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Chemical properties, carotenoid, tocopherol and fatty acid composition of three clones of red fruit (*Pandanus conoideus* Lam.) oil of different ripening stages

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Abstract

The differences in harvest time among clones of red fruit (*Pandanus conoideus* Lam.) could affect their nutritional content and oil quality. The present work was therefore aimed to characterise the chemical properties and fatty acid composition of oil from three red fruit clones (Monsor, Memeri, Edewewits) during four ripening stages (unripe, half-ripe, ripe, over-ripe). The free fatty acid (FFA) in oil of Monsor clone increased along the ripening stage, but there were reductions in iodine, total carotenoids and total tocopherols, and unsaturated fatty acid values. Conversely, the FFA and saponification value in oil of Memeri and Edewewits clones decreased along the ripening stage, with the amounts of iodine, total carotenoids and total tocopherols, and unsaturated fatty acid increased. Maximum total carotenoids (4,090 ± 180 ppm) and total tocopherols (1,468 ± 474 ppm) in oil of Monsor clone were found at the ripe stage. Maximum total carotenoids (6,790 ± 130 ppm) and total tocopherols (1,402 ± 755 ppm) in oil of Memeri clone were found at the over-ripe stage. Maximum total carotenoids (7,723 ± 1,305 ppm) and total tocopherols (1,814 ± 357 ppm) contents in oil of Edewewits clone were also found at the over-ripe stage. Therefore, it could be concluded that the best harvest time for Monsor clone would be at the ripe stage, while for the Memeri and Edewewits clones at the over-ripe stage in order to obtain the maximum quality of oil.

Introduction

Red fruit (*Pandanus conoideus* Lam.) is mostly found in Papua and Papua New Guinea. The Papuans generally consume red fruits with vegetables and tubers. Red fruits can make vegetable delicious because they contain 26.88-30.72% fat (Murtiningrum *et al.*, 2012). The potential health benefits of red fruit oil such as in inhibiting tumour growth and destroying cancerous cells (Mun'im *et al.*, 2006; Surono *et al.*, 2008), providing anti-inflammatory activity and increasing immune system (Khiong *et al.*, 2009), and reducing blood sugar in diabetic rats (Winarto *et al.*, 2009) have been reported. These health benefits are believed to be associated with the high antioxidant content in red fruits (Rohman *et al.*, 2010) such as carotenoids (pro vitamin A) and tocopherol (vitamin E), as well as unsaturated fatty acids (Surono *et al.*, 2008; Sarungallo *et al.*, 2015a; Sarungallo *et al.*, 2015b).

The chemical compositions and active compounds of red fruits and their oil are influenced by environmental factors such as cultivation location, climate and irrigation technique (Murtiningrum *et al.*, 2012), as well as the ripeness of the fruits (Sarungallo *et al.*, 2016). A study on olive (Olea europaea L.) found that the oil content in unripe olives was 24.5% and increased to 40.5% in ripe olives; however the total phenol decreased from 41.3 mg/100 g to 25.3 mg/100 g, and total tocopherols from 32.5 mg/100 g to 22.2 mg/100 g (Shibasaki, 2005; Jiang *et al.*, 2005).

The ripening stages of red fruits are divided into four; unripe, half-ripe, ripe and over-ripe. The texture of the pulp, which becomes softer along the ripening stage, is easy to bruise as a result of physical injury and also susceptible to chemical damage such as hydrolysis and oxidation (Santoso *et al.*, 2011).

The free fatty acid (FFA) is one of the important quality criteria of vegetable oils. Since FFA is very easily oxidised, vegetable oils are associated with the undesirable rancid flavour (hydrolytic rancidity)

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(Bhosle and Subramanian, 2005). As a result, the quality of vegetable oils will decrease. According to Sarungallo *et al.* (2016), the best harvest time of red fruits is at ripe or over-ripe since at these stages their nutrient content is higher. However, the chemical properties, antioxidant compounds and fatty acid composition during the ripening stages of red fruit oil have never been reported before. The present work was therefore aimed to characterise the chemical properties and fatty acid composition of oil from three red fruit clones (Monsor, Memeri, Edewewits) during four ripening stages (unripe, half-ripe, ripe, over-ripe).

Materials and methods

Materials

Three clones of red fruit (Monsor, Memeri, Edewewits) were obtained from the Experimental Farm of Papua University (UNIPA) Manokwari, West Papua Province, Indonesia. Two fruits from different trees of four ripening stages were harvested twice in July and August. The determination of the four ripening stages was (Santoso *et al.*, 2011) based on the changes in fruit colour, dryness of leaves, position of fruits on the tree, as well as shape of grains (*drupa*) and whole fruits (*cepallum*). For unripe red fruits, the grains are formed but not completely filled, the position of the fruits is rather low and the leaf sheath is a little open. For half-ripe red fruits, the grains are completely formed and embedded tightly on the *pedicel* (pith), the position of the fruits is at 160o angle and the leaf sheath is a little open. For ripe red fruits, the grains are completely filled and softer and will excrete oil when pressed, the position of the fruits is at 180o angle. For over-ripe red fruits, the grains are not strongly attached on the pedicel which makes it falls easily and the leaf sheath is dry.

Extraction of oil

The oil was extracted from the red fruits using ethyl acetate at 1:2 (v/v) fruit:solvent ratio at room temperature for 15 h with continuous shaking. The oil extract was filtered using a vacuum pump, evaporated with a rotary evaporator at 30°C, transferred into dark bottles and refrigerated until analysed. The yield of oil extract was calculated as percentage of fresh weight (Jiang *et al.*, 2005).

Analysis of chemical properties

The chemical properties of oil extract were analysed which included water content using oven method (AOAC, 2005), free fatty acid using titration method (AOCS, 2003), iodine value using Wijs method (AOAC, 2005) and saponification value (AOAC, 2005).

Analysis of total carotenoids and total tocopherols

Total carotenoids of red fruits were determined according to PORIM (2005). Briefly, 2 mg sample was dissolved in 10 mL hexane. The absorbance of the sample solution was measured with a spectrophotometer at 446 nm.

Total tocopherols of red fruits were determined according to Wong *et al.* (1988). Briefly, 25 mg sample was dissolved in 5 mL toluene. The solution was diluted with 3.5 mL 2,2-bipyridine (0.07% w/v in 95% ethanol) and 0.5 mL $FeCl_3.6H_2O$ solution (0.2% w/v in 95% ethanol). Next, 95% ethanol was added to reach 10 mL. The solution was then incubated for 1 min in the dark. Its absorbance was measured with UV-VIS spectrophotometer (Shimadzu UV-2450, Kyoto, Japan) at 520 nm. The final total tocopherols were calculated against a standard curve.

Analysis of fatty acid composition

The analysis of fatty acid (FA) composition of the oils was done by trans-esterification of triglycerides into fatty acid methyl esters (FAME) according to AOCS (2003). Briefly, 0.025 g oil was added to 1 mg internal standard solution (C17:0, Sigma Co.; St Louis, MI, USA) in 10 mL hexane. Next, 1.5 mL 0.5 N NaOH in methanol was added, exhaled with N_2 gas and heated for 5 min at 85°C. After cooling at room temperature, 2 mL 14% BF3-methanol was added and heated at 85°C for another 30 min. After cooling, 1.5 mL hexane and 3 mL saturated NaCl were added, mixed gently and the upper layer (FAME) was collected. The FAME (10 µL) was injected into a gas chromatography system (GC-2100 Series; Shimadzu Corp.; Kyoto, Japan) equipped with a flame ionisation detector and a column (DB-23; 30 m × 0.25 mm and 0.25 µm thickness). The conditions for the analysis were: (a) injector at 250°C; (b) oven at 120°C to 230°C for 6 to 25 min, at a rate of 3°C/min; and (c) detector at 260°C. Individual peaks of the FAME were identified by comparing their retention times with those of the standards (FAME Mix C8-C22; Bellefonte, PA USA). Each individual FA composition was calculated using the peak areas of the FA species that appeared in the chromatogram of the total peak areas of all the FAs in the oil samples.

Statistical analysis

Data were shown as tables of mean values and standard deviation from average of triplicate. A one-way Analysis of Variance (ANOVA) was performed followed by the Duncan Multiple Region

Table 1. Yields and chemical properties of three clones of red fruit oil during four stages of maturation

Clone of red fruit*	Ripening stage	Yield of oil (%)	Free fatty acid (%)	Iodine value	Total carotenoids (ppm)	Total tocopherols (ppm)
Monsor	Unripe	7.19 ± 0.27^{d}	1.75 ± 0.32^{b}	73.14 ± 0.5^{a}	257 ± 4^{c}	993 ± 500^{c}
	Half-ripe	10.74 ± 0.20^{c}	2.19 ± 0.45^{ab}	70.97 ± 0.8^{b}	3,110 ± 100^{b}	1,128 ± 875^{b}
	Ripe	20.85 ± 0.86^{b}	2.82 ± 0.53^{ab}	64.45 ± 0.1^{c}	4,090 ± 180^{a}	1,468 ± 474^{a}
	Over-ripe	27.02 ± 0.33^{a}	3.00 ± 0.33^{a}	51.78 ± 0.9^{d}	3,430 ± 110^{a}	1,195 ± 270^{b}
Memeri	Unripe	1.54 ± 0.20^{c}	2.79 ± 0.08^{a}	24.74 ± 1.5^{d}	264 ± 44^{d}	744 ± 119^{d}
	Half-ripe	5.58 ± 0.85^{b}	2.25 ± 0.86^{a}	64.12 ± 0.3^{b}	1,440 ± 150^{c}	1,073 ± 159^{c}
	Ripe	17.70 ± 0.42^{a}	2.25 ± 0.52^{a}	58.03 ± 1.1^{c}	4,300 ± 220^{b}	1,133 ± 106^{b}
	Over-ripe	17.14 ± 0.20^{a}	2.25 ± 0.03^{a}	69.17 ± 0.9^{a}	6,790 ± 130^{a}	1,402 ± 755^{a}
Edewewits	Unripe	0.13 ± 0.01^{d}	1.56 ± 0.19^{a}	22.04 ± 0.7^{d}	958 ± 95^{d}	881 ± 121^{d}
	Half-ripe	11.89 ± 0.52^{c}	1.23 ± 0.93^{a}	58.01 ± 3.0^{c}	3,849 ± 229^{c}	1,189 ± 469^{c}
	Ripe	15.13 ± 0.38^{b}	1.14 ± 0.32^{a}	65.33 ± 3.2^{b}	5,247 ± 473^{b}	1,302 ± 149^{b}
	Over-ripe	22.51 ± 0.93^{a}	0.74 ± 0.08^{a}	66.90 ± 1.0^{a}	7,723 ± 1,305^{a}	1,814 ± 357^{a}

*Different letters within a column indicate a significant difference ($p < 0.05$)

Test (DMRT) with the level of significance at $p < 0.05$. Statistical analyses were performed using the Statistical Product and Service Solutions 8.0 (SPSS 8.0) software.

Results and discussion

The yield of oil

The yield of red fruit oil depends on the fat content of the fruits during maturation. Sarungallo *et al.* (2016) reported that the fat content of red fruits will increase along the ripening stage where the highest level of fat content occurs during the ripe stage. Based on Table 1, the yield of oils seemed to increase from unripe to over-ripe stage; Monsor (7.19 - 27.02%), Memeri (1.54 - 17.14%) and Edewewits (0.13 - 22.51%) clones, reaching the maximum percentage at over-ripe stage for all clones analysed. This might be explained by the fact that the texture of red fruit flesh became softer along the maturation stages which in turn enabled efficient oil extraction (Santoso *et al.*, 2011). Several other oil plant sources also showed similar trend; avocado (Yousef and Hassaneine, 2010), olive (Shibasaki, 2005; Jiang *et al.*, 2005; Desouky *et al.*, 2009) and peanut (Hertiningsih, 2003).

Chemical properties of oil

In the present work, the red fruit oils of three clones at four ripening stages included free fatty acid (FFA), iodine, total carotenoids and total tocopherols (Table 1). The FFA is one of the important quality criteria of vegetable oils. Since FFA is very easily oxidised, vegetable oils are associated with the undesirable rancid flavour (hydrolytic rancidity) (Bhosle and Subramanian, 2005). As a result, the quality of vegetable oils will decrease. The FFA in oils analysed in the present work varied among the four ripening stages of the three clones. The FFA in oil of Monsor clone was found to increase along the ripening stages. In contrast, that of Memeri and Edewewits clones decreased (Table 1). According to Nawar (1996), FFA in oil or fat may be formed by the enzyme (lipase) catalysed hydrolysis after harvesting in the presence of water. This has also been stated by other researchers (Abdalla *et al.*, 2008; Yousef and Hassaneine, 2010; Atinafu and Bedemo, 2011). The FFA in oil of Monsor clone increased along the ripening stage might be due to the increase in water content which was not observed in Memeri and Edewewits clones (Sarungallo *et al.*, 2016). Table 1 also shows that the FFA in oil of Monsor clone was higher than the others Edewewits clone yielded the lowest. This might mean that each clone of red fruits has different endogenous lipase activity. Sambanthamurthi *et al.* (1991) reported that an increase of FFA in palm oil was due to the action of an endogenous lipase. Sambanthamurthi and Kushairi (2002) further confirmed that endogenous lipase activity is genotype-dependent.

Iodine value indicates the degree of unsaturation in fatty acids. Oil with high degree of unsaturated fatty acids is easy to be degraded by oxidation thus decreasing the oil quality (Scrimgeour, 2005; Basizron, 2005). Table 1 shows the iodine values of red fruit oils at different ripening stages which varied among the tested clones. The iodine value of Monsor clone decreased along the ripening stages, while Memeri and Edewewits clones increased (Table 1). This is supported by the fact that among these clones,

Monsor had higher level of unsaturated fatty acids (USFA) as indicated in Table 2 which in turn indicates that Monsor oil is easily degradable or oxidised.

The ripening stages of red fruits also had significant influence ($p < 0.05$) on total carotenoids of the three clones (Table 1). The total carotenoids of Monsor clone oil increased until ripe stage and decreased at over-ripe stage. Conversely, Memeri and Edewewits clones oil increased in total carotenoids until over-ripe stage. Sarungallo *et al*. (2016) reported that the total carotenoids of all clones of red fruit tend to increase along the ripening stages. The result in the present work might indicate that the carotenoid content in oil of Monsor clones at over-ripe stage was easier to degrade than other clones.

The ripening stages of red fruits also had significant influence ($p < 0.05$) on total tocopherols of the three clones (Table 1). The total tocopherols of Monsor clone oil increased until ripe stage and decreased at over-ripe stage. Conversely, Memeri and Edewewits clones oil increased in total tocopherols until over-ripe stage. This trend mirrored that of total carotenoids discussed earlier. Another research, which also analysed the total tocopherols in oil of nine clones of red fruit (Sarungallo *et al.* 2015b), found lower values than that of the present work. This could be due to the different ripening levels of the fruit analysed.

Fatty acid composition

The fatty acid (FA) compositions of red fruit oils of three clones at four ripening stages are shown in Table 2. The dominant saturated fatty acid (SFA) in red fruit oils was palmitic acid/C16 (9.02-15.93%), while the dominant unsaturated fatty acids (USFAs) were oleic acid/C18:1 (25-62-46.40%) and linoleic acid/C18:2 (2.36-5.72%). The results of the present work are similar to those of Sarungallo *et al*. (2015b) and Rohman *et al.* (2012) which reported that the FA composition of red fruit oils were dominated by oleic, linoleic and palmitic acids.

From Table 2, it can be seen that the USFA compositions of red fruit oils of three clones at four ripening stages had fluctuating patterns. The USFAs in oil of Monsor clone decreased along the ripening stages, while that of Memeri and Edewewits clones increased. The decrease in USFAs might indicate that oxidation only occurred in *drupa* of Monsor clone. In Table 1, increasing oxidation correlates with the increase in FFA levels and also a decrease in the iodine value of the Monsor clone oil. Oxidation is easier to occur in USFAs, resulting in peroxides due to the breaking of double bonds of fatty acids, then forming smaller molecules such as aldehydes, ketones, alcohols and short-chain organic acids (Atinafu and Bedemo, 2011). The fraction of peroxides and short-chain organic acids with lower molecular weight than triglycerides makes oils have a high FFA content and low iodine value. Based on the chemical properties of the oil of three clones of red fruit, it is apparent that each clone had different ability in maintaining hydrolysis and oxidation along the ripening stages. Memeri and Edewewits clones were better at maintaining hydrolysis and oxidation as compared to Monsor clone, as shown by the decrease in FFA, increase in iodine number, increase in USFAs, and increase in total carotenoids and total tocopherols of the oils along the ripening stage. Therefore, Monsor clone fruits should be harvested at ripe stage, while Memeri and Edewewits clone fruits should be harvested at over-ripe stage to obtain better quality of oil.

The results also show that the chemical properties, bioactive components and fatty acid compositions of the red fruit oils of all the clones tested were greatly influenced by the ripening stages. When referring to oxidative stability and relative compounds, the best harvesting time of red fruit would be at ripe stage. The present work also indicates that the content of bioactive components in red fruit oils was closely related to harvest time and the optimal harvest time was quite different on each clone in view of the highest content of bioactive components. Therefore, the optimum harvest time should be determined according to the dynamics of accumulation of target bioactive compounds in selected red fruit for oil production. This may also be very important for the quality control of red fruit cultivation.

Conclusion

The FFA contents in red fruit oil of Monsor clone increased along the ripening stages but decreased in iodine value, total carotenoids and total tocopherols as well as unsaturated fatty acid. Conversely, the FFA contents in red fruit oil of Memeri and Edewewits clones decreased along the ripening of the fruits but increased in iodine value, total carotenoids and total tocopherols as well as unsaturated fatty acid. The dominant unsaturated fatty acid in red fruit oil was palmitic acid 9.02-15.93%, while the dominant unsaturated fatty acids were oleic and linoleic acids at 25.62-46.40% and 2.36-5.72%, respectively. Maximum total carotenoids (4,090 ± 180 ppm) and total tocopherols (1,468 ± 474 ppm) in oil of Monsor clone were found at the ripe stage. Maximum total carotenoids (6,790 ± 130 ppm) and total tocopherols (1,402 ± 755 ppm) in oil of Memeri clone were found

Table 2. Fatty acid compositions of three clones of red fruit oil during four stages of maturation.

Fatty acid composition (%)*			Clone of Red Fruit Oil											
			Monsor				Memeri				Edewewits			
			Unripe	Half-ripe	Ripe	Over-ripe	Unripe	Half-ripe	Ripe	Over-ripe	Unripe	Half-ripe	Ripe	Over-ripe
Saturated fatty acid		C12	0.07	0.09	0.1	0.11	0.11	0.09	0.12	0.16	0.12	0.15	0.15	0.2
		C14	0.05	0.05	0.06	0.07	0.07	0.05	0.06	0.1	0.1	0.09	0.09	0.13
		C16	9.02	10.2	12.55	9.79	9.5	9.6	10.16	14.08	11.48	11.01	14.1	15.93
		C18	0.79	0.79	0.74	0.68	0.75	0.59	0.58	0.83	2.01	1.33	1.58	1.48
		C20	0.12	0.12	0.11	0.1	0.09	0.06	0.06	1.1	0.16	0.1	0.12	0.11
		C22	0.03	0.02	0.02	0.02	0.03	0.01	0.01	0.01	0.06	0.02	0.02	0.02
		Total	10.08	11.27	13.58	10.77	10.55	10.4	10.99	16.28	13.93	12.7	16.06	17.87
Unsaturated fatty acid	MUFA	C16:1	1.06	0.95	0.85	0.62	1.15	1.31	1.32	1.11	0.38	0.71	0.8	0,65
		C18:1	46.4	43.49	41.86	33.06	38.64	36.57	37.14	39.87	25.62	33.46	39.2	35,29
		C20:1	0.18	0.18	0.18	0.16	0.1	0.09	0.11	0.15	0.06	0.06	0.06	0,07
		Total	47.64	44.62	42.89	33.84	39.89	37.97	38.57	41.13	26.06	34.23	40.06	36.01
	PUFA	C18:2	4.26	3.75	3.08	2.65	2.07	2.7	2.36	2.46	2.84	5.28	5.39	5,72
		C20:2	0.09	0.09	0.07	0.07	0.08	0.05	0.06	0.06	0.05	0.02	0.02	0,02
		C18:3	0.8	0.78	0.68	0.6	0.89	0.61	0.57	0.71	0.44	0.65	0.7	0,96
		Total	5.15	4.62	3.83	3.32	3.04	3.36	2.99	3.23	3.33	5.95	6.11	6,7
Total			52.79	49.24	46.72	37.16	42.93	41.33	41.56	44.36	29.39	40.18	46.17	42.71

*MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; C8:0 = caprilic acid; C10:0 = capric acid; C12:0 = lauric acid; C14:0 = miristic acid; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid; C18:3 = linolenic acid;C20:0 = arachidic acid; C20:1 = arachidonic acid.

at the over-ripe stage. Maximum total carotenoids (7,723 ± 1,305 ppm) and total tocopherols (1,814 ± 357 ppm) contents in oil of Edewewits clone were also found at the over-ripe stage. Therefore, it could be concluded that the best harvest time for Monsor clone would be at the ripe stage, while for the Memeri and Edewewits clones at the over-ripe stage in order to obtain the maximum quality of oil.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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