# 2D INFRARED CHARACTERISTIC SPECTRA PATTERN OF TYPE B PORCINE AND BOVINE BONE GELATINE IN LOW SCAN

Himmatul Barroroh[1], Abdul Hakim[1]

[1]Chemistry Department, Faculty Science and Technology
UIN Maulana Malik Ibrahim Malang
Jl. Gajayana No.50 Malang

*Abstract* - The use of second derivative method (2D) in the FTIR spectroscopy data processing is often done with the use of a large number of scans. The use of a large number of scan makes greater operating expense, it is not expected. Therefore in this study will be assessed the quality of 2D infrared characteristic spectra pattern of type B gelatin from porcine and bovine bones from spectral data with low scan. This study is part of efforts to find halal food identification methods, especially gelatin, in fast, accurate and economical.

The sample used is of type B gelatin from porcine and bovine bones are prepared in the laboratory, replication is of 10 data respectively. Infrared spectral data were collected using a Varian 1000 FTIR spectrometer in the wavenumber range 4000-700 $cm^{-1}$, a resolution of 4 $cm^{-1}$, scan 20. The second derivative used Fourier techniques.

The results showed that the 2D infrared characteristic spectra pattern of type B gelatin from the porcine and bovine bones that appear in the wavenumber of 730-740 $cm^{-1}$ and 1030-1080 $cm^{-1}$ can still be identified even though the pattern is clearly disturbed by the appearance of noise. Amplitude of the second derivative curve of value less than 0.01% $T/cm^{-2}$ determined as a noise.

## 1. Introduction

Data processing by using derivative spectroscopy has been conducted since the 1970s. These methods continue to develop until nowadays. Delwiche, et al. Has successfully measured the amount of protein and β-conglicinin glicinin found in soybean seeds using Near Infra Red Spectroscopy (NIR), to the limit screening. Earlier this protein commonly separated by ultracentrifugation and electrophoresis [2].

Has also developed a new rapid quantitative measurement method of *trans* fatty acids through the measurement of the height of the infrared absorption band *of trans* fatty acids at 966 $cm^{-1}$ using the second derivative (2D) with scan number of 256. This method succeeded in identifying and separating of interference bands in the 962-956 $cm^{-1}$ band belongs to the saturated fat onto *trans* fatty acids band at 966 $cm^{-1}$. The successful separation of the interference bands can increases the sensitivity and accuracy of the determination of *trans* fatty acids at low concentrations (≤ 0.5% of total fat) [5].

Typical peak of mutton body fat and lard IR spectra have been identified [3]. In other previous research has found the characteristic pattern of the second derivative FTIR spectra of beef and pork at wave number 1680-1695 $cm^{-1}$ and 1075-1090 $cm^{-1}$ generating spectra performed with the number of scans 256 [1].

Gelatin is a food additive that is used widely in the community. Gelatin which is currently distributing in Indonesia is still one hundred percent of products imported from Europe, China and several other countries. The number of imports is up to 2000-3000 tons per year. BPS Data 2007 mentions, gelatin imports reached 2,715,782 kg with a value of 9,535,128 U.S. dollars. While 44.5 percent of the gelatin of the world (136,000 tons) derived from pig skin, and then 27.6 per cent (84,000 tons) of cow skin and bones from 26.6 percent (81.6 thousand tons) and the rest comes from others (1.3 percent or 4,000 tons). Therefore there is a possibility that distributed gelatin in community is pig-base gelatin that is haram consumed by Muslims.

As one embodiment of the implementation of worship that is fardlu kifayah that one of them is to realize clearly halal status of food for Muslims, especially at the level of identification of the presence of pig-base food, then the research on distinction test methods of pig-base gelatin and the other-base gelatin is required. It becomes a challenge for Moslem chemists to be able to find any distinguishing characteristic of pig-base gelatin and gelatin made from other, so it can be used as markers.

Gelatin is the result of collagen partial hidrolisys of certain parts of animal bodies such as cartilages, bones, tendons, and skin. In terms of physical appearance, gelatin is a solid substance, from colorless to slightly yellowish in color and almost no taste and smell. Collagen is the major structural protein found in skin and bones of animals. Collagen molecule consists of three polypeptide chains (chain-α), which is in a triple helix conformation. Triple helix

is stabilized by hydrogen bonds between two molecules of collagen, which occurs as the increasing age of the animal. Each collagen molecule with 3 alpha-chains has a size of 3000 Å long (0.3 micron) with a diameter of 15 Å. Each α-chain has approximately 1,050 amino acids that bind to one another. Gelatin is very rich in amino acids glycine (Gly) (almost one-third of the total amino acids), proline (Pro) and 4-hydroxyproline (4Hyd). The common gelatin structure is: - Ala-Gly-Pro-Arg-Gly-Glu-Gly-4Hyd-Pro-. Gelatin is deficient in tryptophan (Trp), and low in sistein (Cys) and tyrosine (Tyr). In general there are two types of gelatin. Gelatin is obtained after the acid treatment will have isoeletrik point between pH 6 and 9. This gelatin was classified as Type A. In contrast, gelatin produced by alkaline treatment is known as Type B. Type B gelatin has an isoelectric point in between 4.7 to 5. Type A Gelatin usually is specifically produced from pig skin, whereas type B gelatin is produced from cow leather, goat and buffalo, or of the bones of these animals which have been demineralised [4].

## *2. Materials and Method*

In this study, the research object is of type B gelatin made from porcine and bovine bones. The samples prepared in the laboratory. For better reproducibility, sampling was made of 10 times for each kind of gelatin, each of which the raw material of bone is taken from a different place and time. The research design used was completely randomized design. FTIR spectra analysis of the data obtained processed by making the second derivative of the spectral curve, called the *Second Derivative* method (2D), this method will sharpen and enhance spectral peak resolution. Material from this research is porcine and bovine bones obtained in the market. Materials for solid pellet maker of FTIR instrument is KBr. While the equipment used is Simadzhu FTIR spectrometer. FTIR spectra were collected in the wavenumber range of 4000-700 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ scan performed 20 times. As background reference material used ambient air.

Porcine and bovine bone samples processed into gelatin using traditional methods commonly used in the community, complete technique is taken from www.iptek.net.id . Dry gelatin which has been obtained mashed, and can be made in the form of pellets directly to the identification of infrared spectra. Spectral data collection and the second derivative processing used the help of software from Varian Resolution Pro.

## 3. Result and Discussion

In general, the FTIR spectra of porcine and bovine gelatin have the same pattern as shown in Figure 1. In the wavenumber region in between 2000-4000 $cm^{-1}$ both have an identical pattern of peaks. Peaks at around wavenumber 3200-3500cm $^{-1}$ are broadened. This wavenumber range is associated with stretching vibrations of -OH to the typical width, and a slightly tapered double peak at 3300 $cm^{-1}$ wave number associated with the-NH stretching vibration. Both of these peaks can be thought to have come from the -OH and -NH group of the amino acid of protein constituent of gelatin. Sharp peak at around 2900-3000 $cm^{-1}$ is the vibrational spectra from CH stretching. This peak is commonly present at hydrocarbon compounds and biomolecules.

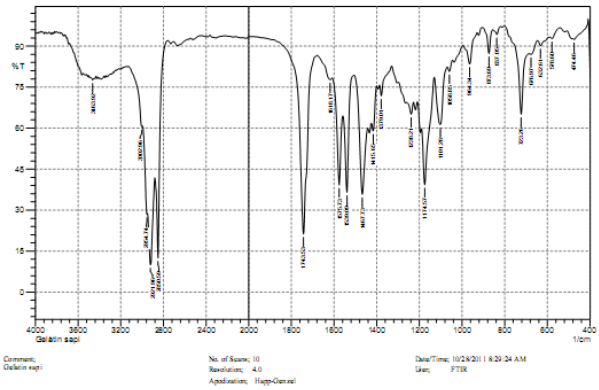


Figure 1. FTIR spectra of bovine gelatin

Both bovine gelatin and porcine gelatin in general have a similar pattern but did not really identical at wavenumber range below 1700 $cm^{-1}$. Peaks at wavenumber region in between 1000-1700 $cm^{-1}$ are usually derived from the bending and the overtone vibration of functional groups. In this area it is possible to find characteristic spectra peaks of each of the different samples. Similarly, the range of 400-1000 $cm^{-1}$ is very characteristic for each of the different compounds, so this area is referred to as the fingerprint region. The specificity of vibrational spectra on the fingerprint region is because the peaks are formed from the breathing vibration of the functional group or molecule as a whole. This resulted in the same group but with different environments and different arrangement of atoms in different molecules will give a different vibrational frequency. Therefore, further detailed analysis of the spectra related to the characteristic porcine and bovine gelatin is projected to be obtained by analyzing the wavenumber region below 1700 $cm^{-1}$ using Second Derivative method.

### 3.1. The Second Derivative IR Spectra

Second Derivative method will essentially increase the resolution of spectra, it will separate the peaks are overlapping. But the use of this method should be done carefully, or it would be able to give a biased analysis results. To the original spectra data which contains noise, it's possible that the noise peaks will be more noticeable when the curve is converted to the second derivative. This seems to occur in our sample. Scan number of FTIR spectra generation in this study is 20, the number of this scans as much as the commonly used in commercial FTIR analysis laboratory. Therefore, the spectra in this study provide a challenge in terms of noise and peak separation. The presence of noise can be seen on the example of Figure 2.

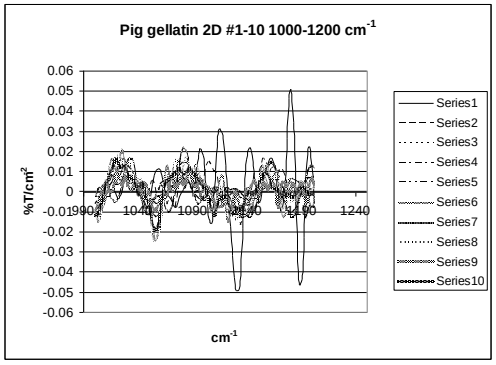


Figure 2. The second derivative FTIR spectra of porcine gelatin at 1000-1200 $cm^{-1}$, the series 1-10 are a repeating sample.

In the wavenumber of 1000-1200 $cm^{-1}$, ten spectra were indeed having 3 valleys and 3 peaks, but there are subtle peaks on each of which is the noise. The noise comes from a non-constant voltage during scanning process.

The second derivative curve made directly from the % transmittance spectra data. Similarity and differences of the 2D peak based on the peak wavenumber position rather than the peak intensity. This is because of the position of peaks wavenumber shows the different vibrational frequencies. Different frequency value is a manifestation of different vibration of a group or a cluster of molecule or molecule as a whole. While the peak intensity shows the relative amount of absorption that indicates the relative amount of the abundance of a group or a cluster of molecule or molecule as a whole which has a vibrational mode that has a certain frequency.

Through the analysis of second derivative on the entire sample of porcine and bovine gelatin there are obtained characteristic peaks for porcine and bovine samples at wavenumber of 730-740 $cm^{-1}$ and 1030-1080 $cm^{-1}$.

### 3.2. Peak and Noise Identification on the Characteristic Second Derivative FTIR Spectra Pattern of Porcine and Bovine Gelatin

The characteristic pattern of the second derivative FTIR spectra of porcine and bovine gelatin showed at Figure 3 to 6.

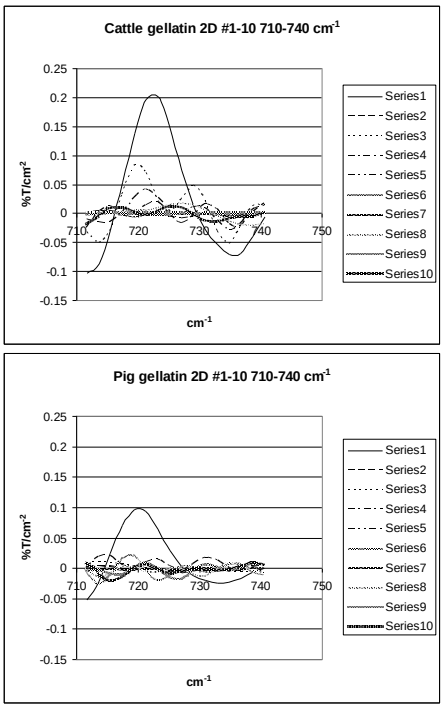


Figure 3. Characteristic second derivative FTIR spectra pattern of porcine and bovine gelatin at 710-740 $cm^{-1}$

There is a different characteristic pattern of the second derivative FTIR spectra of porcine and bovine gelatin at the wavenumber range of 710-740 $cm^{-1}$. In this wavenumber range the bovine gelatin second derivative FTIR spectra curve has two peaks and a valley consistently. Of 10 replicates there is only 1 anomaly data. While, in this wavenumber range the porcine gelatin has no any consistent form of the curve. It seems that the variations curve of second derivative FTIR spectra of porcine gelatin in this wavenumber range are derived from noise.

Detail inspection to comparison of the transmittance curve and the 2D curve at wavenumber range of 710-730 $cm^{-1}$ indicates that the bovine gelatin has two transmittance peaks that are at 720 $cm^{-1}$ and 730 $cm^{-1}$, while the porcine gelatin has no any transmittance peak in the range of this wavenumber. From visual observation on comparison of %T and 2D curve, shows that the amplitude of peak or valley that is smaller than 0.01 $\%T/cm^{-2}$ on the 2D curve is not associated with a certain peak in the transmittance curve, it's mean can be ascribed to noise.

Peak at wavenumber of 710-740 $cm^{-1}$ located at finger print region so possibly it is of a breathing

vibration of a molecular fragment. This peak has a medium intensity, can be derived from the CH rocking vibration, or CS stretching on methyl-sulfide or aliphatic disulfide. This area can also be overlap with $NH_2$ deformation vibrations.

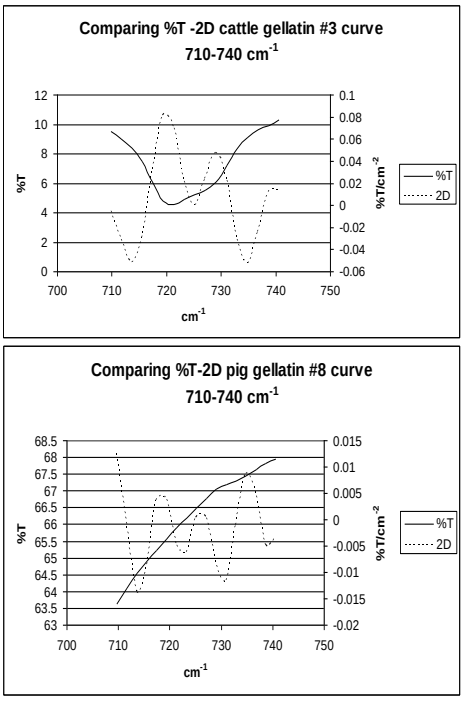


Figure 4. Comparison of %T-2D curves of porcine and bovine gelatin at 730-740 $cm^{-1}$.

The second characteristic peak in the pattern of the second derivative FTIR spectra of porcine and bovine gelatin lies in the range of 1030-1080 $cm^{-1}$. In the second derivative spectra of bovine gelatin appears that there is one peak and two valleys significantly. While the second derivative spectra of porcine gelatin shows only one valley. Noise curve interfere the peak curve significantly in this area.

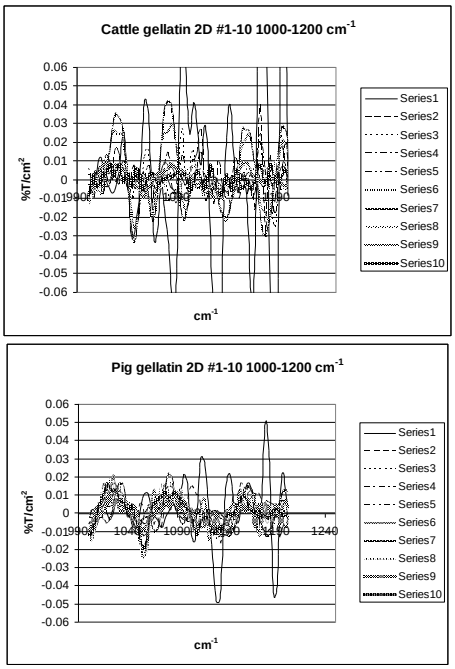


Figure 5. Characteristic pattern of the second derivative FTIR spectra of porcine and bovine gelatin at 1030-1080 $cm^{-1}$

Detailed inspection of the region 1030-1080 $cm^{-1}$ indicates that, precisely at 1060 $cm^{-1}$, there is a transmittance peak on samples contained bovine gelatin while on porcine gelatin does not exist, as can be seen in Figure 6. Visual observation on comparison of %T-2D curves shows that the amplitude of peak or valley that is smaller than 0.01 $\%T/cm^{-2}$ on the 2D curve is not associated with a certain peak in the transmittance curves, it mean can be ascribed as noise.

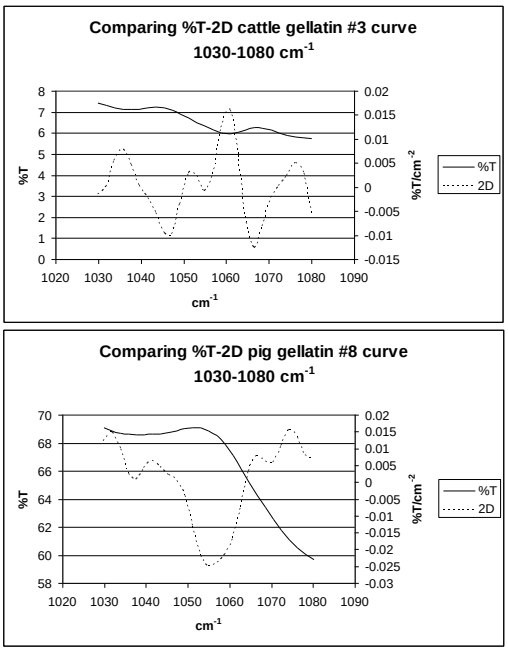


Figure 6. The comparison of %T-2D curves of porcine and bovine gelatin at 1030-1080 $cm^{-1}$

The peak of this area is included in the finger print area so that the vibrations of molecular fragments can be rather complex. The peak is medium to strong (ms), can be derived from stretching vibrations of COC of saturated aliphatic carboxylic acid, or aromatic ring vibration.

## 4. Summary

From these results it can be concluded that in the low-scan FTIR spectra, it can be identified characteristic patterns of the second derivative FTIR spectra of porcine and bovine gelatin in wavenumber of 730-740 $cm^{-1}$ and 1030-1080 $cm^{-1}$. Noise is identified if the amplitude of the second derivative spectra curve is less than 0.01% T /$cm^{-2}$.

## REFERENCES


[1 ] Barroroh, H., 2009, **Identifikasi Pola Spektra Infra Merah Khas protein Daging Sapi dan Babi Menggunakan Metode *Second Derivative* (2D) ,** Research report, Lemlitbang UIN Malang.

[2] Delwiche, S.R., Pordesimo, L.O., Panthee, D.R. and Pantalone, V.R. , 2007, **Assessing Glycinin (11S) and β -Conglycinin (7S) Fractions of Soybean Storage Protein by Near-Infrared Spectroscopy** , Volume 84, Number 12 / December, 1107-1115.

[3] Jaswir, Irwandi, 2006, **Metode Cepat Analisa Lemak** Babi **dengan FTIR,** www.beritaiptek.com

[4] Jaswir, Irwandi, 2007, **Memahami Gelatin** , www.beritaiptek.com.

[5] Mossoba, M.M., Kramer, J.K.G., Milosevic, V., Milosevic, M., and Azizian, H., 2007, **Interference of Saturated Fats in the Determination of Low Levels of *trans* Fats (below 0.5%) by Infrared Spectroscopy** , Journal of the American Oil Chemists' Society, Volume 84, Number 4 / April, 339-342.