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

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

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

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 (R²) 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 R² 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...
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 adulteration 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; Guillon 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 (Guillon 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 v/v). The methanolic extract was then subjected to maceration using ethanol (1:3) for 4 days. The ethanolic extract obtained was evaporated at 70°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°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 mg kg⁻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°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 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°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
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>
<thead>
<tr>
<th>T (ºC)</th>
<th>CF</th>
<th>HF</th>
<th>CF+BHT</th>
<th>HF+BHT</th>
<th>CF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>19.44</td>
<td>18.72</td>
<td>19.65</td>
<td>16.83</td>
<td>19.36</td>
<td>17.46</td>
</tr>
<tr>
<td>100</td>
<td>26.38</td>
<td>19.67</td>
<td>20.15</td>
<td>17.73</td>
<td>20.56</td>
<td>18.02</td>
</tr>
<tr>
<td>180</td>
<td>23.86</td>
<td>22.15</td>
<td>22.15</td>
<td>20.25</td>
<td>23.7</td>
<td>22.85</td>
</tr>
<tr>
<td>200</td>
<td>24.75</td>
<td>24.45</td>
<td>24.62</td>
<td>23.3</td>
<td>24.82</td>
<td>23.28</td>
</tr>
<tr>
<td>300</td>
<td>54.26</td>
<td>65.55</td>
<td>55.36</td>
<td>50.85</td>
<td>65.32</td>
<td>47.68</td>
</tr>
</tbody>
</table>

CF: Chloroform Fraction, HF: Hexane Fraction

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

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

Figure 2. The anisidine 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 chloroform 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.
Table 2. Absorbance changes of FTIR spectra of RFO in hexane fraction at wavenumber 1685 - 1701 cm\(^{-1}\)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Absorbances at wavenumbers of 1685.661 1689.519 1693.376 1697.233 1701.091 1704.948 1708.805 1712.663</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF at 25°C</td>
<td>0.019744 0.023628 0.027841 0.037897 0.058040 0.078616 0.092188 0.092774</td>
</tr>
<tr>
<td>HF at 100°C</td>
<td>0.023603 0.022135 0.026203 0.035862 0.055398 0.075633 0.08916 0.089903</td>
</tr>
<tr>
<td>HF at 150°C</td>
<td>0.018344 0.027525 0.031611 0.041728 0.06218 0.082931 0.096601 0.097139</td>
</tr>
<tr>
<td>HF at 180°C</td>
<td>0.017125 0.020443 0.024155 0.033224 0.051704 0.071089 0.084179 0.084958</td>
</tr>
<tr>
<td>HF at 200°C</td>
<td>0.016334 0.019482 0.022987 0.031532 0.049185 0.067867 0.080561 0.08131</td>
</tr>
<tr>
<td>HF at 300°C</td>
<td>0.010049 0.010947 0.011971 0.014089 0.017776 0.0215 0.02475 0.026429</td>
</tr>
<tr>
<td>HF + BHT at 25°C</td>
<td>0.016335 0.019635 0.023172 0.032007 0.049564 0.067663 0.079827 0.080655</td>
</tr>
<tr>
<td>HF + BHT at 100°C</td>
<td>0.016941 0.020202 0.023767 0.032341 0.049681 0.065749 0.079513 0.080302</td>
</tr>
<tr>
<td>HF + BHT at 150°C</td>
<td>0.016002 0.019227 0.022771 0.031243 0.048408 0.066294 0.078388 0.079287</td>
</tr>
<tr>
<td>HF + BHT at 180°C</td>
<td>0.015217 0.018315 0.021731 0.030023 0.046968 0.064766 0.076856 0.077747</td>
</tr>
<tr>
<td>HF + BHT at 200°C</td>
<td>0.014354 0.017294 0.020564 0.028511 0.044834 0.06218 0.074137 0.075064</td>
</tr>
<tr>
<td>HF + BHT at 300°C</td>
<td>0.012205 0.01441 0.016671 0.022913 0.030315 0.044954 0.053429 0.054407</td>
</tr>
<tr>
<td>HF + α-tocopherol 25°C</td>
<td>0.019424 0.023292 0.027442 0.037294 0.057066 0.077392 0.090893 0.091585</td>
</tr>
<tr>
<td>HF + α-tocopherol 100°C</td>
<td>0.019428 0.023299 0.027462 0.037323 0.057041 0.077233 0.090599 0.091193</td>
</tr>
<tr>
<td>HF + α-tocopherol 150°C</td>
<td>0.019371 0.022997 0.026914 0.036236 0.055141 0.074747 0.087838 0.088545</td>
</tr>
<tr>
<td>HF + α-tocopherol 180°C</td>
<td>0.017622 0.020789 0.0242 0.032476 0.049408 0.067908 0.078952 0.079492</td>
</tr>
<tr>
<td>HF + α-tocopherol 200°C</td>
<td>0.016135 0.019327 0.02282 0.031356 0.049019 0.067674 0.080315 0.081073</td>
</tr>
<tr>
<td>HF + α-tocopherol 300°C</td>
<td>0.009342 0.010182 0.01114 0.013233 0.016995 0.020872 0.024233 0.025831</td>
</tr>
</tbody>
</table>

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°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:

\[
y = 1.00376547 x + 0.17406289 \quad \text{(RFO in hexane fraction)}
\]

\[
y = 0.98778900 x + 0.02306001 \quad \text{(RFO in chloroform fraction)}
\]

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 (R²) 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


