INFRARED SPECTROSCOPY AND MULTIVARIATE CALIBRATION FOR THE RAPID QUANTIFICATION OF FREE FATTY ACID CONTENT IN PANGASius HYPOPTHALMUS OIL

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Received: 06 Aug 2018, Revised and Accepted: 21 Sep 2018

ABSTRACT

Objective: The objective of this study was to evaluate the capability of fourier transform infrared (FTIR) spectroscopy in combination with multivariate calibration for prediction of free fatty acids (FFA) in Pangasius hypopthalmus (P. hypopthalmus) oil.

Methods: FFA content in P. hypopthalmus oil was determined by attenuated total reflectance-FTIR spectroscopy. P. hypopthalmus oil derived from Pangasius's meat (MP), and Pangasius's liver and fat (LFP) were subjected to heat treatments. Determination of FFA content in P. hypopthalmus oil's was performed by gas chromatography-flame ionization detector.

Results: Oleic acid was found to be the main fatty acid component in P. hypopthalmus oil. FTIR spectra of P. hypopthalmus oil has 3 main peaks, C-H bonds of cis-form of fatty acid showed the stretching vibration, symmetric and asymmetric vibrations of the C-H2 and C-H3 aliphatic group and vibrations of the carbonyl (C=O) ester derived from the oil triacylglycerols. Principal component regression (PCR) model showed a better performance than the partial least square (PLS) model. PCR at wavenumbers of 1200-1000 cm-1 with first derivative treatment was chosen for FFA content in P. hypopthalmus oil's liver and fat (LFP) were subjected to heat treatments. Determination of FFA content in P. hypopthalmus oil's was performed by gas chromatography-flame ionization detector.

Conclusion: FTIR spectroscopy combined with PCR can be used as an alternative method for analysis of fatty acid contents.

Keywords: Pangasius hypopthalmus oil, FTIR spectroscopy, Free fatty acid, Principal component regression

INTRODUCTION

Pangasius hypopthalmus (fig. 1) is one of the widely consumed fish commodities in Indonesia which its production has increased significantly over the last few years. In 2004 the production of P. hypopthalmus amounted to 23,962 tons and increased to 52,470 tons in 2008. P. hypopthalmus is also one of a very popular freshwater fish that consumed worldwide [1]. Based on Hwang et al. P. hypopthalmus abdominal part including the gastrointestinal tract, liver, gallbladder, and abdominal fat were the potential source of high omega 3 fat [2]. Panagan et al. [3] reported that omega 3 acids content in P. hypopthalmus oil ranged from 1.16 to 12.44%. The production of P. hypopthalmus in South Kalimantan in 2014 was reached to 70,000 tons per year [4]. Currently, the most widely cultivated catfish species in Indonesia are P. djambal (jambal catfish) and P. hypopthalmus (Siamese catfish).

Fig. 1: Pangasius hypopthalmus

Free fatty acid content (FFA) is one of the parameters that can be used to represent the quality of an edible oil. According to the standard method, FFA is determined by titration method. This procedure required a large number of a chemical solvent which has an adverse impact on the environment. It also required trained laboratory assistant and long processing time, which is laborious and time-consuming. Fourier transform infrared (FTIR) combined with chemometrics are reported to be the alternative method for the determination of oil quality and stability such as acid value [5, 6], free fatty acid value [5], saponification value [7, 8], iodine value [7, 9], peroxide value [10, 11], and anisidine value [12, 13]. Besides, FTIR was also successfully used as an alternative for a quantitative method for pharmaceuticals [14, 15]. It was found to be rapid, easy technique, and in contrary to the standard method it doesn't require a large amount of sample and chemical solvent. Which have made this method less expensive and environmentally friendly.

This study was intended to develop a quantitative method for the FFA content in P. hypopthalmus oil. The developed model would be compared to the standard titration method using chemometric with a set of validation data.

MATERIALS AND METHODS

P. hypopthalmus oil extraction

P. hypopthalmus was divided into meat, liver, and abdominal fat for further processing by dry rendering. Each part was heated to a temperature of 60 °C until the oil stop to comes out and then filtered. The extraction products were labeled as Pangasius's meat (MP) and Pangasius's liver and fat (LFP).

Fatty acid determination

The profiles of fatty acids in P. hypopthalmus oil were determined by gas chromatography-flame ionization detector (GC-FID) according to Rohman and Che Man method [16]. Approximately 50.0 mg of oil was dissolved in 0.8 ml of n-hexane, added with 0.2 ml of sodium methoxide (1 M), and then shook using vortex for 1 minute. Glycerol was precipitated by addition of saturated sodium chloride (5 drops) to the mixture, then mixed for 15 seconds. A-1 μl supernatant was injected into GC using condition as follows, column: RTX-5 capillary column (thickness: 0.2 μm, internal diameter 0.25 mm, length: 30 m); oven: 50 °C (1 min) increased to 240 °C; detector: flame ionization detector; and carrier gas: N2 (6.8 ml/min).
FTIR measurement

*P. hypopthalmus* oil was measured by FTIR spectrophotometer Nicolet 6700 with deuterated triglycine sulphate (DTGS) as a detector and linked with OMNIC software (version 7.0 thermo nicolet). The sample was placed on the attenuated total reflectance (ATR) crystal, and the measurements were performed on 32 scans, a resolution of 4 cm⁻¹ and at a controlled temperature (20 °C). All spectra were recorded in triplicate measurement at wavenumbers of 4000-650 cm⁻¹ using absorbance mode.

Free fatty acid content (FFA) determination

FFA was determined by the standard titration method. FTIR method was developed by modification of Andina et al. [11, 13] for the determination of *P. hypopthalmus* oil’s FFA. Approximately 150 ml of samples in 250 ml beaker glass (Pyrex®) were subjected to heat treatment at 150°C for 15, 30, 60, 120, 360, 480 and 60 0 min. Samples were then measured by ATR-FTIR with the same condition as the previous measurement. Partial least squares regression (PLS) and principal component regression (PCR) were carried out using the software of TQ analyst 9.

RESULTS AND DISCUSSION

The result of fatty acid determination can be seen in table 1. Oleic acid (37.36%) was found to be the main fatty acid. Different habitat water temperature affects the fatty acid profile of fish. Water temperature usually affects polar lipids, but it also increased the changes from unsaturated to the saturated fatty acid ratio in fish [17-20]. The member of the short-chain group C4-C14 of saturated fatty acids was present in large amount consist of butyric, lauric, and myristic acids. The number of short chain fatty acids affect the consistency of *P. hypopthalmus* oil, it appears partly solidified at room temperature, respectively. Fatty acids with the short-chain of carbon were also found in coconut oil, palm kernel oil, and milk fats [21].

| Fatty acid classification | Fatty acid  | (%)     |
|---------------------------|------------|---------|
| Saturated fatty acid (SFA)| BUTYRIC C4:0 | 13.23   |
|                           | LAURIC C12:0 | 0.58    |
|                           | MYRISTIC C14:0 | 3.85    |
|                           | PALMITIC C16:0 | 26.08  |
|                           | Δ SFA       | 43.74   |
| Mono unsaturated fatty acid (MUFA)| ELAIDIC C18:1 9(n-9) | 6.79 |
|                                 | OLEIC C18:1(n-9) | 37.36   |
|                                 | Σ MUFA      | 44.15   |
| Poly unsaturated fatty acid (PUFA)| LINOLEIC C18:2(n-6) | 12.09 |
|                                 | Σ PUFA      | 12.09   |

Table 1: Fatty acid profiles in *P. hypopthalmus* oil

FTIR spectra of *P. hypopthalmus* oil have similarities with other edible oils. As shown in fig 2 the FTIR spectrum of *P. hypopthalmus* oil has 3 main peaks. A peak at 3007.31 cm⁻¹ was derived from a stretching vibration of the carbon and hydrogen bonds in cis double bond [22-26], due to the presence of cis-cieic in *P. hypopthalmus* oil. There peaks at 2921 and 2852 cm⁻¹ are corresponding to the symmetric and asymmetric stretching vibrations of the carbon aliphatic group [22, 27]. The presence of the carbonyl (C=O) ester from the oil tricacylglycerols vibrations shown at 1743 cm⁻¹[28]. The strong intensity due to the big difference of carbon and hydrogen atoms electro-negativity [22, 24, 26].

In the present study, 7 models (normal and derivative) of each PLS and PCR were tested and investigated in terms of coefficient of determination (R²), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP), to obtain the most accurate models to predict FFA. PLS is a multivariate calibration technique intended to reduce data dimensions and to find out the most relevant factors in predicting and interpreting data. By balancing the information between predictors and responses, PLS reduces the impact of many of the predictors irrelevant to the data diversity. Predictors giving high correlation are more concern since it will be more effective for prediction. PCR also used for linear regression models, when the number of independent variables (predictors) is very large, or when interceptors are highly correlated. One of the most important
applications of PCR is multivariate calibration, where the goal is to predict constituent concentrations based on the spectrum. The spectrum analysis can be obtained through various techniques, one of them is FTIR spectroscopy. FTIR spectra typically composed of values that reach a wide range of wave numbers, so that it consists of hundreds of components to be analyzed [33]. Different regions of wavenumber were selected and evaluated to find the suited model which give the optimum model for FFA prediction. PCR at 1.200-1.000 cm\(^{-1}\) with first derivative treatment was selected to predict FFA as shown in table 2.

Table 2: The performance of PLS and PCR models for prediction of fatty acid levels in \textit{P. hypopthalmus} oil.

| Region (cm\(^{-1}\)) | treatment | factors | \(R^2\) | RMSEC | RMSEP |
|-----------------------|-----------|---------|--------|------|------|
| PLS                   | 1425-1105 | Normal  | 1      | 0.4075 | 1.97 | 1.91 |
|                       | 1200-1000 | Normal  | 1      | 0.4018 | 1.97 | 1.94 |
|                       | 3100-2900 | Normal  | 1      | 0.2790 | 2.07 | 2.24 |
|                       | 1392-1382, 1439-1423, 1460-1439,1765-1747 | First derivative | 1 | 0.4198 | 1.95 | 2.16 |
|                       | 1200-1000 and 3100-2900 | First derivative | 1 | 0.3090 | 2.05 | 2.43 |
|                       | 1200-1000 | First derivative | 3 | 0.5496 | 1.81 | 2.17 |
|                       | 3100-2900 | First derivative | 6 | 0.4435 | 1.93 | 3.89 |
| PCR                   | 1425-1105 | Normal  | 1      | 0.7639 | 1.39 | 2.60 |
|                       | 1200-1000 | Normal  | 10     | 0.9276 | 0.804 | 4.50 |
|                       | 3100-2900 | Normal  | 6      | 0.4433 | 3.84 | 7.47 |
|                       | 1392-1382, 1439-1423, 1460-1439,1765-1747 | First derivative | 10 | 0.7246 | 1.48 | 3.24 |
|                       | 1200-1000 and 3100-2900 | First derivative | 10 | 0.8967 | 0.953 | 2.26 |
|                       | **1200-1000** | First derivative | **10** | **0.9417** | **0.725** | **2.40** |
|                       | 3100-2900 | First derivative | 10 | 0.7584 | 1.40 | 2.95 |

The wavenumbers marked with bold was preferred for prediction

As it can be seen in table 2, the PCR model at wavenumbers region of 1200-1000 cm\(^{-1}\) using first derivative treatment (fig. 3) offered the highest \(R^2\) and the lowest results of RMSEC and RMSEP. These findings indicate that the PCR at 1.200-1.000 cm\(^{-1}\) with first derivative treatment was the most accurate among other developed models in this study. Fig. 3 shows the first derivative treatment to the spectra at 1.200-1.000 cm\(^{-1}\), this treatment removes baseline shifts and improves the accuracy of quantification [34].

The scatter plot in fig. 4 (a) showed the relationship between FFA actual and FFA calculated. The FFA actual in the \(x\) values obtained from the standard titration method, whereas the FFA calculated in \(y\) values given by the prediction model generated by FTIR using PCR model. The time required for FFA determination of sample by titration takes 10-20 min, while the time required for FFA determination uses FTIR in less than three minutes. This time comparison is in line with the previous research which stated that FTIR spectroscopy indeed not time-consuming. This method was also not laborious and an easy method. Besides, FTIR spectroscopy also reduced the number of chemical solvents usually used in the standard method for FFA determination, such as ethanol and sodium hydroxide.
At least there are two concerns regarding a developed model; the first is the prediction result by the developed model and the second is residual results generated by the developed model shown in fig. 4 (b). Residual plot aims to analyze whether a regression obtained by the model is suitable to use as a prediction model. It's clearly shown in fig. 4 (b) that the residual plots in regression represent a satisfactory distribution of residuals. It can be seen that such plots are approximately identical across the line, and normally distributed close to zero. The residual analysis gave the conclusion that PCR at 1.200-1.000 cm⁻¹ with first derivative treatment has been used as the correct and suited model for prediction of FFA in *P. hypopthalmus* oil.

**CONCLUSION**

FTIR spectroscopy combined with multivariate calibration of PCR at 1.200-1.000 cm⁻¹ with first derivative treatment can be used as an alternative method for prediction of FFA in *P. hypopthalmus* oil.

**ACKNOWLEDGMENT**

The authors thank the Ministry of Research and Technology of The Republic of Indonesia for the Competitive Grants.

**AUTHORS CONTRIBUTIONS**

LA, RS, and ANP performed research activities and prepared manuscript. LA and AR designed research, analyzed data, and made critical thinking on the manuscript.

**CONFLICTS OF INTERESTS**

All authors have none to declare.

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