

## Characterization, biological activities, and authentication of red fruit (*Pandanus conoideus* Lam) oil

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### Abstract

Red fruit (*Pandanus conoideus* Lam) oil (RFO) is one of high value edible oils having many functional advantages for health. *P. conoideus* is a native plant from Papua province, Republic of Indonesia and Papua New Guinea. RFO is good for diets and served as preventive agents for various diseases such as cancer and coronary disease so that RFO can be considered as functional edible oils. Because of its high quality, in Indonesia fats and oils market, RFO has a more expensive price (10-15 times) than other common edible oils like palm oil and corn oil. This fact attracts some unethical player to adulterate RFO with other lower priced-oils such as palm oil and corn oil. As a consequence, the authentication of RFO from any oil adulterants becomes important to ensure the quality and the authenticity of RFO. This article report some biological advantages of RFO and the characterization of RFO. FTIR spectroscopy in combination with multivariate calibrations has been reported as a powerful technique for the authentication of RFO from other oils and is the most reported techniques in some literature.

## 1. Introduction

Red fruit (*Pandanus conoideus* Lam) is a special and typical plant (endemic) which grow at Papua Island, i.e. Papua (Indonesia) and Papua New Guinea. The shape of the fruit is oval with bright maroon red color. The fruit has 55 cm - 100 cm long, 10-15 cm diameter, and 2-3 kg weight (Bourke, 2005). RFO is known as functional oils, having relatively complete essential vitamins and minerals. RFO also contains high doses of beta-carotene, tocopherol, and essential fatty acids of oleic and linolenic acids (Budi and Paimin, 2004). The high quality of RFO not only served as a diet but also as preventive agents of various degenerative diseases. Nowadays, RFO is widely distributed over the world, also in other provinces of Indonesia. RFO is labeled as daily oil diet which is good for health. The price of RFO is approximately 10-15 times higher than other vegetable oils such as palm, soybean, and canola oil. RFO has been considered as functional oil having a beneficial effect on human health (Rohman and Che Man, 2011).

Because of its prices, the authentication of RFO becomes more important to verify and to ensure the originality of RFO. RFO is potential to be adulterated with other lower price oils such as palm oil, corn oil,

sesame oil and others (Setyaningrum *et al.*, 2013). Adulteration of high quality oils with lower oils is a serious problem not only for producers but also for consumers. The adulteration practice was related to health aspects, and in some cases, adulteration can be related to certain religion if adulterant is non-halal components for Muslim like lard (Rohman and Che Man, 2008). In order to assure the quality and the safety of RFO, the oil characteristics must be defined via determining several physico-chemical properties in order to assure the quality of RFO. This review aims to highlight some physico-chemical properties of RFO along with the biological activities and to describe FTIR spectroscopy combined with chemometrics for authentication of RFO from potential adulteration with low priced oils.

## 2. Characterization of red fruit oil

RFO is a vegetable oil obtained from the extraction process of red fruit. Due to the increasing numbers of RFO products in Indonesia fats and oils market, it is important to define the standard characteristics of RFO. Some quality parameters and requirements of RFO are proposed, including organoleptic (smell, taste, colour),

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microbial contamination, and other physico-chemical properties including acid value, iodine value, saponification value, conjugated diene and triene values, anisidine values, coefficient of absorptivity, volatile compounds, and fatty acid composition (Mousavi *et al.*, 2012).

Arumsari *et al.* (2013) have compared physico-chemical properties of RFO obtained using hexane and chloroform solvents, to obtain hexane fraction and chloroform fraction. Acid value represents the free fatty acids present in fats and oils. The lower the acid value, the better the fats and oils. RFO in hexane fractions has the lower acid value ( $100.63 \pm 3.36$  mg KOH/g RFO) than chloroform fractions ( $77.55 \pm 6.87$  mg KOH/g RFO). Therefore, hexane fraction is subjected to lower hydrolytic and lipolytic degradation than that of chloroform fraction. Saponification value is used to estimate the molecular weight of fatty acids in RFO. The saponification value of RFO in chloroform fraction ( $155.01 \pm 0.52$  mg KOH/g RFO) is higher than in hexane ( $127.30 \pm 1.36$  mg KOH/g RFO). The iodine value indicates the unsaturation degree of fats and oils. The higher level of iodine value, the more unsaturated fatty acids in RFO. RFO contains more unsaturated fatty acid in hexane fraction ( $81.48 \pm 4.59$  g<sub>I<sub>2</sub></sub>/100 g RFO) than in chloroform fraction ( $70.73 \pm 3.38$  g<sub>I<sub>2</sub></sub>/100 g RFO).

The conjugated diene (CDs) and triene (CTs) values at 232 and 270 nm, respectively in the UV spectrum is related to the formation of peroxides (Wanasundara *et al.*, 1995). The content of CDs and CTs can serve as a relative measurement of oxidation (Pegg, 2005). The CDs value of RFO in hexane fractions ( $3.03 \pm 0.07$  g/100 mL cm<sup>-1</sup>) is not statistically different with CDs value from chloroform fractions ( $3.02 \pm 0.008$  g/100 mL cm<sup>-1</sup>). However, the CTs value of RFO in chloroform fraction ( $2.56$  g/100 mL.cm<sup>-1</sup>) was higher than in hexane fraction ( $1.68$  g/100 mL.cm<sup>-1</sup>) (Arumsari *et al.*, 2013). Anisidine values measure the formation of 2-alkenals which is used to measure the extent of secondary oxidation products in fats and oils (Laguerre *et al.*, 2007). Anisidine values of RFO in hexane fraction (21.37) was higher than that of RFO in chloroform fraction (19.46).

Fatty acids are an essential components of edible fats and oils. The quantitative analysis of fatty acids is important in fats and oils because fatty acid composition can be used as characteristics property and nutritional information of edible fats and oils (Carrosco-Pancorbo *et al.*, 2009). Rohman *et al.* (2012) have measured the fatty acids compositions of RFO, as determined using gas chromatography with flame ionization detector. Table 1 detailed fatty acid composition of RFO. The main fatty acids composed of RFO were oleic acid (C18:1) and linoleic (C18:2) acids with the concentration of 68.80% and 8.49%, respectively. It is believed that the high

presence of monounsaturated fatty acid (oleic acid) in RFO can contribute to the beneficial effects to human health (Fomuso and Akoh, 2002).

Table 1. Fatty acid composition of red fruit oil (Rohman *et al.*, 2012).

Fatty acid	Concentration (%)
C12:0	0.06 ± 0.02
C14:0	0.07 ± 0.00
C14:1	0.15 ± 0.00
C16:0	20.05 ± 0.09
C16:1	0.15 ± 0.01
C18:0	0.18 ± 0.01
C18:1	68.80 ± 1.29
C18:2	8.49 ± 0.02
C18:3n6	0.17 ± 0.00
C20:0	0.13 ± 0.00

The volatile compounds of RFO also have been evaluated by Rohman *et al.* (2012) using gas chromatography-mass spectrometry using headspace analyzer (GC-MS-HS). There are 35 volatile compounds identified with the main components are 1,3-dimethylbenzene (27.46%), N-glycyl-L-alanine (17.36%), trichloromethane (15.2%), and ethane (11.43%).

### 3. The biological activities of RFO

RFO has been known as a natural food supplement containing medicinal qualities and as a dye. RFO can be considered as functional food oils due to its ability to give several biological activities (Rohman and Che Man, 2012). Several studies have been reported on the biological activities of RFO. RFO has an effect on anticancer activity. Local communities in Indonesia believed that RFO can treat several degenerative diseases such as cancer, arteriosclerosis, rheumatoid arthritis, and stroke (Budi and Paimin, 2004) as well as HIV, malaria, cholesterol, and diabetes mellitus (Limbongan and Malik, 2009).

RFO was found to inhibit the growth of lung cancer cell A549 at doses of 500 mg of fruit extract/mL dissolved in dimethylsulfoxide (DMSO). The presence of β-cryptoxanthin could cause the growth inhibition of lung cancer cells associated with smoking (Lim, 2013). Another study showed that RFO exhibited antitumour effects in either tumour volumes or tumour weights against mouse Sarcoma-180, Lewis lung cancer and human nonsmall lung cancer A549 bearing mice (Lim, 2013). Animal studies showed that RFO could reduce the serum level of proinflammatory cytokines, such as TNF-α (tumour necrosis factor-α), IFN-γ (interferon-γ) and IL-1 (interleukin-1) in experimental colorectal cancer model mice (Oeji and Khiong, 2010). RFO with different concentrations has been reported to significantly decrease the rate of reactive oxygen

intermediate (ROI) production of endothelial cells. RFO can neutralize oxidative stress of cells by reducing ROI production of endothelial cells exposed to severe malaria patient serum and neutrophils from healthy donor *in vitro* (Armiyanti *et al.*, 2007).

Another study reported that RFO has a hypoglycaemic activity. A combination of RFO and glibenclamide produced a significant reduction in blood glucose in diabetic-treated rats. The results showed that RFO could increase the hypoglycaemic effect of glibenclamide on diabetic rats (Winarto *et al.*, 2009). RFO at dose of 0.9 mL/kg b.w. significantly inhibited inflammation in female wistar rats after carrageen induced inflammation with inhibition rates of 72.5, 69.2, and 85% at 3, 4, and 24 hours respectively, compared to control rats (Sukandar *et al.*, 2005). With its high presence of monounsaturated fatty acid (oleic acid), RFO is believed to have beneficial effects for human health such as preventing cardiovascular diseases, reducing plasma triacylglycerol (TAG), increasing high density lipoprotein (HDL)-cholesterol levels, improving the postprandial lipoprotein metabolism, reducing blood pressure and the risk of hypertension, having anti-cancerogenic effects in animal models and in human cell lines, not promoting obesity and increasing the lipolytic activity in adipose tissue, and preventing age-related cognitive decline and dementia (García-González *et al.*, 2008).

#### 4. Authentication of RFO

Due to the high-priced value of RFO in the Indonesian fats and oils market, some unethical players try to blend RFO with other vegetable oils having low price value such as palm oil and corn oil. This is based on fact that the economic profit obtained from fats and oils industry is relatively narrow, compared to pharmaceutical industry (Asensio *et al.*, 2008; Schieber, 2008; Arlorio *et al.*, 2010). Authentication analysis of RFO becomes important in order to ensure the quality of RFO from adulteration practices. Some analytical methods have been developed, and the most reported are Fourier transform infrared (FTIR) spectroscopy. Due to its property as fingerprint technique, FTIR spectroscopy is widely used for the authentication of RFO. FTIR spectroscopy in combination with chemometrics of multivariate calibration and classification technique such as principal component analysis (PCA) and discriminant analysis (DA) becomes a powerful method for authentication purposes (Rohman, 2012; Rohman and Che Man, 2012).

The authentication of RFO from corn oil (CO) is performed by spectroscopy FTIR combined with chemometrics of multivariate calibration of partial least square (PLS). Some wavenumbers regions were

optimized to analyze CO in RFO. The combined wavenumbers y region of 1600-1800  $\text{cm}^{-1}$  and 800-1200  $\text{cm}^{-1}$  were selected for quantification of CO in RFO. During PLS calibration models, these wavenumbers regions showed the lowest values of root mean error of calibration (RMSEC) and root mean square error of prediction (RMSEP) and the highest values of coefficient of determination ( $R^2$ ) using FTIR normal spectra. The RMSEC and  $R^2$  values obtained are 0.987% and 0.998, respectively. In order to validate the developed model, some independent samples comprising the mixture of CO and RFO are measured using the same condition used for determining calibration samples. The RMSEP value of CO in RFO obtained was 1.195% (Setyaningrum *et al.*, 2013). The high value of  $R^2$  and low values of RMSEC and RMSEP obtained indicated that the developed PLS calibration model exhibited acceptable accuracy and precision, therefore, the developed model can be used for authentication of RFO from CO.

Rohman *et al.* (2011) have developed the model for authentication of RFO from canola oil (CaO) using FTIR spectroscopy in combined with multivariate calibration of partial least square. Using the optimization process, the frequency region of 1200-1050  $\text{cm}^{-1}$  was used for quantification of CaO due to the high correlation between actual value and FTIR measured values of CaO in RFO. The coefficient of determination ( $R^2$ ) value obtained was 0.999. The error during calibration as expressed as root mean square error of calibration (RMSEC) was 0.812% (v/v). The PLS model was further used to predict the level of CaO using laboratory prepared samples. The  $R^2$  value for correlation between actual and measured values of CaO in prediction models is 0.996 with a root mean square error of prediction (RMSEP) of 1.05% (v/v). In order to validate the performance of the PLS model, leave-one-out-technique was used. The root mean square error of cross validation (RMSECV) obtained was 2.28% with an  $R^2$  of 0.990. Discriminant analysis (DA) is a chemometric technique with supervised pattern recognition type which allocates a new object of the unknown group to the correct group (Miller and Miller, 2005). DA using frequency regions of 1200-1050  $\text{cm}^{-1}$  can discriminate pure RFO samples and those adulterated with CaO and RBO with no misclassification reported during the study (Rohman *et al.*, 2011).

The authentication of RFO from soybean oil (SO) is performed using FTIR spectroscopy in combined with chemometrics multivariate calibrations. It can be shown that the normal spectra in combination with PLS calibration technique at two frequencies of 1600-1800  $\text{cm}^{-1}$  and 800-1200  $\text{cm}^{-1}$  is the better model, in terms of the highest  $R^2$  (0.997), the lowest values of RMSEC (1.195% v/v) for the relationship between the actual

value and the predicted value. The validation/prediction model showed that the RMSEP value obtained was 1.063% (v/v) (Setyaningrum *et al.*, 2013).

The presence of rice bran oil (RBO) in RFO also has been exploited using FTIR spectroscopy in combined with partial least square. The two combined frequencies of two region ranges (1207-1078 and 1747-1600  $\text{cm}^{-1}$ ) were selected for this analysis. The coefficient of determination ( $R^2$ ) value obtained was 0.996. The root mean square error of calibration (RMSEC) value obtained was 0.957% v/v. The root mean square error of prediction (RMSEP) and the  $R^2$  value for prediction was 0.991. Using two principal components, the RMSECV value obtained was 1.91% (v/v) (Rohman *et al.*, 2011).

FTIR spectroscopy coupled with multivariate calibration of PLS is also used for quantitative analysis of sunflower oil (SFO) and palm oil (PO) as oil adulterants in RFO for authentication purposes. Some wavenumbers are optimized to seek the best wavenumbers region, FTIR spectral treatment including derivatization and its combination capable of the best predicting of adulterant levels in RFO. Based on  $R^2$  and RMSEC values obtained, the wavenumber regions 1780–1680  $\text{cm}^{-1}$  using FTIR normal spectra was more preferred than its derivative for quantitative analysis of PO in RFO. The  $R^2$  and RMSEC values obtained are 0.9991 and 0.98% v/v, respectively. FTIR normal spectra at 1200–1000  $\text{cm}^{-1}$  was used for quantification of SFO in RFO with  $R^2$  and RMSEC value of 0.991 and 1.09% v/v, respectively (Rohman *et al.*, 2014).

## 5. Conclusion

RFO has been recognized as functional food oils because of its beneficial effects on human health. The high quality of RFO served as preventive agents of various diseases, therefore RFO has high price oils in the market. RFO is potential to be adulterated with other lower quality and lower price oils, therefore the authentication of RFO is very important. FTIR spectroscopy combined with chemometrics multivariate calibrations is a powerful method for the detection and quantification the adulteration practices in RFO.

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