

Determination of peroxide value of red fruit oil by FTIR spectroscopy and multivariate calibration

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Abstract

Red fruit oil (RFO) is a rapidly growing commodities, which have comparatively high price than other oils. RFO was obtained through maceration process of red fruit using methanol as solvent, followed by the partition using n-hexane and chloroform to obtain hexane fraction (HF) and chloroform fraction (CF). Antioxidant Butylated Hydroxytoluene (BHT) and α -tocopherol of 200 mg.Kg⁻¹ were added to both fraction respectively. RFOs was subjected at a 100, 150, 180, 200, and 300°C of heat temperature. These RFOs were further subjected to determination of peroxide value (PV) using titrimetric method and Attenuated (ATR-FTIR) spectrophotometry combined with chemometric multivariate calibration. Based on the optimization processes, it can be shown that Attenuated Total Reflectance –Fourier Transformed Infra Red (ATR-FTIR) method combined with Partial Least Square (PLS) can be used for PV determination at 3085-3610 cm⁻¹ with R² of 0.997, and standard error calibration of 0.14%. This study indicates that ATR-FTIR spectroscopy can be used to determine PV. While the conventional method were time consuming in sample preparation, ATR-FTIR spectroscopy is capable of determining PV accurately in less than 3 minutes for each sample, it was simple and environmentally friendly.

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Introduction

Red fruit (*Pandanus conoideus*, Lam) is a plant found in Papua New Guinea, Indonesia. This plant sporadically began to be planted in some areas such as Maluku, Sulawesi, Kalimantan, Java, and Sumatra. Spread all over area in Papua, including Wamena Baliem valley, Tolikara, Mountains, Yahukimo, Jayapura, Sorong and Manokwari (Wamaer and Malik, 2009). Red fruit oil (RFO) known as it's high oleic acid content (Rohman *et al.*, 2012; Arumsari *et al.*, 2013). In 2009, Wijaya and Pohan reported about RFO quality, the research could be used as a reference for consumers on choosing products contained red fruit oil on the market (Wijaya and Pohan, 2009). Studies have also been carried out on the RFO adulteration with other cheaper oil (Rohman *et al.*, 2011). Spectral characterization of RFO, analysis of carotenoids and tocopherol content and fatty acid composition of RFO also have been reported (Rohman *et al.*, 2012).

Number of methods have been developed for evaluation of the oxidative stability of edible oils under the condition of accelerated oxidation (Velasco *et al.*, 2004). Various parameters have been used

to determine the oxidation of oils and fats, such as peroxide. Standard method for the determination of PV according to Association of Official Analytical Chemistry (AOAC) was using volumetric technique. This technique require a long analysis time and use a variety of toxic reagents. Therefore, there are various methods proposed by the researchers to determine PV, such as infrared spectrophotometry especially in combination with chemometric multivariate calibrations method such as Partial Least Square (PLS) (Van De Voort *et al.*, 1992).

In the last few years, as presented by Bendini *et al.*, 2007, spectroscopic method such as Fourier Transformed Infra Red and Fourier Transformed Near Infra Red (FTIR and FTNIR) combined with chemometric has been widely used in the analysis of oils and fats associated with the production process. FTIR spectrophotometry was used to set the parameters of the oil stability, such as PV determination in vegetable oils (Van de voort *et al.*, 1994; Li *et al.*, 2000; Ruiz *et al.*, 2001; Guillen and Cabo, 2002; Bendini *et al.*, 2007). It was also used for PV determination in palm oil (Setiowaty and Che Man, 2003), as well as PV determination in crude palm oil (Moh *et al.*, 1999). ATR- FTIR was also

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successfully used to determine the Iodine Value, Acid Value, Anisidine Value and Saponification Value of RFO (Triyasmono *et al.*, 2013; Andina *et al.*, 2014; Abdul Rohman *et al.*, 2015). In this study, we analyzed PV of RFO using FTIR spectrophotometer combined with chemometric through a process of accelerated oxidation under elevated temperature conditions.

Materials and Method

Red Fruit, methanol, hexane, chloroform, sodium tiosulfate, potassium bicromate, HCl, KI, glacial acetic acid, (p.a. quality reagents from E. Merck, Darmstat, Germany).

Preparation of red fruit oil

Small pieces of Red fruit subsequently blended with methanol (1:1). Furthermore, the methanolic extract was then subjected to maceration process using methanol (1:3) for 4 days. Methanol extract obtained was evaporated at 70°C and was fractionated using hexane (1:1) for three times. The residue of methanolic extract was also partitioned with chloroform under the same condition as hexane fraction. The hexane (HF) and chloroform fraction (CF) were evaporated at 60°C and then subjected for the oxidation process.

Oxidation process of RFO

Initially, RFO in hexane (HF) and chloroform fraction (CF) directly added with BHT and α -tocopherol at each concentration of 200 mg kg⁻¹. Six samples of HF and CF without antioxidant and six samples of HF and CF contained BHT and six samples HF and CF contained α -tocopherol were placed in a separate 100 mL open beaker. Each sample treated with the elevated heating temperature as without heated (room temperature), treated at 100±1°C, 150±1°C, 180±1°C, 200±1°C, 300±1°C for 90 minutes in an oven (Memmert, Germany). After each treatment period, oil samples were analyzed immediately.

PV determination

PV was determined by the standard AOAC iodometric method (AOAC, 2005).

FTIR studies

Absorption spectra of all oil samples were measured in ABB FTIR spectrophotometer MB 3000 (Canada) equipped with deuterated triglycine sulphate (DTGS) detector connected to the Horizon MB software. Attenuated total reflectance (ATR) was applied as a sampling technique tool. The samples

were placed in ATR at controlled temperature (20°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 sample. The instrument was kept at a constant humidity to minimize disruption from air vapor. All spectra were recorded as absorbance at 4000 to 650 cm⁻¹, and performed in two replicates.

Result and Discussion

Oxidation process of RFO

RFO spectral profile in the 4000-650cm⁻¹, which does not undergo oxidation processes can be seen in Figure 1. The region 1750-1700 cm⁻¹ was related to the stretching vibration of carbonyl groups (C=O), RFO has two peaks in the 1744 and 1709 cm⁻¹. FTIR spectra provides information about the functional groups. Guillen and Cabo (2000) examines some of the significant changes in the FTIR spectra of oil under oxidative conditions. They reported that the changes in the infrared spectra of all samples of the oil showed a similar pattern. As the RFO spectra were observed, the spectral region between 1650 and 1750 cm⁻¹ encountered some changes during the oxidation process. Peaks at wave numbers around 1709 cm⁻¹ continued to decline during the oxidation process and almost disappeared in the spectra of RFO treated at 300°C. As the oxidation process continued, the hydroperoxide degraded into secondary oxidation products such as aldehydes and ketones. The presence of aldehydes and ketones will cause broadening and decreasing peak at a frequency of 1744 cm⁻¹. Hydroperoxide formation generate the loss of cis double bond and a change in the cis to trans double bond during the formation of hydroperoxide. Peak appears at the frequency of 3007 cm⁻¹ derived from the CH stretching vibration of the cis double bond (=CH) of fatty acid structure (Guillen and Cabo, 2002; Vlachos *et al.*, 2006; Rohman *et al.*, 2012). Unsaturated fatty acids were damaged, this is due to the form of oleic hydroperoxide and linoleic hydroperoxide from oleic and linoleic acid (Frankel, 1980; Ketaren, 2008; Abdul Rohman *et al.*, 2015). During changes in the concentration of oleic and linoleic acid, it is also generating peak changes at 3007 cm⁻¹ wavenumber region, respectively. Development of methods for the determination of FTIR spectra peroxide on some oil has been reported by Van De Voort *et al.* (1994). FTIR spectrophotometric method combined with a PLS multivariate calibration method

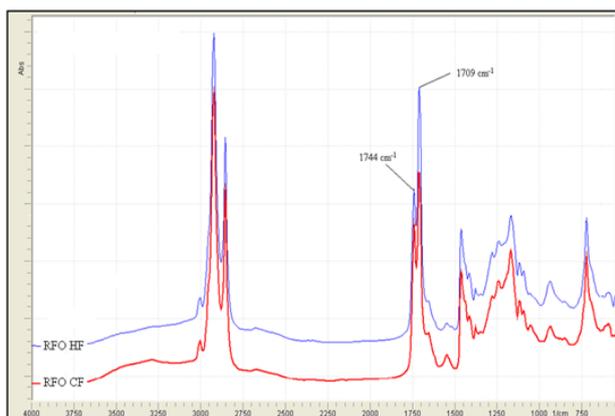


Figure 1. Spectra profile of RFO (RFO HF=Hexane Fraction; RFO CF=Chloroform Fraction) with no heat treatment at 4000-650 cm^{-1}

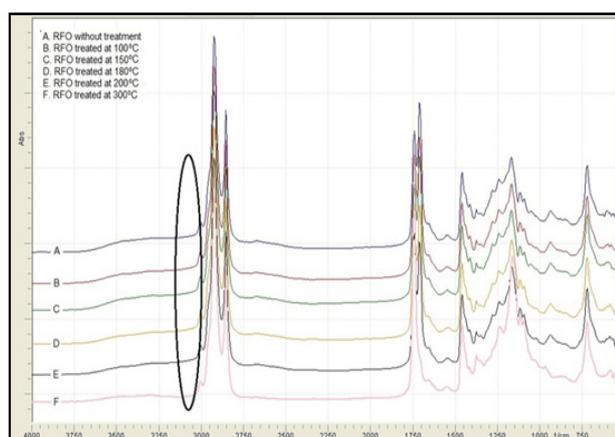


Figure 2. Spectra of RFO under elevated temperature. Region assigned with circle (3085-3610 cm^{-1}) were selected to develop PV calibration model.

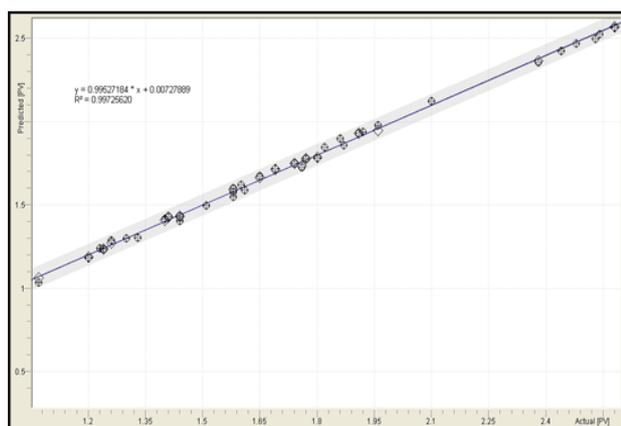


Figure 3. Calibration plot of FTIR-predicted PV vs. actual PV of RFO for region 3085-3610 cm^{-1}

in the wave number region 3750-3150 cm^{-1} gives better results for PV determination.

PV determination and FTIR studies

Based on the results of the determination of PV, it is known that PV increased as a function of the higher temperatures (Table 1). Development of FTIR

Table 1. The anisidine value of of Red Fruit Oil with and without the addition of antioxidant

Temperature ($^{\circ}\text{C}$)	RFO HF (meq/Kg)			RFO CF (meq/kg)		
	I	II	III	I	II	III
Without treatment	1.25	1.03	0.94	1.29	1.11	1.21
100	1.28	1.25	1.18	1.43	1.31	1.25
150	1.48	1.57	1.74	1.68	1.60	1.47
180	1.98	1.88	1.72	1.86	1.76	1.67
200	1.81	2.05	1.91	1.82	1.76	1.82
300	2.46	2.68	2.59	2.32	2.56	2.73

The Results of PV (meq/Kg) determination using AOAC method, RFO HF is Red Fruit Oil Hexane Fraction, RFO CF is Red Fruit Oil Chloroform Fraction

spectrophotometric method for the determination of PV is also applied to the RFO. According Fukuzumi and Kobayashi, the-OH stretching vibration of hydroperoxide can be observed at 3450 cm^{-1} region (Moh *et al.*, 1999), while Van de voort observed hydroperoxide vibration at 3444 cm^{-1} region (Van de voort *et al.*, 1992; Van de voort *et al.*, 1994). The observations are then reinforced by research conducted by Hayati *et al.* (2005) who observed hydroperoxide vibration at 3471 cm^{-1} region. Yildirim (2009) investigated the widening of the peaks in the area around 3445 and 3468 cm^{-1} , it shows the presence of hydroperoxide and alcohol. Peroxide as the primary oxidation products generally appears in the wave numbers 3435 or 3450 cm^{-1} (Guillen and Cabo, 2000), thus changes in the spectral region showed a hint of oil oxidation. However, these differences is difficult to analyzed visually, and it is very difficult to analyzed the difference. The difficulties, as reported by Innawong *et al.* (2004) possibly because the profile of FTIR spectra was very similar, the heat treatment and the addition of antioxidants did not provide any noticeable effect on the formation of oil peroxide. Therefore chemometric is required to determine the differences of intensity of each RFO sample quantitatively, by selecting the PLS multivariate calibration. PLS calibration will automatically incorporate the contribution of each variable to the calibration samples (Van de voort *et al.*, 1994). Under these conditions, changes in absorbance at wave numbers 3085-3610 cm^{-1} (Figure 2) optimized to determined PV, by developing the relationship between the absorbance (Y axis) and the actual peroxide (X axis). The calibration plot can be seen in Figure 3. Based on the calibration equation, $Y = 0.995x + 0.007$ with $R^2 = 0.997$ and RMSEC (Root Mean Square Error Calibration) = 0.1471% (v/v), it can be concluded that the FTIR spectrophotometry can be used to determine peroxide. There is a good correlation between the predicted value peroxide

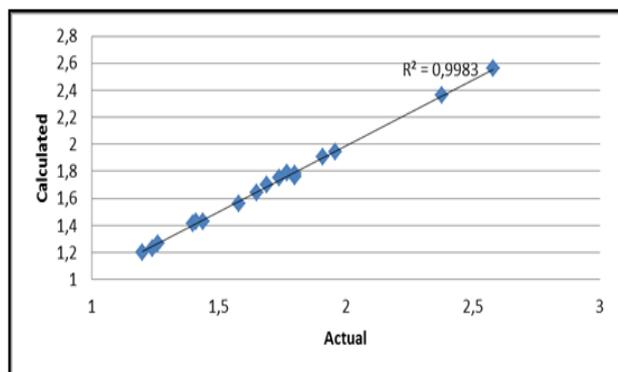


Figure 4. Validation plot of FTIR-predicted PV vs. actual PV of RFO for region 3085-3610 cm^{-1}

with the results obtained by standard AOAC methods. Validation of the calibration model has been developed to ensure that the measurement results of each measurement with the existing model can provide a quite close value to the actual value. Figure 4 is a validation plot which shows the correlation between the actual value (actual) with the calculated value (calculated). R^2 obtained for validation of the model was 0.998, which means that the model can measure peroxide with the 99.8% of accuracy. This shows the high accuracy of the developed model for the determination of PV. Validation is also performed by calculating the RMSEP, which is 0.00532, respectively. RMSEP value is an error in the validation of the developed models in predicting PV. The small value of RMSEP indicates that the developed model have a high precision to determine PV of RFO.

Conclusion

Based on studies, it can be concluded that the ATR-FTIR method combined with the multivariate calibration can be use to measure PV of RFO rapidly, accurately, and without a lot of harmful chemical reagent, thus supporting the campaign Green Chemistry.

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