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Determination of Buffalo and Pig "Rambak" Crackers Using FTIR Spectroscopy and Chemometrics

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ABSTRACT

This study aimed to identify the type of "rambak" (cracker) by comparing rambak made from buffalo skin and that made from pig skin using fourier transformed infrared spectroscopy (FTIR) method. Samples of lipid obtained during Soxhlet extraction from buffalo skin, pig skin, rambak from buffalo skin and rambak from pig skin was analyzed. The lipid was scanned using FTIR spectrophotometer aided with chemometrics of Partial Least Square (PLS) and Principle Component Analysis (PCA). After optimization procedure, wave number of 1200-1000 cm^{-1} was selected for analysis. The results showed that the relationship between the predicted value to the true value of pig skin in rambak has coefficient of determination (R^2) of 0.96, root mean square of calibration (RMSEC) of 2.56 and Root Mean Square Error of Prediction (RMSEP) of 1.10. The PCA models successfully classify types of buffalo skin, pig skin and commercial rambak. The PLS calibration model and PCA can be used to classification and quantification of the various types of used skin lipid.

Key words: Rambak, lard, FTIR spectroscopy, partial least square, principle component analysis

INTRODUCTION

Skin derived from animals such as cows, buffaloes and pigs can be processed as food products such as rambak. Rambak or cracker a traditional food that is favored by most people of Indonesia (Nurhayati, 2007). Rambak is easily obtained in traditional market with various labels and types. Source of rambak in traditional market are so abundant, therefore, it created some opportunities for counterfeit labels such pig rambak labelled with buffalo rambak. The presence of pig derivatives including pig skin in any products is not allowed for followers of Islamic religion. Not only in Islamic religion which forbids to consume pork and its derivatives, but also Jewish people (Regenstein *et al*., 2003). Adulteration of food has serious case of contamination with harmful substances (Defernez and Wilson, 1995). This proves the higher consumer awareness toward basic material in food contained. Therefore, it is important to control the processing of food in order to know the origin of the products Halal (lawful or permitted) (Aida *et al*., 2005).

Halal products become a very serious concern in the food industry. Due to the advance of food technology, some producers can mix their products with nonhalal components derived from pigs like pork, lard and meat forgery. Therefore, to avoid the counterfeiting of food products, it is a need to ensure the halalness and safety for food products.

Various methods or techniques have been used for analysis of pig derivatives, namely gas chromatography-mass spectrometry (Nizar *et al*., 2013), liquid chromatography-mass spectrometry (Czerwenka *et al*., 2010), Gas Chromatography Tandem Mass Spectrometry (GC-MS) (Oliveira *et al*., 2009). Differential Scanning Calorimetry (DSC) (Marina *et al*., 2009; Nurrulhidayah *et al*., 2015), high pressure liquid chromatography (Saeed *et al*., 1989; Marikkar *et al*., 2005), electronic nose (Nurjuliana *et al*., 2011) and DNA-based methods using polymerase chain reaction (Man *et al*., 2007; Erwanto *et al*., 2014; Maryam *et al*., 2015). Some of the methods that have been conducted have weaknesses because it takes a long time in detecting the adulteration in food stuffs. Therefore, the routine method needs fast, accurate and easy to use and inexpensive. One of ideal method to be used in routine analytical laboratory is Fourier transform infrared (FTIR) spectroscopy (Rohman *et al*., 2014).

The FTIR Spectroscopy is a versatile method widely used for analysis of pig derivatives (Syahariza *et al*., 2005). Fast spectrum acquisition, easy to operate and needing no complex sample preparation are its advantages of FTIR spectrophotometer (Maggio *et al*., 2009). Currently, the application of FTIR spectroscopy has emerged as the main tool used in food science, especially its combination with chemometrics. This is due to its properties of FTIR spectroscopy as fingerprint technique, which can be used for qualitative and quantitative analyses (Guillen and Cabo, 1997). With the advancement in technology and research machinery, there are many tools that can be utilized for analysis and quality control of food products. Analysis of adulteration in food products using FTIR spectroscopy has been reported by Xu *et al*. (2012) for rapid discrimination of pork in Halal and non-Halal Chinese ham sausages. Our group also developed FTIR spectroscopy in combination with chemometrics for analysis of pork in beef meatball (Rohman *et al*., 2011), lard in meatball broth (Kurniawati *et al*., 2014), wild boar meat in meatball (Guntarti *et al*., 2015) and rat's meat in beef meatball (Rahmania *et al*., 2015). Hashim *et al*. (2010) used FTIR spectroscopy for differentiation of porcine gelatin and bovine gelatin successfully.

This study aimed to identify the type of rambak by comparing rambak made from buffalo skin and that from pig skin using FTIR spectrophotometer. Using literature review, there is no publication reporting the employment of FTIR spectroscopy for identification and quantification of crackers made from buffalo skin adulterated with pig skin.

MATERIALS AND METHODS

The skin of buffalo and pig was randomly obtained from some slaughter houses in Jogjakarta, Indonesia during February-April, 2014. The materials used for making crackers formulation were purchased from traditional market. All solvents used for analysis were of pro analytical grade.

Sample preparation: Rambak crackers were prepared by fresh skin from slaughter house such as cow, buffalo and pig. Skin used must be cleaned before frying. Skin was soaked overnight with composition of 1000 g skin, 400 g $CaCO_3$ and 5 L of water. The function of soaking is to make hide swelling and easily to unhearing. After soaking is complete, the skin is washed by running water until clean, no flavor and pH of 7. Subsequently, skin is boiled at 100°C for 2 h. After that, skin is cut into small size and steamed with flavor until 1 h. The skin is dried using sunlight dry for 2-3 days. The final product is ready to be frying process.

Extraction of lipid fraction from Rambak crackers: Rambak crackers were purchased from traditional market in Yogyakarta. Rambak crackers are smooth such as powder before Soxhlet

extraction. The extraction process involved the use of hexane as an extracting solvent as described by Association of Official Analytical Chemists, AOAC (1995). The lipid fraction yielded was further used for FTIR spectral measurement.

Calibration and validation samples: Sixteen fat samples extract from skin and rambak crackers have been used in this study. Sample of pig skin is mixed with buffalo skin and used to prepare calibration models with different level concentration, namely 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% of pig skin. Five independent samples which covers the whole range of concentration were used for validation. The lipid fraction obtained was scanned using FTIR spectrophotometer. The spectral regions where the variations were observed were chosen for developing calibration model.

Analysis using FTIR spectrophotometer: Lipids obtained are read by spectrophotometer in the mid infrared region (650-4000 cm^{-1}). This instrument is equipped with deuterated triglycine sulphate (DTGS) detector and KBR as beam splitter, with a resolution of 8 cm^{-1} and 32 scanning. After every scan, a new reference air background spectrum was taken. The ATR (Attenuated Total Reflectance) plate was carefully cleaned in situ using hexane twice followed by acetone and dried with a soft tissue before filling with next sample.

Statistical analysis and validation: The statistical analysis using chemometric was aided by software Horizon MB (Canada) for analysis Partial Least Square (PLS) and Principal Component Analysis (PCA). Calibration model was verified using leave one out technique. The values of Root Mean Standard Error of Calibration (RMSEC) and coefficient of determination (R^2) were used as the validity criteria for the calibration. While, Root Mean Square Error of Prediction (RMSEP) and R^2 was used for validity criteria of validation model (Paradkar *et al*., 2002).

RESULTS AND DISCUSSION

FTIR spectral analysis: In the analytical field, there were many principal techniques that have been successfully applied to detect and identify adulteration in food. Man and Mirghani (2001) have developed a Fourier-transform infrared (FTIR) spectroscopic method for detecting lard in mixtures with other animal fats, such as chicken, lamb and cow. The infrared spectroscopy have been widely used to determine fats. Lipid fraction obtained during Soxhlet extraction was analyzed using FTIR spectrophotometer at mid infrared region (4,000-650 cm^{-1}). The FTIR spectroscopy can be an ideal technique for analysis of lipids, due to its property as fingerprint technique allowing an analyst to differentiate among samples. The IR spectra can be used as means for identification (qualitative analysis) and quantitative analysis (Guillen and Cabo, 1997).

The importance of IR spectroscopy for the qualitative analysis comes from much information contents obtained and the possibility to assign certain absorption bands related to the functional groups. In fats and oils, most of the peaks and shoulders of the spectrum are attributable to speci c functional groups (Bendini *et al*., 2007). Figure 1 show FTIR spectra of lipid fraction extracted from Rambak cracker containing 100% buffalo skin (buffalo fat) and 100% pig skin (lard). Both spectra look very similar and show a typical absorption bands of edible fats and oils (Man *et al*., 2011). The assignments of major peaks and shoulders were shown in Table 1. Upon a closer scrutiny, the peaks at fingerprint regions (1500-1650 cm^{-1}) showed minor differences (peak heights), especially at wavenumbers of 1118 and 1096 cm^{-1} (assigned with j and k in Fig. 2) corresponding to the vibrations of C-H bending and C-H deformation of fatty acids, respectively.

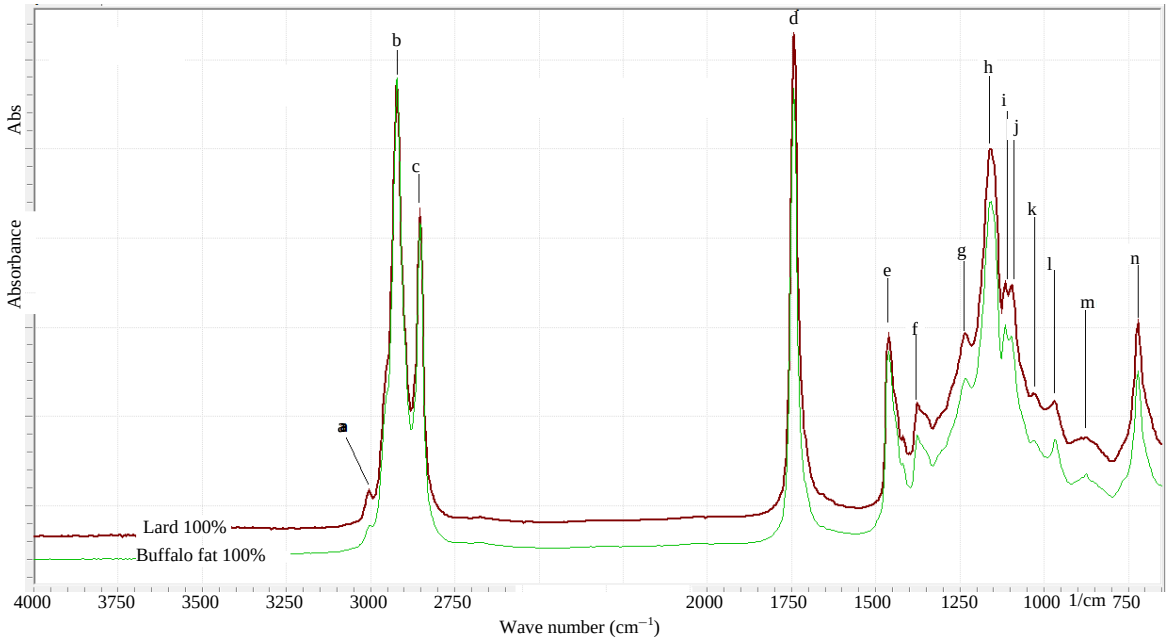

Fig. 1: FTIR spectra of lipid fraction extracted from Rambak cracker containing 100% buffalo skin (buffalo fat) and 100% pig skin (lard) at mid infrared region (4,000-650 cm^{-1})

Table 1: Model and functional group lard and buffaloes fat

Assignment	Wave numbers (cm^{-1})	Functional group responsible for IR absorption*
a	3.007	Cis-ole nic C = H
b	2970	-CH_3 stretching asymmetric
c	2925	-CH_2 stretching asymmetric
d	2875	-CH_3 stretching asymmetric
e	1715	-C = O carbonyl stretching
f	1650	Cis C = C
g	1462	-CH_2bending
h	1418	-CH rocking (bending) from cis-disubstituted alkenes
i	1375	-CH_3 bending
j	1226	-C-O (eter) stretching
k	1160	-C-O (eter) stretching
l	1.117	-C-O (eter) stretching
m	1.098	-C-O (eter) stretching
n	1031	-C-O (eter) stretching
o	962	= CH from isolated trans-ole n

*Vlachos *et al*. (2006)

Figure 2 showed the enlarged FTIR spectra at fingerprint regions. The different peaks in terms of peak intensity was used as a means for selecting the spectral regions for the quantification and classification of lard in rambak crackers samples.

Quantification of lard in rambak crackers: Quantification of lard and buffalo fat was carried out with the aid of multivariate calibration. The PLS were used to evaluate the relationship between actual value (x-axis) and value (y-axis). Absorbance of lard and buffalo fat with level concentrations from 0-100% was used as a calibration model. The PLS was used for making a relationship between actual and predicted values of lipid (%v/v) skin. Figure 3 shows the overlay spectra of lard mixed into buffalo fat at concentration range of 0-100.0% (v/v).

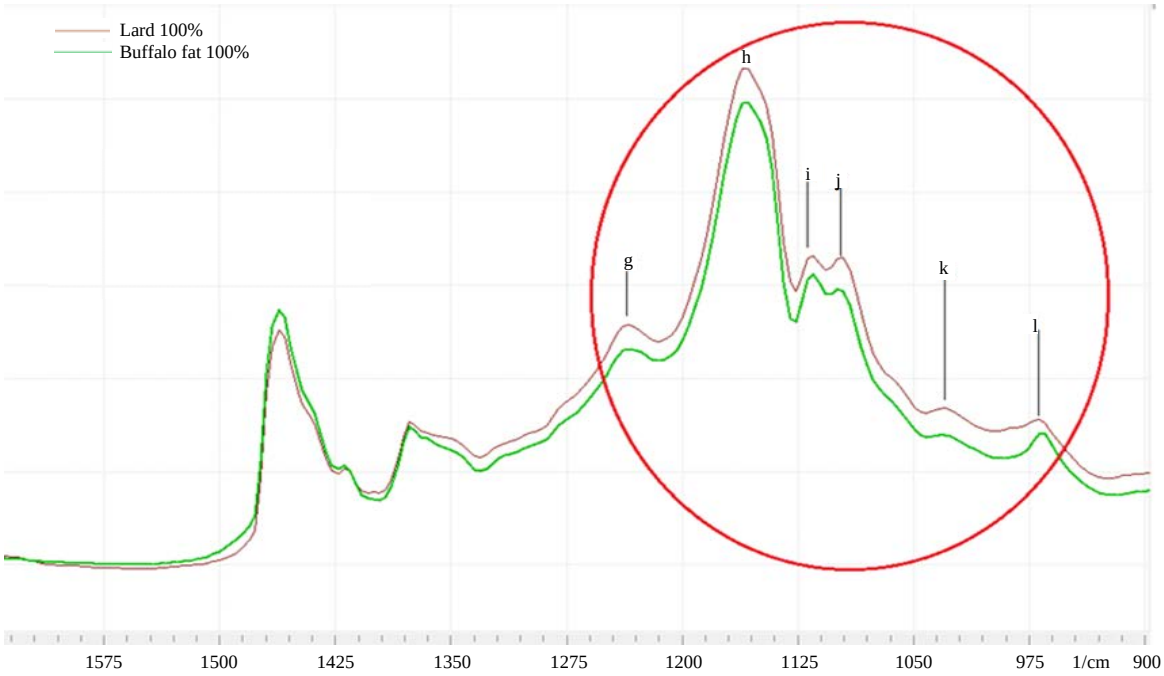

Fig. 2: Enlarged spectra for the differentiation of peak intensity in lard 100% and buffalo fat 100%

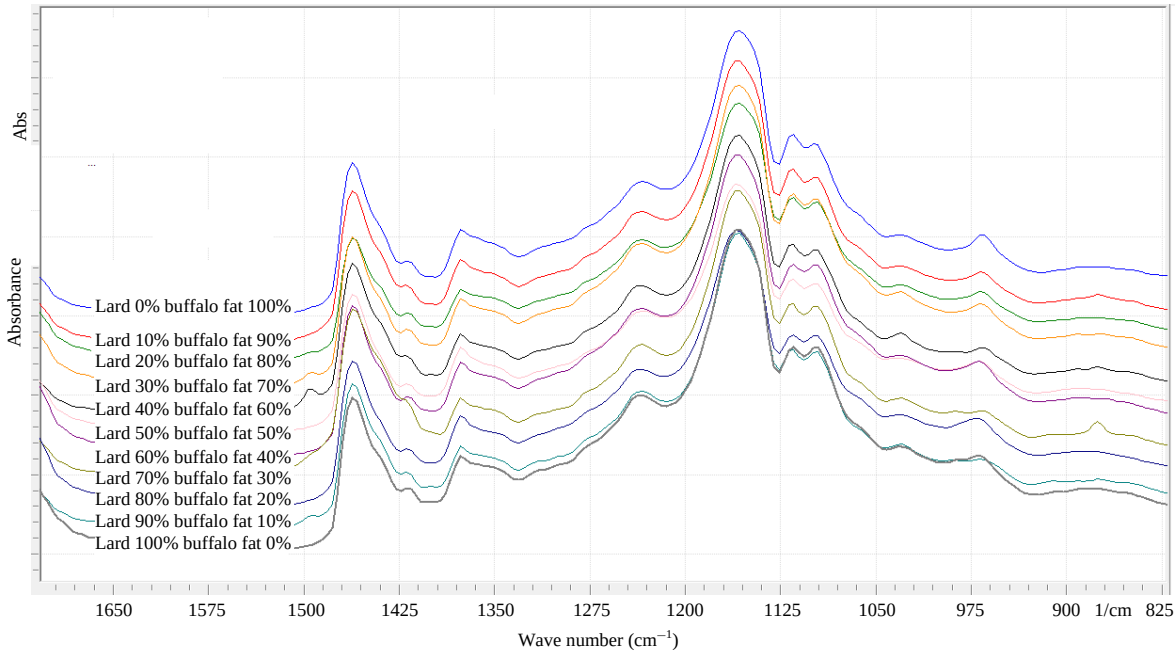

Fig. 3: Overlay spectra of lard mixed into buffalo fat at concentration range 0-100.0% (v/v)

Quantification of lard (lipid obtained from rambak crackers containing pig skin) in calibration and validation samples is performed with the aid of PLS. Some wave numbers are optimized in order to find the optimum wave numbers offering good correlation between actual value of lard and

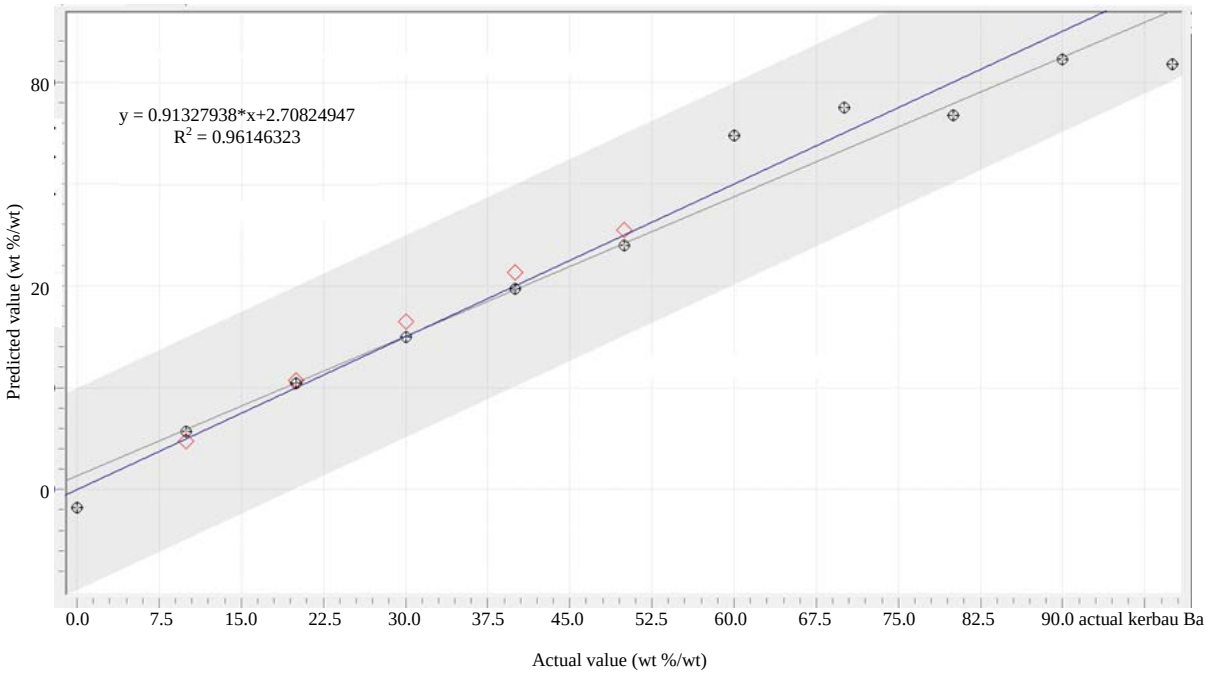

Fig. 4: Calibration model of PLS for the relationship between actual and FTIR predicted value of buffalo fat adulterated with lard using spectra 1200-1000 cm^{-1}

FTIR predicted value. Finally, we used wavenumbers region of 1,200-1,000 cm^{-1} for quantification of lard due to its capability to offer the best prediction model for the relationship between actual value of lard and FTIR predicted values. Besides, this wavenumber also offer the highest coefficient of determination (R^2) and the lowest values of errors in calibration (RMSEC) and prediction (RMSEP). Figure 4 exhibited the calibration model for the relationship between actual value of lard (x-axis) and FTIR predicted value (y-axis), as determined using multivariate calibration of PLS using normal spectra at wavenumbers of 1,200-1,000 cm^{-1}. The coefficient of determination obtained is high, i.e., 0.961, meaning that the calibration models can describe the accuracy of 96.1%. In addition, the calibration error expressed with RMSEC is low 2.56. The calibration model was further evaluated using validation or validation samples. The values of R^2 (0.994) and RMSEP of 1.10 were obtained. From this result, it is obvious that FTIR spectroscopy combined with multivariate calibration of PLS provide the accurate and precise results with high R^2 values and low errors (RMSEC and RMSEP values) for analysis of lard in rambak crackers.

The confirmation and validation of the analysis region used for developing the PLS model were performed by computing the Predicted Residual Error Sum of Squares (PRESS) values for different factors or Principal Components (PCs). The PRESS is a value direct measure on how well a calibration can predict the concentration left out during a cross validation (Smith, 2002), PRESS informed that the optimal factor number is 8, as revealed in Fig. 5, which illustrates how the RMSEC obtain a stable value, minimally after eight factor. This confirms that the spectral region used for developing the PLS model for the quantification of rambak significant correlation with it's concentration.

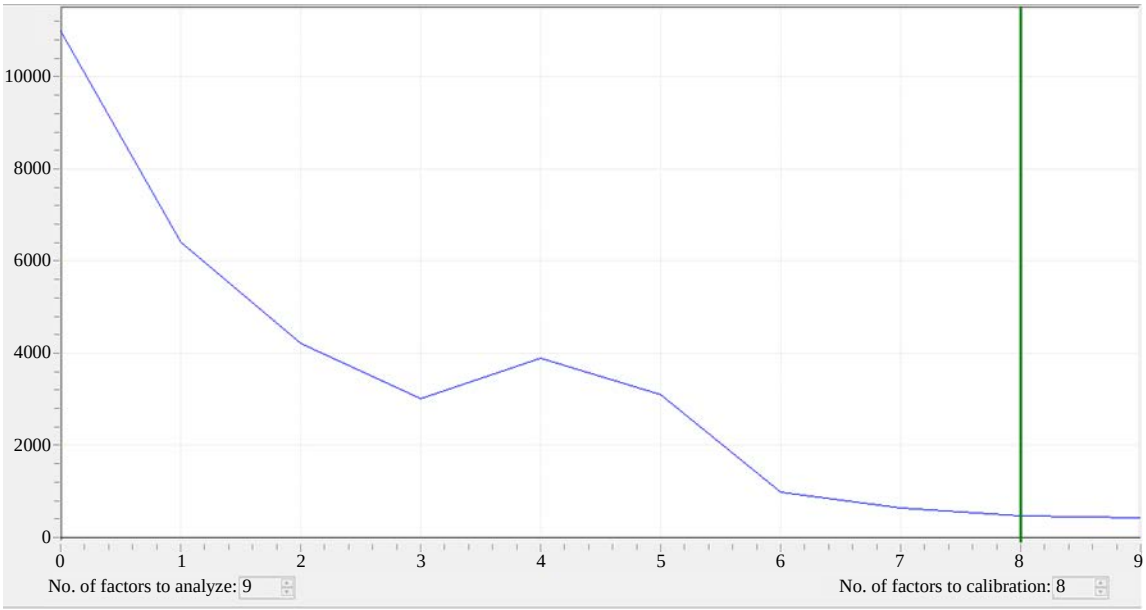

Fig. 5: Number of factor for modeling PLS Calibration

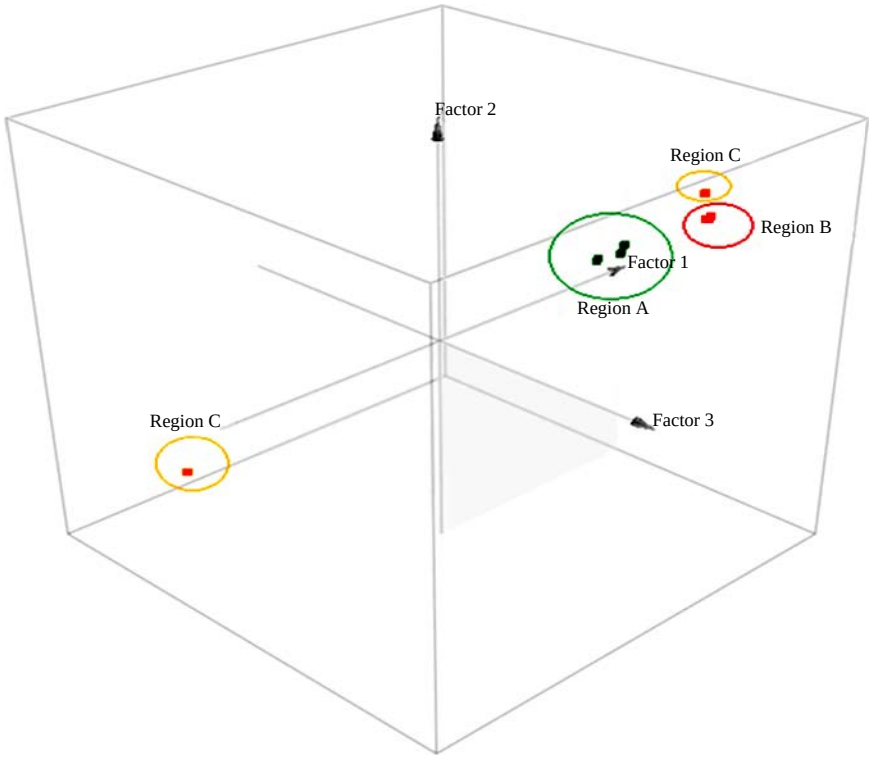

Fig. 6: PCA score plot (3 Dimension), expressed as first principal component (PC1) and second principal component (PC2) for classification of rambak with lard, buffalo and commercial sample

Classification of rambak crackers with pig skin and cow skin: Rambak crackers with lard and without lard were classified using chemometrics of Principal Component Analysis (PCA). The wave number regions for PCA were also optimized based on its capability to separate between pig skin and pig buffalo present in rambak crackers. The optimal wave numbers used for quantitative analysis (1200-1000 cm^{-1}), was chosen for PCA.

Figure 6 show result score plot of PCA of pig skin, buffalo skin contained in ramback. Principal component describing where the position of the sample. There is two principle component describing the projection of sample. First Principle Component (PC1) and the second Principle Component (PC2). Using this projection, rambak crackers containing pig skin, buffalo skin and commercial rambak crackers are well separated. This means that PCA can accomplish the classi cation among them. Based on this profile, it can be stated that commercial samples (region C) do not contain pig skin in the products.

CONCLUSION

The FTIR spectra combined with chemometric method are successfully used to classify and to quantify lard in rambak crackers at wavenumber regions of 1,200-1000 cm^{-1}. With the aid of Partial Least Square (PLS), the correlation between actual value of lard and FTIR predicted value has R^2 value of 0.961 with low errors in calibration and validation models. The chemometrics of Principal Component Analysis (PCA) can be successfully used for pig skin, buffalo skin and commercial rambak crackers.

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