

Determination of iodine value and acid value of red fruit oil by infrared spectroscopy and multivariate calibration

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Abstract

A rapid method for the quantitative determination of iodine value (IV) and acid value (AV) of red fruit oil (RFO) using FTIR spectroscopy and multivariate calibration of partial least square (PLS) is described. A calibration standard was developed using a series of RFO subjected to thermal treatment at without heating (ambient temperature); 100°C, 150°C, 180°C, 200°C and 300°C, respectively. Based on optimization processes, the FTIR spectra of RFO samples were measured in the frequency region of 1080 - 1114 cm⁻¹ for IV determination, and at frequency region of 1731 - 1762 cm⁻¹ for AV determination. PLS calibration model was developed for the prediction of IV and AV in RFO samples. The relationship between actual values of IV and AV as determined using AOCS method and FTIR predicted value as determined with PLS calibration model show a close relationship with coefficient of determination (R^2) of 0.998 for IV and 0.998 for AV. The values of standard error of calibration were 0.70% for IV determination and 0.49% for AV using FTIR spectroscopy. This study concludes that ATR- FTIR spectra can be used to determine the IV and AV values of RFO. The developed method is simple, rapid with a total analysis time per sample of less than 2 min, and environmentally friendly.

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Introduction

Red fruit, or known as Buah merah in Indonesia, received great attention among the scientists worldwide since 2003. Red fruit (*Pandanus conoideus* Lam) is a plant, endemic to Papua Indonesia and Papua New Guinea. Some studies have been carried out regarding the biological activity of red fruit. Budi and Paimin (2004) have done a preliminary study of red fruit. Testimony regarding the efficacy of red fruit has been published, one of them as the *in vitro* anticancer (Mun'im *et al.*, 2006). Furthermore, Rohman *et al.* (2010) also evaluated the antioxidant activity of extract and fractions of red fruit *in vitro*.

The valuable part of red fruit is its oil, known as red fruit oil (RFO). RFO is expensive oil in the market. The price of RFO is 10 – 15 times more expensive than that of common oils like palm oil and corn oil. As a consequence, the quality of RFO must be assured by determining some physico-chemical properties as reported by Rohman *et al.* (2012) and Novitasari *et al.* (2013). Iodine value (IV) and acid value (AV) is one of the oil quality parameters used to asses fats and oils, including RFO. Usually, the determination of these values 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, carcinogenic, and environmentally unfriendly chemicals. These methods are also time consuming, costly, and largely dependent on the skills of the analyst. 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.

Due to its properties as fingerprint technique, FTIR spectroscopy is promising approach for quality assurance of edible fats and oils. The fact that FTIR spectroscopy especially in combination with chemometrics technique can be used for qualitative and quantitative analyses is proven, as can be seen from several publications reporting that matter. FTIR spectroscopy assisted by chemometrics of multivariate calibration in the analysis of data has been successfully used for the determination of quality parameters of fats and oils namely for determination of iodine number in palm oil (Che Man *et al.*, 1999), vegetable oils (Kampars and Kronberga, 2003), coconut oil (Hendl *et al.*, 2001) and in the pure triglycerides (Sedman *et al.*, 2000).

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FTIR spectroscopy has also been successful for determining the acid number in lubricant (Li *et al.*, 2009), free fatty acids in palm oil (Che Man *et al.*, 1998), acid value in vegetable oil (Al-Alawi *et al.*, 2006), fish oil (Alberta *et al.*, 2009) and in olive oil (Maggio *et al.*, 2009). However, using literature searching, the application of FTIR spectroscopy for determination of quality parameters of RFO has not been reported yet. Therefore, in this study, FTIR spectroscopy in combination with chemometrics of partial least square is developed for determination of iodine value and acid value in RFO.

Materials and Method

Materials

Red fruit was taken from Papua, Indonesia. Botanical identification was carried out in Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. 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 previously reported by Rohman *et al.* (2012). Briefly, Red fruit was cut into small pieces using a commercial cutter and subsequently subjected to commercial blender containing methanol (one part of fruit was added with one part of methanol). The methanolic extract obtained was further macerated with methanol (1: 3 volume/volume) 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 of chloroform fraction. The oil obtained was further used to determine iodine value and acid value by titration method according to AOCS and FTIR spectroscopy method.

Treatment of RFO samples on accelerated temperature

Approximately 50 gram of each sample is introduced into 100 mL Beaker glass. The samples are divided into 6 groups, each was labeled according to the name of oil and temperature treatment used, namely: without heating (ambient temperature); 100°C, 150°C, 180°C, 200°C and 300°C. Each group was subjected to thermal treatment in conventional oven for 90 minutes. Furthermore, the oil sample is cooled and prepared for further analysis (the determination of iodine and acid values with the

chemical method and FTIR spectrophotometry in combination with chemometrics methods.

Reference method

Determination of iodine value and acid value in the treated RFO samples was performed according to the Standard Methods for the Analysis of Fats, Oils in Official Methods of Analysis of AOAC International (2005).

Measurement of FTIR spectra

All the samples were measured using FTIR spectrophotometer FTIR MB3000 (Clairet 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 - 400 cm⁻¹, as reported previously by Lukitaningsih *et al.* (2012). Spectra were scanned 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, at each scanning of the sample. The spectrum was recorded as absorbance value at each frequency point data conducted in three replicates.

Data analysis

Multivariate calibration of partial least square (PLS) was used to construct the correlation model between actual value (from the determination of iodine value and acid value using standard AOAC methods) 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

The absorption of infrared radiation can cause a molecule to vibrate. However, only those vibrations that are accompanied by a change in the electric dipole moment cause absorption of infrared light. Vibrations which do not change the dipole moment, do not result in the absorption of infrared radiation. The intensity of absorption depends on the square of the change in dipole moment, thus there can be large differences in absorption intensity. The vibration of polyatomic molecules is much more complicated. Fortunately, different parts of a molecule can vibrate independently (Stuart, 2004). The concept of group frequencies is basic to the understanding of infrared spectra and their use in molecular identification (Che

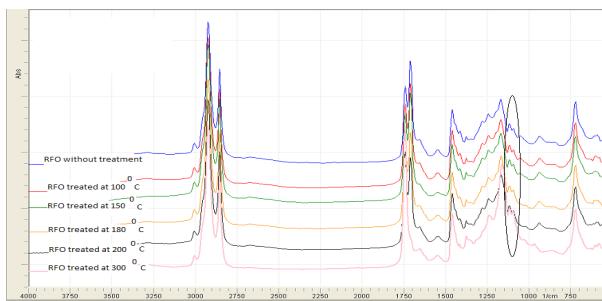


Figure 1. ATR-FTIR spectra of red fruit oil during high temperature treatment for iodine value determination. The frequency region assigned with circle was used for partial least square (PLS) calibration model.

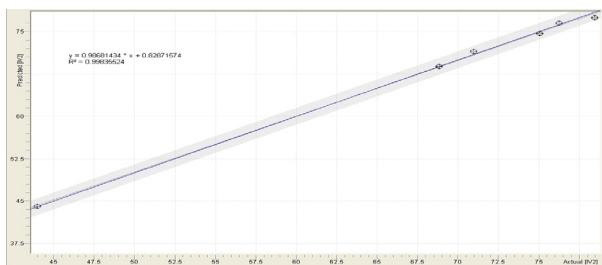


Figure 2. PLS calibration model for correlation between actual and FTIR predicted value of iodine value.

Man *et al.*, 1999).

Figure 1 presents the typical spectrum of RFO from 4000 - 400 cm⁻¹ at several temperature treatment. This spectrum illustrates the dominant spectral features associated with the oil absorption regions: the CH stretching absorption in region from 3050 - 2800 cm⁻¹ (*cis* C=CH, CH₂, CH₃ and CH₂/CH₃ stretching bands), the -C=O stretching absorption of the triglyceride ester linkage around 1800 - 1700 cm⁻¹, and the fingerprint region of 1500 - 1000 cm⁻¹ (Guillen and Cabo, 1997). During the thermal treatment, some peak intensities changed; therefore, this change can be exploited for quantitative analysis, as the peak intensities is proportional with the concentration of analyte of interest (Rohman, 2012). As shown in Figure 1, some peak intensities especially at frequency region of 1750 cm⁻¹ (indicated with circle) changed with the temperature used for RFO treatment.

For determination of iodine value, some peaks due to -CH stretch in *cis* HC=CH, *cis* C=C stretch, and CH stretch in trans HC=CH regions at around 3006, 1650, and 968 cm⁻¹ can be exploited, respectively (Guillen and Cabo, 1997). For determination of acid value, the regions of the wavelength of 3050 - 2800, 1740 and 1500 - 650 cm⁻¹ indicate the C-H stretching vibrations, C=O double bond stretching vibrations, deformations and bending of C-H, and stretching vibrations of C-O can be exploited, respectively (Che Man and Moh, 1998; Yildirim, 2009; Li *et al.*, 2009).

For spectral regions where there are absorptions

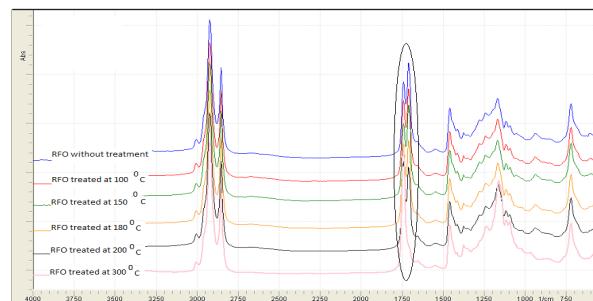


Figure 3. ATR-FTIR spectra of red fruit oil during high temperature treatment for acid value determination. The frequency region assigned with circle was used for partial least square (PLS) calibration model.



Figure 4. PLS calibration model for correlation between actual and FTIR predicted value of acid value.

caused by the component of interest, there is a positive deflection of the correlation coefficient (Fuller *et al.*, 1988). Both variance and correlation spectra can provide clues to the spectral regions that provide the best calibration model in IV determination. After the optimization process, finally the frequency region of 1080 - 1114 cm⁻¹ was used for prediction of iodine value. The correlation between actual value (determined with AOCS method) and FTIR predicted value was shown in Figure 2. The following equation was obtained, IV predicted = 0.98 IV actual + 0.82. The coefficient of determination (R²) obtained was higher than 0.998 indicating that both actual and FTIR predicted values have close relationship.

For determination of acid value, the frequency region of 1731 - 1762 cm⁻¹ was preferred (Figure 3). The selection of this frequency region was relied on its capability to provide good relationship between actual acid value of RFO as determined using ACS method and FTIR predicted value. Besides, this frequency region also offers the low standard error of calibration and prediction. Figure 4 revealed the correlation between actual value of acid value (AV) and FTIR predicted value having R² of 0.998. The following equation was obtained, AV predicted = 0.98 AV actual + 1.12. 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 iodine and acid values in RFO.

The low values of errors obtained during

determination of iodine value and acid value, either in calibration or validation samples, indicated that the developed method is reproducible enough to be used in routine analysis for assuring the quality of RFO. An analytical method in the development phase must be reproducible.

Based on the result obtained, it can be stated FTIR spectroscopy in combination with multivariate calibration is proven technique capable of determining IV and AV values in red fruit oil. This finding support some previously reports regarding the successfull of FTIR spectroscopy for analysis of quality parameters of fats and oils such as in Che Man *et al.*, 1999; Kampars and Kronberga, 2003; and Hendl *et al.*, 2001.

Conclusion

From this study, it can be concluded that FTIR spectroscopy at frequency region of 1080 - 1114 cm⁻¹ can be used for estimation of iodine value (IV) of RFO treated with some temperature. Furthermore, the frequency region of 1731 - 1762 cm⁻¹ is suitable for estimation of acid value (AV). The rapid determination of IV and AV by FT-IR spectroscopy is therefore suitable and practical for process control. Another advantage of FT-IR method is that it is environmentally friendly as no chemical is needed. By utilizing this method, chemical cost is negligible as compared by AOAC standard method.

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