carbohydrate of peanut initially refuse to maximum limitation and then increased again. Nevertheless, according to PCA (Figure 2), at unripe and half ripe stages all clones are containing carbohydrates higher than ripe and overripe, except Edewewits clone in half ripe stage not include in this group. Edewewits clone converts carbohydrate faster in half ripe stage and then slowly in the next stage. This result indicates that carbohydrates content of red fruit could be used as indicator of maturity fruit. Kansci *et al.* (2003) also reported that the ripe pulps of four mango varieties contain more soluble sugars than the unripe ones. In addition, there was no starch in the ripe Keitt mangoes, indicating total transformation into soluble sugars during ripening. This indication is related to the characteristics of biochemical and physiological components of the genetic influence of specific chemical compound in each clone.

The content of carbohydrates of these red fruit pulps at ripe stage (37.49-46.3%) was lower than previous study of Murtiningrum *et al.*, (2012), which reported that carbohydrate content of 16 clones of red fruits from five regions in Papua at the ripe stage was about 43.86 to 79.66%. The differences were caused by genetic, maturity, geographical difference and soil composition that were characterized by rain intensity and diverse vegetation.

Protein is one of important nutritional component that are synthesized plant. The content of protein of red fruit is tend to decline during the stage of maturation (Table 2). As shown in Figure 2, the protein content of red fruit located in quadrant II but far from the centre of biplot, because contribution of protein in red fruit nutrition component only 2.55-2.91%. Ghasemnezhad and Honermeier (2007) reported that the protein content of seed of *Oenothera*

*biennis* L. was significantly influenced by the harvest time. Moreover, the reduction of the protein content during the late harvest could be explained by negative correlation between oil and protein content in oilseed. Fandi *et al.* (2011) also state that continuous but non-uniform disappearance of some proteins and formation of new proteins were observed at the early stages of mesocarp development and during certain periods of oil synthesis and palm fruit ripening. In addition the results of their study indicate that developing mesocarp revealed significant changes in the protein profiles during fruit development.

Based on the data on Table 2, the fat content of both unripe and half ripe of Monsor and Memeri clones are not optimal (2.16-11.0%), but Edewewits clone has a higher fat content. This data in line with the PCA (Figure 2) that at ripe and overripe stages of all clones are containing fat higher than unripe and half ripe, except Edewewits clone in half ripe stage include in this group. This result indicates that the harvest must be executed on the ripe stage. Connell *et al.* (2000) reported that the fat content of almond increased by the increasing of length of time of the harvest. Basiron and Chan (2004) determined that the unripe palm fruits contain very little oil, but the mesocarp of ripe fruits has an oil content of 70 to 75% of its total weight. Hertiningsih (2003) also reported that fat content of peanut increased to maximum level by the increasing of seedling age, but it decreased at the age of 61 days after flowering. Decreasing of the fat content might be caused by reorganization of the reserves of food in the cotyledons, whereas the transfer and its formation have been discontinued. In addition, the fat content of the third clones of the red fruits (48-55.8%) in the ripe stages was higher than the previous study (11.8-30.7%) which conducted by Murtiningrum *et al.* (2012). The differences may have been caused by either a different genetic of the plants or environmental effects.

Natural colors of red fruit mainly cause by carotenoids that having at least seven conjugated double bonds, therefore it can produce a red color, consisting of  $\alpha$ - and  $\beta$ -carotenoids, and  $\beta$ -cryptoxanthin, which is converted to vitamin A and essential for the body (Surono *et al.*, 2008).

The content of total carotenoids of red fruit tends to increase during maturation process (Table 3). Carotenoids content of *Solanum indicum* L. (N'Dri *et al.*, 2010) and some citrus clones (Kato *et al.*, 2004) were also reported that increased during ripening. Generally, the chlorophyll in the fruit will be degraded during the ripening process as well as produces large amounts of carotenoids. Me'ndez *et al.* (2000) reported that the ripening fruit of the five

clones showed the typical and characteristic pattern of carotenoid biosynthesis for the *Capsicum* genus, where lutein and neoxanthin, both characteristic chloroplast pigments, decreased in concentration with ripening and eventually disappeared.

In this study, a large number of carotenoids have been formed since the unripe stage, so it was a good source of carotenoids. Thus, the level of maturity was one important factor affecting the level of carotenoids, which were formed in the chloroplast cells chromoplast in red fruit. The PCA results shows that total carotenoids content of three clones red fruit at ripe and overripe stages are grouping in one group, except Edewewits clone in half ripe stage include in this group (Figure 2). This result indicates that total carotenoids and fat content of red fruit could be used as indicator of maturity fruit. According to Me'ndez *et al.* (2000), either higher or lower content of carotenoid for a given clone depends on various factors such as greater or lesser expression of the genes governing carotenogenesis, physiological and morphological characteristics intrinsic to the cultivar, and growth conditions. Although the last factor is very important in field trials because of its effect on the agronomic yield of the plant, in the present work it can be ignored because the orchard conditions are the same and all clones of red fruit originated from the same area.

The total tocopherols content of three red fruit clone tendency to increase during maturation stage. Results of PCA shows that total tocopherol content of three clones red fruit at ripe and overripe stages are grouping in one group, except Edewewits clone in half ripe stage include in this group (Figure 2). This result indicates that total tocopherol as well as total carotenoids and fat content of red fruit could be used as indicator of maturity fruit. Beltra'n *et al.* (2010) also explained that tocopherols decreased during the ripening process of olive fruit, and although  $\gamma$ -tocopherol showed an increase for the last harvesting dates, this trend was related to the chlorophyll that losses in the oil. Furthermore, during chloroplast senescence, tocopherols levels can increase several fold; this was directly related to the breakdown and subsequent increased availability of phytol from chlorophyll (Rise *et al.*, 1989). Therefore, the tocopherols increase observed in this study may be explained by the decrease and breakdown of chlorophyll. The content of total tocopherol of three red fruit clone in this study was similar to Murtiningrum *et al.* (2012) reported, that the range of total tocopherols content of 16 red fruit clones on the ripe stage were 285.3-1191.8 ppm, that was influenced by genetic and environmental effect.

In this experiment, the content of vitamin C of the three clones on the ripening stage (23.75-44.5 mg per 100 g) was higher than the previous study was conducted by Murtiningrum *et al.* (2012) which reported that 16 red fruits clones from five regions in Papua contained from 3.78 to 21.88 mg per 100 g. When fruit become ripe, vitamin C content tend to declines; this decline may be concurrent with the degradation of fruit tissue. Simonne *et al.* (1997) reported that among different types of colored peppers, ascorbic acid increased, decreased, or remained the same during ripening. According to Lee and Kader (2011), the content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypes differences, pre harvest climatic conditions and cultural practices, maturity and harvesting methods, and post harvest handling procedures. The higher intensity of light during the growing season, the greater was vitamin C content in plant tissues. Among pre harvest factors, light intensity and temperature were the most important in determining the final vitamin C content of the commodity. Then, vitamin C losses continue through post harvest handling, processing, cooking, and storage of fruits and vegetables.

## Conclusions

Maturity level of red fruit significantly affects the nutrients of red fruit clones, namely Monsor, Edewewits and Memeri. Nutritional components in the red fruit were fat, carbohydrate, vitamin C, phosphorus (P), calcium (Ca), carotenoids and tocopherols. Changes in nutrient composition of red fruits vary in each clone during ripening process. Monsor clone reaches maximum levels content of fat, vitamin C, phosphorus and calcium on the ripe; while, total carotenoids and total tocopherols was on the overripe stage. Edewewits and Memeri clones achieve maximum levels of fat, vitamin C, total carotenoids and total tocopherols on the overripe; while the optimum level of phosphorus and calcium reaches on the ripe stage. Using the principal component analysis (PCA), commonly red fruit in unripe and half ripe stages could be characterized by high content of ash, calcium, phosphor, and carbohydrate, while red fruit in maturity level of ripe and overripe were characterized by high content of fat, total carotenoids and total tocopherol content. The best harvest time of red fruit clones was on the ripe or overripe stage since their nutrient content is higher, except carbohydrate and phosphorus.

## Acknowledgements

The authors would like to thank to the Directorate for Research and Community Service, Directorate General of Higher Education, Ministry of Education Republic of Indonesia for funding through Competitive Research Grants National Priorities, No. 546/PP/SP2H/DP2M/VII/24. Thanks to Armiati, STP for assistance in the study.

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