Simple combination method of FTIR spectroscopy and chemometrics for qualitative identification of cattle bones

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Abstract. This research aims to identify Acehnese, Bali and Brahman cattle bones using Fourier-Transform Infrared (FTIR) spectroscopy combined with chemometrics through Principal Component Analysis (PCA). Cattle bone samples were obtained from Lambaro and Lampulo traditional market in Aceh Besar. Firstly, each bone sample was analyzed using FTIR and then followed by FTIR spectra analysis using PCA. FTIR spectra showed that inorganic samples produced from these cattle bones consisting of hydroxyl, carbonate and phosphate functional groups. Further inspection using the PCA plot, the cattle bones were separated into three groups with its difference of 90.03%. Three types of cattle bone (Aceh, Bali dan Brahman) are separated into different quadrants. Inspite of both Aceh cattle data are close each to others and are located in the same quadrant. This experiment proved that the cattle bones of Aceh, Bali dan Brahman can be well distinguished by using the combination method of FTIR and PCA.

1. Introduction

It known generally that Acehnese people highly consumed beef, normally at the special moment such Ramadhan, Eid Al-Fitr, and Eid Al-Adha. Most of Acehnese people consume the beef produced from

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the specific local cattle which was popular as Aceh cattle. However, based on the Central Bureau of Statistics (BPS) data, the beef production in Aceh was around of 9.137 tons in 2018, it was more lower than the market demands. Therefore, to fulfill the beef demand in Aceh, the different beef products such as Bali and Brahman beef cattle were supplied to Aceh market from other provinces as well as from foreign country like India.

Economically, the Aceh local beef has higher price than beef produced from Bali and Brahman cattle. This condition assist fraud activity by some beef traders in Aceh market, namely some merchants would label Bali and Brahman beef as Aceh local beef and sold it expensively. Physically, there is no different appearance among Aceh local beef, Brahmana and Bali beef products, so that its interesting for providing the simple identification method to differ those beef products.

Spectroscopy has been widely used to identify organic material such as coal and food with laser [1,2], fish meat and protein with Uv-Vis [3,4], bone with FTIR [5]. For this purpose, the FTIR spectroscopy has been noticed as an efficient, cheap, and easy method for chemical analysis, so that it has been also popular methods used to distinguish samples of organic material like fish and meat products [6]. Depending on the chemical composition of samples, the FTIR resulted specific spectra of the samples [7]. However in practical application, the FTIR existing weak point on interpreting data of the analysis process, namely it has complex spectra profile because the overlapping of the many absorption spectra come from the similar samples which leading the difficulty for visual interpretation of obtained FTIR spectra [8].

Chemometrics is the application of mathematics and statistics procedures to process, evaluate, and interpret data from chemical measurement. Recently, a combination of chemical measurement and chemometrics has been used to identify and authenticate medicinal plants classification, geographical origin, study of drug and protein interaction, grouping of chromatographic data, and detection of counterfeit material. The FTIR has been combined with chemometrics for distinguishing gelatin source between bovine or pork [9], distinguishing turmeric, temulawak, and bangle in plant identification[10], solubility process of bamboo powder [11] study of fatty acid and epidermal fatty acid binding protein interaction [12], grouping of similarity of index retention data [13] and distinguishing Carob from different geographical origin [14].

Principal Component Analysis (PCA) is mathematic technique to recognize frequently patterns without supervision based its frequently data, which making it possible to group samples into suitable categories with maximizing variance between categories and minimizing variance in group. Principal component analysis changes initial variable to new variable named Principal Component (PC). These components are linear combination from original variable with the first principal component having the largest variance, second principal component having second largest variance, and so on [10,15]. Information that could be retrieved from PCA are sample pattern, groups, difference, and similarity in which if there are two objects with similar characteristic, it will be portrayed as two dots in adjacent position. In PCA, new principal component (PC) score would prove the correlation between spectrums (or samples). Similar samples will appear as group in score plot, meanwhile different samples will appear separate from each one [9]. Based on information explained above, this research proposed for combining FTIR spectroscopy with PCA to differ the chemical of cattle bones found at beef traditional market in Aceh.

2. Methodology

2.1 Tools and materials

Tools used in this research are grinder, mortar, analytical scale, spatula, Furnace and FTIR Spectrophotometer, while materials are cattle bone from three different breeds: Aceh, Bali, Brahman. Cattle bone retrieval location can be seen in Table 1.
2.2. Bone preparation
The preparation method is adopted from previous procedure [10]. Cattle bone is washed and cleaned to remove dust, meat, fat, and cartilage attached to the bone. Next, cattle bone dried in the oven at 110°C for 1 hour. The bone then crushed with steel grinder and mashed up with mortar. Cattle bone powder sifted with 100 mesh sift. Then, cattle bone fine powder calcinated at 900°C for 4 h.

| Cattle bone type          | Sample label | Source                  |
|---------------------------|--------------|-------------------------|
| Aceh Cattle Bone          | Aceh 1       | Lambaro, Aceh Besar     |
|                           | Aceh 2       | Lampulo, Aceh Besar     |
| Bali Cattle Bone          | Bali         | Lambaro, Aceh Besar     |
| Brahman Cattle Bone       | Brahman      | Lambaro, Aceh Besar     |

2.3 Bone characterization
Prepared powder samples measured with *Fourier-Transform Infrared* (FTIR) spectrophotometer. The FTIR spectra were recorded as pellet sample with KBR using a Shimadzu IR-Prestige-21 spectrometers in the range of 400–4500 cm⁻¹. FTIR spectra data saved in Microsoft Office Excel format (.xls) to be analyzed chemometrically using Principal Component Analysis (PCA).

2.4 Data analysis
Before PCA analysis, the signal from FTIR spectra is processed beforehand to mineralized light dispersion effect and removing noise. Signal processing is carried out using baseline and SNV. Multivariate analysis from FTIR absorption data (*Excel file*) carried out with Principal Component Analysis (PCA). Software used in the analysis is XL Stat Software.

3. Results and discussion

3.1 *Fourier-transform infrared* (FTIR)
The FTIR spectra is useful to have entire bond information in a molecule based on its vibrational and rotational motion. This phenomena making FTIR spectroscopy very useful for qualitative analysis such as distinguishing samples. Particularly, FTIR spectroscopy was used to investigate the kind of functional groups existing the cattle bone samples. The profile of FTIR spectra obtained from four cattle bones in this experiment are shown in figure 1. It can be seen that there are overlappings and similarity between the spectra profiles of the bone samples resulted from FTIR analysis, so that it is difficult to use for distinguishing the bone samples.

The FTIR spectra of the calcined bones exhibited the characteristic absorption peaks come from hydroxyapatite compound. As it can be observed on FTIR spectrum analysis shown in Figure 1, those four cattle bone samples had similar functional groups, in which the peaks around 3500 cm⁻¹ corresponded to the stretching mode of vibrations of OH functional group. The band at frequency number of around 1400 cm⁻¹ shows the asymmetric stretching vibration between the carbon and oxygen (CO₂³⁻), while the band around 875 cm⁻¹ shows the asymmetric bending mode of vibrations for CO₂²⁻. The bands at a wavelength around 1070 and 980 cm⁻¹ indicate the asymmetric stretching mode of vibrations between phosphorus and oxygen in PO₄³⁻ group [16,17], as it is shown in Table 2. Based on the band characteristic of the FTIR spectra, the cattle bones resulted in this experiment was form as a hydroxyapatite compound (Ca₁₀(PO₄)₆(OH)) [18,19].
A

B

C

D

Figure 1. FTIR Spectra resulted from cattle bones of; a) Aceh 1, b) Aceh 2, c) Bali and d) Brahman.

Table 2. FTIR identification of Aceh, Bali and Brahman cattle bones.

| Functional Group      | Wave number (cm$^{-1}$) | Cattle bone Aceh 1 | Cattle bone Aceh 2 | Cattle bone Bali | Cattle bone Brahman |
|-----------------------|--------------------------|---------------------|---------------------|------------------|---------------------|
| Hydroxyl (OH)         |                          | 3566.36             | 3562.52             | 3566.38          | 3566.38             |
| Carbonate (CO$_2^-$)  |                          | 1442.75             | 1440.83             | 1460.11          | 1460.11             |
|                       |                          | 883.40              | 881.47              | 881.47           | 883.40              |
|                       |                          | 646.15              | 634.58              | 646.15           | 638                 |
| Phosphate (PO$_4^{3-}$)|                          | 1070.49             | 1062.78             | 1029.99          | 1076.28             |
|                       |                          | 991.41              | 996.27              | 983.70           | 985.63              |

3.2 Principal component analysis (PCA)

The main purpose of Principal Component Analysis (PCA) analysis is to find out a pattern similarity, or the difference between samples in a variable data. As mentioned in the introduction of this study, FTIR spectroscopy data combined with principal component analysis were used to identify and distinguish the three species of cattle bones. Before the obtained FTIR spectra combined with PCA, some typical pre-processing techniques of FTIR spectrum is necessary to be done for reducing or removing any systematic variation in the data. Furthermore, the obtained FTIR spectra was processing using PCA software, without compromising important information in total variance calculation. FTIR spectrum pre-processing is a standard procedure in chemometrics, signal processing is carried through baseline and SNV. FTIR spectrum pre-treatment using baseline dan SNV were applied for correcting the baseline, smoothing the data points and scaling the data before subjecting the spectra to PCA[20].
Figure 2 showed the results of PCA analysis of Aceh, Bali and Brahman cattle bones which used in this experiment. As can be seen in Figure 2, the examined samples were clustered into three different groups. The PCA results confirmed that the cattle bones has been classified successfully into three species based on different their cattle species. The PCA plot explained data variation of 90.03%, in which PC1 with 57.81%, and PC 2 with 32.22%. This meant that it is clearly showed that 90.03% of the original groups were correctly classified into its own group. In the grouping process of the data, the PCA reduced the spectrum variables to only the main variable and grouped the data into different groups depending on the variable information correlation. Data processing using PCA analysis had been separating and grouping three specific groups of the bone samples which labeled as Aceh, Bali, and Brahman. Based on PCA data, the similarity of two Aceh cattle bones can also be seen clearly. It was showed that both Aceh cattle data are close to each other and are located in the same groups. This result proved that the combination method of FTIR and PCA has been useful to distinguish bone samples from different breeds of cattle.

4. Conclusion
A combination method using FTIR and PCA is demonstrated successfully to identify and distinguish bone samples in its specific group as Aceh, Bali, and Brahman cattle bones. The difference between three cattle bones can be well distinguished and the similarity of two Aceh cattle bones can also be seen clearly. This combination method is needed to be continually investigated so that it can reliably validate other kinds of samples.

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