

Liling_Rapid Determination

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Rapid Determination of Saponification Value in Red Fruit Oil by Infrared Spectroscopy and Partial Least Square Calibration

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ABSTRACT

A simple, rapid and reproducible method for determining the Saponification Value (SV) of Red Fruit Oil (RFO) was developed using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy in combination with multivariate calibration of Partial Least Square (PLS). A calibration model was developed using a series of RFO subjected to several thermal treatments, namely at ambient temperature 28, 100, 150, 180, 200 and 300°C, respectively. Based on the optimization processes, the FTIR spectra of RFO samples were measured in the frequency region of 1145-1168 cm⁻¹ for SV determination. The relationship between actual values of SV as determined using AOCS method and FTIR predicted value as determined with FTIR spectroscopy in combination with PLS calibration model showed a close relationship with coefficient of determination (R²) of 0.998 and the value of Standard Error of Calibration (SEC) was 0.79%. This study concluded that ATR-FTIR spectra can be used to determine SV of RFO. The developed method is simple, rapid with a total analysis time per sample of less than 2 min and environment friendly.

Key words: ATR-FTIR spectroscopy, saponification value, red fruit oil, partial least square

INTRODUCTION

Pandanus conoideus Lam. is endemic plant in province of Papua, Indonesia and in Papua New Guinea. The local name of *P. conoideus* is red fruit. This fruit has uncommon shape with 68-110 cm length and with diameter of 10-15 cm, red in color and contains large amount of oil, known as Red Fruit Oil (RFO) (Rohman *et al.*, 2012a). Some local industry exploits RFO as an important commodity to be distributed around Indonesia or exported in some countries. Oil extracted from red fruit known as Red Fruit Oil (RFO) is used as valuable oil sources in oily food because RFO contains high level of beta-carotene (Limbongan and Malik, 2009).

The increasing number of RFO products in the market will require a standard of RFO to ensure its quality and safety. Wijaya and Pohan (2009) have reported RFO standards such as characterization and saponification number of RFO. This parameter can be used as a reference in determining the quality of RFO. Determination of quality parameter of edible fats and oils is commonly performed by determining some physico-chemical values such as acid value, iodine value and saponification value. Some researchers have also performed the characterization and physico-chemical properties of RFO as reported by Rohman *et al.* (2012a) and Arumsari *et al.* (2013).

The determination of saponification value was carried out using the titration method according to AOCS procedure (Che Man *et al.*, 1999). The titration methods involve the use of highly toxic reagents, carcinogenic and environmentally unfriendly chemicals. These methods are also time consuming, costly and largely dependent on the skills of the analyst (Rohman *et al.*, 2012b; Aparicio and Aparicio-Ruiz, 2000). Therefore, some new techniques have been developed for determination of quality parameters of fats and oils. One of the developed methods is Fourier Transform Infrared (FTIR) spectroscopy, especially in combination with multivariate calibrations such as Partial Least Square (PLS) and Principle Component Regression (PCR) (Miller and Miller, 2010).

Fourier transform infrared spectroscopy is an ideal instrumental technique due to its rapidity and ease in sample preparation. Besides, FTIR spectroscopy is considered as fingerprint technique. Some investigators have used FTIR spectroscopy for monitoring saponification value. Van de Voort *et al.* (1992) employed FTIR-Attenuated Total Reflectance (ATR) spectroscopy in combination with PLS calibration for the evaluation of iodine value and saponification value in fat and oil and compared their results to AOAC standard method. Li *et al.* (1999) have also examined the use of FTIR spectroscopy combined with PLS for saponification number analysis in selected edible oils.

However, the application of FTIR spectroscopy for determination of quality parameters especially saponification value of RFO has not been reported yet. Therefore, FTIR spectroscopy in combination with partial least square is developed for determination of saponification value.

MATERIALS AND METHODS

Materials: Red fruit was taken from Papua, Indonesia. Botanical identification was carried out in Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia under supervision by Prof. Dr. Wahyono. All chemical and reagents used were of analytical grade.

Preparation of red fruit oil: Red Fruit Oil (RFO) was obtained using solvent extraction according to procedure previously reported by Rohman *et al.* (2012a). Briefly, Red fruit was cut into small pieces using a commercial cutter and was subsequently subjected to commercial blender containing methanol (one part of fruit was added with one part of methanol). The ethanolic extract obtained was further macerated with methanol (1:3 v/v) for 4 days. The extract was evaporated at 70°C and subjected to partition 3 times using hexane (1:1 volume extract/volume hexane). The hexane extracts containing RFO were evaporated at 60°C. For preparation of chloroform extract containing RFO, the residue of methanolic extract after being partitioned using hexane, was further partitioned with chloroform to get RFO. The oil obtained was further used for characterization and for determining of saponification value using Titration method according to AOCS and FTIR spectroscopy method.

Characterization of RFO: Red Fruit Oil (RFO) obtained was further subjected to physico-chemical analysis of carotenoid content and fatty acid composition. Determination of carotenoid content was performed as previously reported by PORIM (1995), while determination of fatty acid composition was carried out according to Rohman and Che Man (2009).

Treatment of RFO samples on accelerated temperature: Approximately 50 g of each RFO sample was taken in 100 mL Beaker glass. The samples were divided into 6 groups, each was

labeled according to the name of oil and temperature treatment used namely, without heating (room temperature), 100, 150, 180, 200 and 300°C. Each group was subjected to thermal treatment in conventional oven for 90 min. Furthermore, the oil sample was cooled and prepared for further analysis (the determination of saponification value with the chemical method and FTIR spectrophotometry in combination with partial least square). Determination of saponification value in the treated RFO samples was performed according to the standard methods for the analysis of fats, oils official methods of analysis of AOAC (2005).

Measurement of FTIR spectra: All samples were analyzed using FTIR spectrophotometer FTIR MB 3000 (Clair Scientific, Northampton, UK), equipped with a DTGS detector with a resolution of 4 cm^{-1} , number of scanning of 32 at mid infrared region of $4000\text{-}400\text{ cm}^{-1}$, as reported previously by Lukitaningsih *et al.* (2012). Spectra was acquired using Horizon MB FTIR software version 3.0.13.1 (ABB Canada). The oil samples were placed in Horizontal Attenuated Total Reflectance (HATR) consisting of ZnSe crystal at a controlled temperature (20°C). All spectra were measured and subtracted with background spectrum of air. The spectrum was recorded as absorbance value at each frequency point data conducted in three replicates.

Data analysis: Multivariate calibrations of Partial Least Square (PLS) were used to construct the correlation model between actual value (from the determination of saponification value using AOAC) and FTIR predicted value. The values of coefficient of determination (R^2) and errors during calibration and validation were used for evaluation the correlation model. All data analyses were performed using Horizon MB software included in FTIR spectrophotometer.

RESULTS AND DISCUSSION

Fatty acid composition and carotenoid content in RFO: Before being treated with several temperature and used for determination of saponification value, RFO was subjected to analysis of carotenoid total and fatty acid composition. Fatty acid composition of the RFO is determined using gas chromatography with flame ionization detector. Firstly, RFO was esterified to obtain derivate of fatty acid methyl ester. The main fatty acid of RFO is oleic acid accounting of 80.12% followed by palmitic acid accounting of 20%. This result was in agreement with that of Rohman *et al.* (2012a) and Nishigaki and Wasposito (2010) that oleic acid is fatty acid dominant in RFO. Furthermore, changes in concentration of RFO fatty acids i.e., oleic acid and linoleic acid due to heat treatment (Fig. 1 and 2) occurs due to the oxidation process resulting into linoleic hydroperoxide (Frankel, 1993). Hydroperoxide formation leads to loss of *cis* double bonds and changing the *cis* into *trans* double bonds during the formation of hydroperoxide. In FTIR spectra of RFO, the intensity of the peak at 3007 cm^{-1} also changed as shown Fig. 2 in which it associated with CH stretching vibration of the *cis* double bond (=CH) of fatty acids (Guillen and Cabo, 2002; Vlachos *et al.*, 2006; Rohman *et al.*, 2012a).

Total carotenoid content in RFO with and without heating was calculated based on PORIM (1995) and given in Table 1. Declining levels of carotenoids due to the heating effect is associated with the defect in double bond leading to decrease in the color formation of carotenoids (Elbe and Schwartz, 1996). The total carotenoid content of RFO in this study are consistent with those previously done by Satriyanto *et al.* (2012), which total carotenoid levels in the heating temperature of 85°C for 360 min is 19387.40 ppm, while Budi (2001) showed that the level of total carotenoids of RFO at 100°C is 12233.34 ppm.

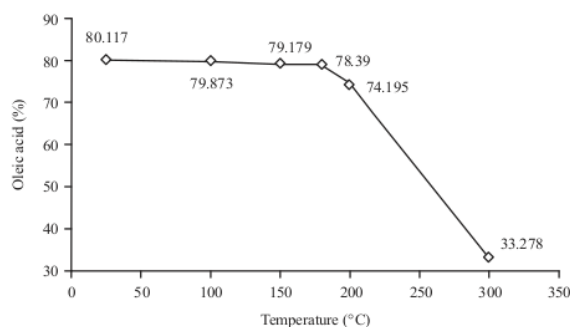


Fig. 1: Changes in oleic acid concentration of red fruit oil as the heating temperature increased

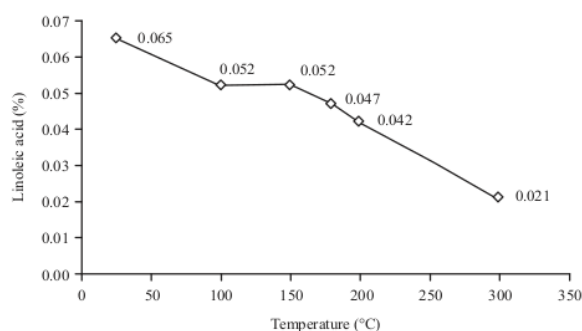


Fig. 2: Changes in linoleic acid concentration of red fruit oil as the heating temperature increased

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Table 1: Levels of total carotenoids of red fruit oil fractions of hexane and chloroform fractions without and with thermal treatment

RFO hexane fraction	Total carotenoid content (ppm)	RFO Chloroform fraction	Total carotenoid content (ppm)
Without heating	17030.51	Without heating	13136.72
100°C	10889.82	100°C	10366.39
150°C	9294.01	150°C	8259.92
180°C	4085.28	180°C	3459.72
200°C	2170.30	200°C	2055.40
300°C	548.95	300°C	408.52

Determination of saponification value using FTIR spectra: The FTIR spectra in the mid-infrared region consist of fundamental and characteristic bands whose frequencies and intensities can clearly determine the relevant functional groups in RFO as a consequence, if the composition of samples change, FTIR spectra will alter (Guillen and Cabo, 1997). Indeed, FTIR spectra of RFO subjected to high temperature will differ from that not treated at elevated temperature. Figure 3 represents the typical FTIR spectrum of RFO from 4000-400 cm^{-1} at several temperature treatments. The FTIR-ATR spectra obtained have strong C-H absorption between 3000 and 2850 cm^{-1} . The RFO samples also showed two strong bands at 1749 and 1710 cm^{-1} , respectively indicating the presence of carbonyl group from triacylglycerols. The FTIR spectra of RFO samples at ambient temperature and treated with elevated temperature showed very similar. However, the peak intensities (absorbance) of some specific bands were noticeable which indicate

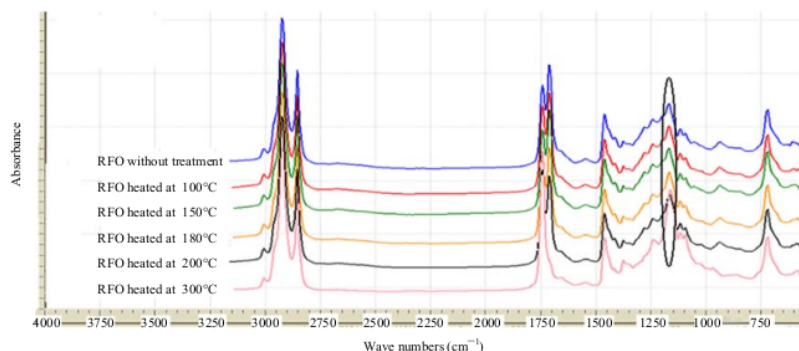
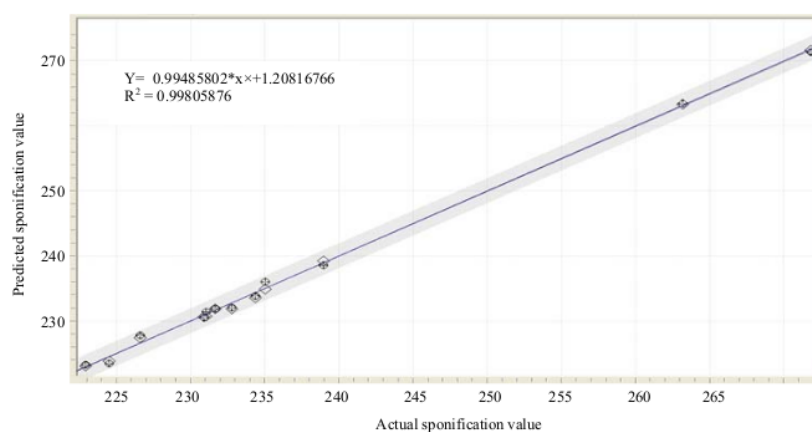


Fig. 3: ATR-FTIR spectra of red fruit oil during high temperature treatment for determination of saponification value. The frequency region assigned with circle was used for partial least square calibration model



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Fig. 4: PLS calibration model for correlation between actual and FTIR predicted value of saponification value

the differences among FTIR spectra (Innawong *et al.*, 2004). Therefore, FTIR spectra change can be exploited for quantitative analysis, as the peak intensities are proportional with the concentration of analyte of interest.

For determination of saponification value, some peaks related to the stretching vibrations of CH_3 in fatty acid hydrocarbon chain can be exploited. Van de Voort *et al.* (1992) has used wave numbers of 1850-000 cm^{-1} for determination of saponification value using FTIR spectrophotometer and Partial Least Square (PLS). After the optimization process, finally the frequency region of 1145-1168 cm^{-1} was used for determination of Saponification Value (SV). The selection of this frequency region to be used for estimation of saponification value is relied on its ability to provide the highest coefficient of determination and the lowest errors.

The correlation between actual value (determined with AOCS method) and FTIR predicted value was shown in Fig. 4. The following equation was obtained, $\text{SV predicted} = 0.99 \text{ SV}$

actual+1.20. The coefficient of determination (R^2) obtained was higher than 0.998 and standard error of calibration is 0.79%, indicating that both actual and FTIR predicted values have close relationship. The FTIR-ATR technique would eliminate the use and disposal of hazardous solvents and reagents required by the chemical methods and average time of analysis to produce saponification value data for RFO is ~2 min per sample. Based on this result, it can be stated that FTIR spectroscopy in combination with multivariate calibration of PLS can be an alternative technique for determination of saponification value in RFO.

CONCLUSION

The FTIR spectroscopy at frequency region of 1145-1168 cm^{-1} can be used for estimation of Saponification Value (SV) of RFO treated with some temperature. The rapid determination of SV by ATR-FTIR spectroscopy is therefore suitable and practical option for process control. Another advantage of FTIR spectroscopy method is that it is environmentally friendly as no chemical is needed except hexane for cell cleaning. By utilizing this method, chemical cost is negligible as compared by AOAC standard method.

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