International Food Research Journal 21(6): 2325-2330 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Determination of anisidine value of red fruit oil under elevated temperature using FTIR spectroscopy and multivariate calibration

[1,2]Andina, L., [2]Riyanto, R. and [2,3,4*]Rohman, A.

[1]*Vocational School of Health Analyst of Borneo Lestari, Banjarbaru, South Kalimantan, 70712, Indonesia*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281, Indonesia*
[3]*Research Center of Halal Products, Gadjah Mada University, Yogyakarta, 55281, Indonesia*
[4]*Center of Research for Fiqh Science and Technology (CFirst), Universiti Teknologi Malaysia, Malaysia.*

Article history

Received: 26 April 2013
Received in revised form: 9 April 2014
Accepted: 11 April 2014

Keywords

Red fruit oil
FTIR spectroscopy
Partial least square
p-anisidine value
BHT
α-tocopherol

Abstract

This Study was conducted to evaluate anisidine value (AV) of red fruit oil (RFO) obtained from hexane fraction and chloroform Fraction of RFO in elevated temperature. RFO with and without butylated hydroxytoluene (BHT of 200 mg/Kg) and α-tocopherol (BHT of 200 mg/Kg) were subjected to elevated heating temperature (room temperature, treated at 100 ± 1°C, 150 ± 1°C, 180 ± 1°C, 200 ± 1°C, and 300 ± 1°C) for 90 minutes in the oven. A band shift was observed at wavenumbers of 1689 cm^{-1} and 1716 cm^{-1}, assigned to the stretching vibrations of C=O and C-O from ester triglyceride, respectively. Besides, the absorbance change at wavenumber region of 1330 - 1454 cm^{-1} was also observed, indicating the possible presence of aldehydic or ketonic groups with isolated cis double bonds in RFO. Based on the optimization processes, the best coefficient of determination (R^2) value for the relationship between actual value of AV as determined by AOCS method and FTIR predicted value on determination AV was in the range of 1685 - 1712 cm^{-1}. The R^2 value obtained was 0.9998 for RFO in hexane fraction, and 0.9989 for RFO in chloroform fraction. In this study, the effect of antioxidants on AV of RFO in hexane fraction and chloroform fraction was also investigated.

Introduction

Red fruit (*Pandanus conoideus* Lam) comes from Pandanus plant family. This plant is commonly found in Papua, Republic of Indonesia, New Guinea, and sporadically began to be planted in some areas such as Maluku, Sulawesi, Kalimantan, Java and Sumatra (Wamaer and Malik, 2009). Some biological activities have been evaluated for red fruit. Mun'im *et al*. (2006) have reported that red fruit extract inhibited lung carcinogenesis in rat female. Rohman *et al.* (2010) have also studied the antioxidant activities of red fruit extracts and its fractions in vitro. These beneficial effects may be attributed from the several active compounds such as β-carotene and α-tocopherol contained in red fruit oil. Today, red fruit oil (RFO) is widely distributed in Indonesia and marketed as daily oil in diet. In the market, the price of RFO is approximately 10 – 15 times of common vegetable oils like corn, palm and canola oils, and can be potential to be used as functional food oils. Functional food can be defined as food or part of food which have the beneficial effect to human health (Rohman and Che Man, 2011).

Lipid oxidation is probably the single most important factors affecting the shelf life of edible oils. The hydroperoxides formed by lipid oxidation degrade into various smaller molecules such as aldehydes, ketones, alcohols, and acids. Some of these volatile lipid oxidation products affect flavor at extremely low concentrations (Richards *et al*., 2005; Choe and Min, 2006). Primary and secondary oxidation products can affect the smell and taste of oil (off flavor) and also important to provide an overall picture of the oil and fat oxidation status (Velasco *et al*., 2004). Rohman *et al.* (2012) and Arumsari *et al.* (2013) characterized RFO in term of FTIR spectra, fatty acid composition, and volatile compounds.

Edible oils naturally contain antioxidants such as tocopherols, tocotrienols, carotenoids, phenolic compounds, sterols, and antioxidants. The group of compounds is sometimes intentionally added to oil in order to improve oxidative stability. Tocopherols, particularly α-tocopherol, act as prooxidants when present in high concentrations in vegetable oils through the propagation of free radicals (Choe and Min, 2006). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole and propyl gallate are primary antioxidants which interrupt the free-radical chain during the oxidative reactions by contributing hydrogen from the phenolic hydroxyl groups. Besides, radical antioxidants

*Corresponding author.
Email: *abdulkimfar@gmail.com*
Tel: +62274-546868

themselves form stable free radicals which do not initiate or propagate further oxidation of lipid (Domingos *et al*., 2007).

Wijaya dan Pohan (2009) has reported the quality requirement of RFO. The another study has been evaluated for the possibility of RFO to be subjected of aldulteration with the lower price oils (Rohman *et al*., 2011). The last few years as Bendini et al. (2007) reported, the method of Fourier transformed spectroscopic techniques (FTIR and FTNIR) has been widely used in the analysis of oils and fats associated with the oils production process. FTIR spectroscopy have been used to determine the oil quality parameters such as acidity (Bertran *et al.*, 1999; Iñón *et al.*, 2003; Al-Alawi *et al.*, 2004), iodine value (Li *et al*., 1999) and fatty acid composition (Maggio *et al*., 2009). FTIR method has also been used for the determination of oil stability parameters, including the determination peroxide value in vegetable oils (Van de voort, 1994; Li *et al.*, 2000; Ruiz *et al.*, 2001; Guillen and Cabo, 2002; Bendini *et al*., 2007) as well as in crude palm oil (Moh *et al.*, 1999), and for determination of anisidin value in vegetable oils (Guillen and Cabo, 2002). However, using literature searching, there are no reports related to the determination of anisidine value (AV) in RFO. Therefore, in this study, we evaluate the anisidine value (AV) of RFO and the influence of antioxidant added to RFO toward AV using FTIR spectroscopy in combination with multivariate calibration.

Materials and Method

Red fruit was taken from Papua, Indonesia. Botanical identification was done in Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. All chemicals and reagents used were of analytical grade.

Red fruit oil preparation

Oil from red fruit was obtained using solvent extraction method. Red fruit was cut into small pieces using a commercial cutter and subsequently blended with methanol (1:1 wt/v). The methanolic extract was then subjected to maceration using ethanol (1:3) for 4 days. The ethanolic extract obtained was evaporated at 70^{o}C and partitioned using hexane for three times (1:1 v/v). The residue of ethanolic extract was also partitioned with the same condition as hexane partition using chloroform. The hexane and chloroform fractions were evaporated at 60^{o}C and then subjected to determine the influence of α-tocopherol and BHT on the anisidine value of red fruit oil by FTIR spectral data.

Sample preparation

Initially, RFO in hexane and chloroform fractions was treated without antioxidant (control). Furthermore, RFO in hexane and chloroform fractions was directly added with BHT and α-tocopherol, each at concentration of 200 mgkg^{-1} before being subjected to accelerated oxidation process.

Sample oxidation

Six samples of RFO namely (i) RFO control obtained from hexane fraction (ii) RFO control obtained from chloroform fraction; (iii) RFO in hexane fraction contained BHT; (iv) RFO in chloroform fraction contained BHT (v) RFO in hexane fraction containing α-tocopherol; and (vi) RFO in chloroform fraction containing α-tocopherol (50 g of each oil treatment) were placed in a separate 100 mL opened Beaker. Each samples were treated with the elevated heating temperature, as without being heated (room temperature), treated at 100 ± 1^{o}C, 150 ± 1^{o}C, 180 ± 1^{o}C, 200 ± 1^{o}C, 300 ± 1^{o}C for 90 minutes in an oven (Memmert, Germany). After each treatment period, oil samples were immediately analyzed.

AV determination

AV was determined spectrophotometrically using the standard method 2504 IUPAC (IUPAC, 1987) using an Genesys 10 UV-Vis Spectrophotometer.

FTIR studies

Absorption spectra of all oil samples were measured on ABB FTIR spectrophotometer MB 3000 (Canada) equipped with deuterated triglycine sulphate (DTGS) detector connected to Horizon MB software. Sampling technique used was attenuated total reflectance (ATR). The samples were placed in ATR at controlled temperature (20^{o}C). Measurements were taken at 32 scanning, and at 8 cm-1 resolution. After each scanning, ATR crystal was cleaned two times using n-hexane and acetone, and dried with soft tissue. To avoid spectral variation across time, the basic spectra (background) was measured before the measurement of each samples. Instrument is kept at a constant humidity to minimize disruption of air vapor. All spectra were recorded as absorbance at 4000 to 650 cm^{-1}, and performed in two replicates.

Result and Discussion

Anisidine Value (AV) represents the content of secondary oxidation products such as β-alkenals. These compounds able to react with p-anisidine

Table 1. The anisidine value of red fruit oil with and without the addition of antioxidants

T (ºC)	CF	HF	CF+BHT	HF+BHT	CF +α-tocopherol	HF +α-tocopherol
25	19.44	18.72	19.65	16.83	19.89	17.46
100	20.38	19.67	20.15	17.73	20.56	18.02
150	21.64	20.07	21.24	18.76	21.25	19.7
180	23.86	22.15	22.15	20.25	23.7	22.85
200	24.75	24.45	24.62	23.3	24.82	23.28
300	54.26	65.55	55.36	50.85	65.32	47.68

*CF: Chloroform Fraction, HF: Hexane Fraction

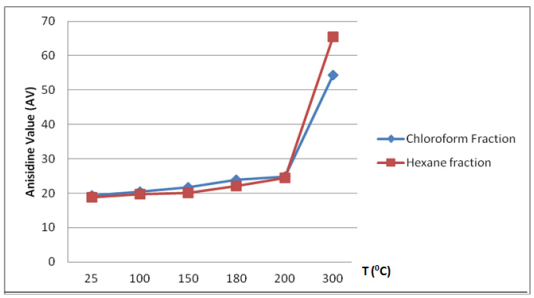

Figure 1. The anisidin value of red fruit oil (RFO) without antioxidant versus temperature (ºC)

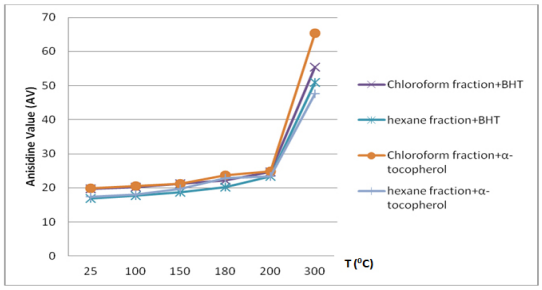

Figure 2. The anisidin value of red fruit oil (RFO) contained antioxidant versus temperature (ºC)

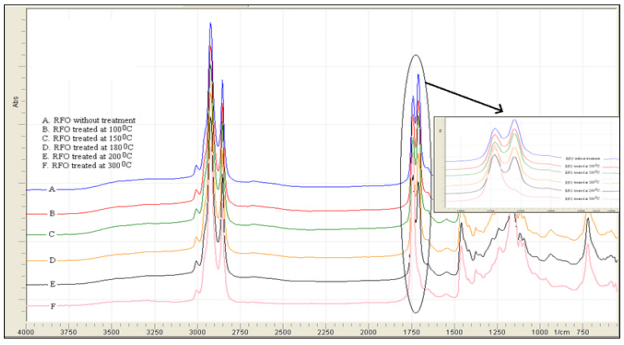

Figure 3. Absorbance changes in the region between 1650 and 1800 cm^{-1} of the infrared spectrum of RFO under elevated temperature.

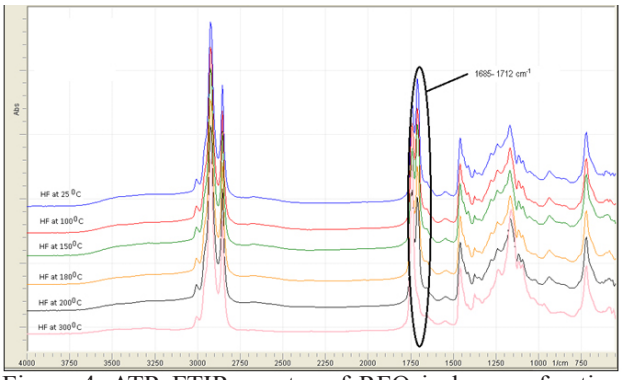

Figure 4. ATR FTIR spectra of RFO in hexane fraction under elevated temperature condition. Region assigned with circle were selected to develop anisidine value (1685 - 1712 cm^{-1}) in the calibration model.

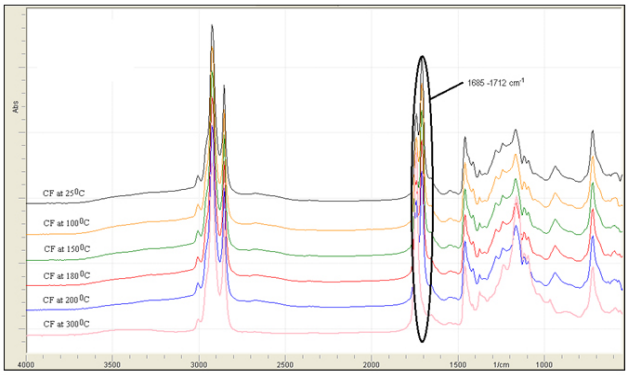

Figure 5. ATR FTIR spectra of RFO in chlorofrom fraction under elevated temperature condition. Region assigned with circle were selected to develop anisidine value (1685 -1712 cm^{-1}) in the calibration model.

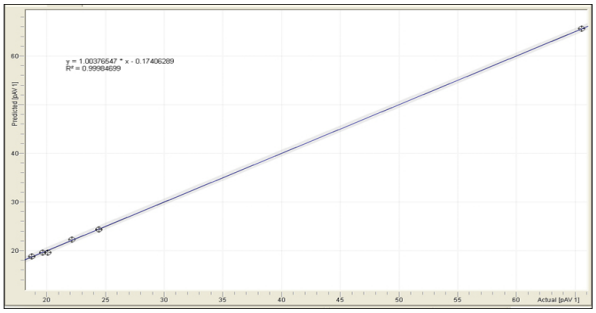

Figure 6. The calibration plot of FTIR-predicted anisidine value at region of 1685 - 1712 cm^{-1} vs. actual value of AV of RFO as determined by IUPAC method in hexane fraction

reagent (Guillen and Cabo, 2002). AV is particularly appropriate for the heated oils, because most of peroxides are destroyed during thermal oxidation using high temperature. Figure 1 shows AV of all oil samples obtained from hexane and chloroform fractions throughout the oxidation process at various temperatures. In two different fraction of RFO (hexane and chloroform fractions), we can see that AVs increase as a function of temperature. AV of RFO in chloroform fraction was higher than that in hexane fraction at any temperature. It means that the secondary products observed during thermal oxidation in chloroform fraction were higher than that in hexane fraction, as shown in Table 1. Figure 2 shows AV of RFO containing BHT and α-tocopherol as antioxidants. The influence of antioxidant of BHT and α-tocopherol on AV of RFO in hexane and chloroform fraction was lowering AV, compared with AV without the addition of antioxidant. AV of RFO containing BHT was lower than that of RFO containing α-tocopherol. Therefore, it can be stated that BHT was better than α-tocopherol on retarding the production of secondary products on RFO.

Table 2. Absorbance changes of FTIR spectra of RFO in hexane fraction at wavenumber 1685 - 17012 cm^{-1}

Samples	Absorbancies at wavenumbers of							
	1685.661	1689.519	1693.376	1697.233	1701.091	1704.948	1708.805	1712.663
HF at 25^{0}C	0.019744	0.023628	0.027841	0.037897	0.05804	0.078616	0.092188	0.092774
HF at 100^{0}C	0.023603	0.022135	0.026203	0.035862	0.055398	0.075633	0.08916	0.089893
HF at 150^{0}C	0.018344	0.027525	0.031611	0.041728	0.06218	0.082931	0.096601	0.097139
HF at 180^{0}C	0.017125	0.020443	0.02415	0.033224	0.051704	0.071089	0.084179	0.084958
HF at 200^{0}C	0.016334	0.019482	0.022987	0.031532	0.049185	0.067867	0.080561	0.08131
HF at 300^{0}C	0.010049	0.010947	0.011971	0.014089	0.017778	0.0215	0.02475	0.026429
HF + BHT at 25^{0}C	0.016335	0.019635	0.02327	0.032007	0.049564	0.067663	0.079827	0.080655
HF + BHT at 100^{0}C	0.016941	0.020202	0.023767	0.032341	0.049681	0.067549	0.079513	0.08032
HF + BHT at 150^{0}C	0.016002	0.019227	0.022771	0.031243	0.048408	0.066294	0.078388	0.079287
HF + BHT at 180^{0}C	0.015217	0.018315	0.021733	0.030023	0.046968	0.064766	0.076856	0.077747
HF + BHT at 200^{0}C	0.014354	0.017294	0.020564	0.02851	0.044834	0.06218	0.074137	0.075064
HF + BHT at 300^{0}C	0.012305	0.01441	0.016677	0.022013	0.033015	0.044954	0.053429	0.054407
HF + α-tocopherol 25^{0}C	0.019424	0.023292	0.027442	0.037294	0.057066	0.077392	0.090893	0.091585
HF + α-tocopherol 100^{0}C	0.019428	0.023299	0.027462	0.037323	0.057041	0.077233	0.090599	0.091193
HF + α-tocopherol 150^{0}C	0.019371	0.022997	0.026914	0.036236	0.055141	0.074747	0.087838	0.088545
HF + α-tocopherol 180^{0}C	0.017622	0.020789	0.0242	0.032476	0.049408	0.067098	0.078952	0.079492
HF + α-tocopherol 200^{0}C	0.016135	0.019327	0.02282	0.031356	0.049019	0.067674	0.080315	0.081073
HF + α-tocopherol 300^{0}C	0.009342	0.010182	0.01114	0.013233	0.016995	0.020872	0.024233	0.025831

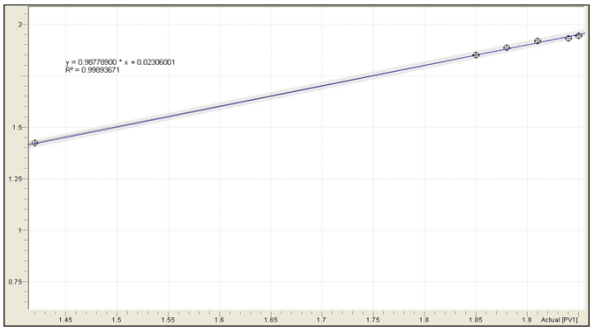

Figure 7. The calibration plot of FTIR-predicted anisidine value at region of 1685 - 1712 cm^{-1} vs. actual value of AV of RFO as determined by IUPAC method in chloroform fraction.

FTIR studies

The FTIR spectra give the information on the functional groups of the sample. Rohman *et al.* (2012) have investigated the differences of FTIR spectra of RFO compared with other vegetable oils. Guillen and Cabo (2000) have investigated some of the most significant changes in FTIR spectra under oxidative condition, and they have reported that changes in infrared spectra of all oil samples during the oxidation process show very similar pattern. As shown in Figure 3, FTIR spectra of RFO in region between 1800 and 1650 cm^{-1} has two peaks. The spectral region between 1800 and 1650 cm^{-1} (due to the ester carbonyl functional group of the triglycerides) undergoes several changes during the oxidation process. A band at 1654 cm^{-1}, associated with the stretching vibration of the carbon-carbon double bonds of cis-olefins, usually disappear in the transition of oxidation stage. The faster the oxidative process, the faster the disappearance of this band will be (Guillen and Cabo, 2000).

During the oxidation process, hydroperoxides degrade into secondary oxidation products such as aldehydes and ketones, which give bands near 1728 cm^{-1}. These bands overlap with that of ester group at 1746 cm^{-1} which result in the broadening of the band and a decreasing of its frequency (Guillen and Cabo, 2002). It is also occurred in FTIR spectra of RFO, the band approximately at 1709 cm^{-1} decreased as the temperature increased, and almost disappeared in FTIR spectra of RFO treated at 300^{o}C. At band 1744 cm^{-1}, the opposite was observed, where the peak intensities increased and become sharper as the temperature elevated. FTIR spectra of RFO in chloroform fraction exhibited the same profile. The hexane fraction added with BHT has lowered the decreasing of peak at wavenumber of 1709 cm-1 rather than hexane fraction containing α-tocopherol, meaning that BHT can retard the production of secondary products of oxidation which is better than α-tocopherol, meanwhile for FTIR spectra of RFO in chloroform fraction, the addition of BHT nor α-tocopherol did not cause any absorbance changes. The absorbance changes of FTIR spectra of RFO in hexane fraction at band 1685-1712 cm^{-1} is given in table 2.

In order to propose that bands between 1685 cm^{-1} and 1712 cm^{-1} (Figures 4 and 5) can be used as an alternative method for the measurement of AV of RFO rather than conventional technique using visible Spectroscopic technique, the relationship between absorbance changes at selected band (x-axis) and AV (y-axis) was built. The equations obtained were as follows:

$$y = 1.00376547\ x + 0.17406289 \text{ (RFO in hexane fraction)}$$
$$y = 0.98778900\ x + 0.02306001 \text{ (RFO in chloroform fraction)}$$

Figures 6 and 7 shows the correlation plot of RFO at region of 1685-1712 cm^{-1} using the actual value of AV as determined by IUPAC method versus FTIR predicted value. The correlation gave R^2 of 0.9998 for RFO in hexane fraction, and R^2 of 0.9989 for RFO

in chloroform fraction. There is a good correlation between FTIR predicted value with the results obtained by the standard method. The coefficient of determination (R^2) obtained was higher than 0.998 indicating that both actual and FTIR predicted values have close relationship.

Conclusion

The present study indicates that the spectral changes appeared at wavenumbers of 1685 cm^{-1} and 1712 cm^{-1} region can be useful to determine the AV of RFO in hexane and chloroform fractions. BHT as antioxidants added to RFO was better in retard the production of secondary products of RFO. FTIR spectroscopy could be useful to determine AV in a simple, fast and accurate way.

References

Al-Alawi, A., van de Voort, F.R., Sedman, J. and Ghetler, A. 2006. Automated FTIR analysis of free fatty acids or moisture in edible oils. Journal of the African Literature Association 11: 23-29.

Arumsari, N.I., Riyanto, S. and Rohman, A. 2013. Some physico-chemical properties of red fruit oil (*Pandanus conoideus* lam) from hexane and chloroform fractions. Journal of Food and Pharmaceutical Sciences 1(2): 30-34.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A. and Lercker, G. 2007. Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules 12: 1679–1719.

Bertran, E., Blanco, M., Coello, J., Ituriga, H., Maspoch, S. and Montoliu, I. 1999. Determination of olive oil free fatty acid by Fourier transform infrared Spectroscopy. Journal of the American Oil Chemists' Society 76: 611–616.

Budi, M. and Paimin, F.R. 2004. Red fruit (*Pandanus conoideus* L). pp. 3-26, 47-56, 67-68, Penebar Swadaya, Jakarta.

Choe, E. and Min, D.B. 2006. Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety 5: 169-186.

Dubois J., Van de Voort F.R., Sedman J., Ismail A.A. and Ramaswamy H.R. 1996. Quantitative Fourier transform infrared analysis for anisidine value and aldehydes in thermally stressed oils. Journal of the American Oil Chemists' Society 73: 787-794.

Guillen, M.D. and Cabo, N. 2000. Some of the most significant changes in the Fourier transform infrared spectra of edible oils under oxidative conditions. Journal of the Science of Food and Agriculture 80: 2028-2036.

Guillen, M.D. and Cabo, N. 2002. Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. Food Chemistry 77: 503–510.

Inon, F. A., Garrigues, J. M., Garrigues, S., Molina, A. and de la Guardia, M. 2003. Selection of calibration set samples in determination of olive oil acidity by partial least squares-attenuated total reflectance-Fourier transform infrared spectroscopy. Analytica Chimica Acta 489: 59–75.

IUPAC. 1987. Standard Methods for the Analysis of Oils. Fats and Derivatives. 7th Ed. International Union of Pure and Applied Chemistry Blackwell Scientific Publications. Oxford. UK. Methods 2.501, 2.505.

Li, H., van de Voort, F.R., Ismail, A.A. and Cox, R. 2000. Determination of peroxide value by Fourier transform near-infrared spectroscopy. Journal of the American Oil Chemists' Society 77: 137-142.

Li H, van de Voort, F.R., Sedman, J. and Ismail, A.A. 1999. Rapid determination of cis and trans content, iodine value, and saponification number of edible oils by Fourier transform near-infrared spectroscopy. Journal of the American Oil Chemists' Society 76(4): 491-497.

Maggio, R.M., Kaufman, T.S., Del Carlo, M., Cerretani, L., Bendini, A., Cichelli, A. and Compagnone, D. 2009. Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. Food Chemistry 114: 1549-1554.

Moh, M.H., Tang, T.S., Che Man, Y.B. and Lai, O.M. 1999. Rapid determination of peroxide value in crued palm oil product using Fourier transform infrared spectroscopy. Journal of Food Lipids 6: 261-270.

Mun'im, A., Andrajati, R. and Susilowati, H. 2006. Tumorigenesis inhibition of water extract of red fruit (*Pandanus conoideus* Lam.) on Sprague-Dawley rat female induced by 7,12 dimetilbenz(a)antrasen (DMBA). Indonesia Journal of Pharmaceutical Science 3: 153 – 161.

Richards, A. Wijesundera, C. and Salisbury, P. 2005. Evaluation of oxidative stability of canola oils by headspace analysis. Journal of the American Oil Chemists' Society 82: 869–874.

Rohman, A., Riyanto, S., Yuniarti, N., Saputra, W.R., Utami, R. and Mulatsih, W. 2010. Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). International Food Research Journal 17: 97-106.

Rohman, A., Che Man, Y.B. and Riyanto, S. 2011a. Authentication analysis of red fruit (*Pandanus conoideus* Lam) oil using FTIR spectroscopy in combination with chemometrics. Phytochemical Analysis 22 (5): 462-467.

Rohman, A., Che Man, Y. B., Ismail, A. and Hashim, P. 2011b. Monitoring the oxidative stability of virgin coconut oil during oven test using chemical indexes and FTIR spectroscopy. International Food Research Journal 18: 303-310.

Rohman, A., Riyanto, S. and Che Man, Y.B. 2012. Characterization of red fruit (*Pandanus coneideus*

Lam) oil. International Food Research Journal 19(2): 563-567.

Ruiz, A., Canada, M.J.A. and Lendl, B. 2001. A rapid method for peroxide value determination in edible oils based on flow analysis with Fourier transform infrared spectroscopic detection. Analyst 126: 242-246.

Van de Voort, F.R., Ismail, A., Sedman, A., Dubois, J. and Nicodemo, T. 1994. The determination of peroxide value by Fourier transform infrared spectroscopy. Journal of the American oil Chemists' Society 71(9): 921-926.

Velasco, J. Andersen, M.L. and Skibsted, L.H. 2004. Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the rancimat method and differential scanning calorimetry. Food Chemistry 85: 623-632.

Wamaer, D. and Malik, A. 2009. Analisis finansial pascapanen buah merah (*Pandanus conoideus* Lamk.). Jurnal Tambue Universitas Moh. Yamin Solok VIII(1): 96−100.

Wijaya, H. and Pohan, H.G. 2009. Kajian Teknis Standar Minyak Buah Merah (*Pandanus conoideus* Lam) (Eng: technical study of red fruit standard). Prosiding PPI Standardisasi 2009: 1 -12.