PORK AND BEEF PROTEIN
INFRA RED CHARACTERISTIC SPECTRA PATTERN IDENTIFICATION
USING SECOND DERIVATIVE (2D) METHOD

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ABSTRACT

Fast and cheap qualitative identification method of pork contamination on beef had been develop using second derivative (2D) technic of pork and beef FTIR spectra. The sample include: pure beef, pure pork, and prok-beef blended meat of concentration 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% (b/b pork). FTIR spectra collected using Varian FTIR Spectromoter 1000. The second derivative was generated by Fourier technic. The result showed that there were two characteristic molecular vibration peak of pork rised from infra red second derivative spectra on 1680-1695 cm^{-1} and 1075-1090 cm^{-1}. The least pork concentration contamination on beef stil detected by this method was up to 0.1% (b/b). Two kind characteristic molecular vibration of pork protein on 1680-1695 cm^{-1} due to general amide C-O stretching (1670-1690 cm^{-1}) and R-S-CO-N group C-O stretching (1700-1680 cm^{-1}) of peptide bond. Meanwhile, the two kind characteristic molecular vibration of pork protein on 1075-1090 cm^{-1} due to primary aliphatic amine C-N stretching with secondary α-carbon (1090-1065 cm^{-1}) and tertiary α-carbon (1140-1035 cm^{-1}).

Key word: infra red spectra, second derivative, beef, pork, protein.

INTRODUCTION

Food products containing pork ingredients can be identified through the fat, protein or DNA. Several methods have been developed to test the presence of pork

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contaminants in food products through the identification of various typical proteins include the method of High Performance Liquid Chromatography (HPLC) (Boes, 2000) and Electrophoresis (Aning, 2005), while pig DNA testing should be done with the help of PCR equipment (Protein Chain Reaction) (Boes, 2000). This method is fairly reliable for identifying the presence of pork contaminants in a variety of food products to the extent of contamination above 5% (Boes, 2000), while the electrophoresis method is good enough for contaminant above 50% (Aning, 2005). PCR method for identification of DNA can be more accurate at a low level of contamination, but the cost is very expensive. HPLC method is still cheaper than DNA testing method, but still quite expensive, while the electrophoresis is inexpensive, but it is quite complicated and time-consuming and require a lot of chemicals.

High performance liquid chromatography (HPLC) was used for protein analysis of raw pork mixed, qualitative analysis of the specific component which is owned by the pig has only relative retention time from 1.74 to 1.77. HPLC method was developed to analyze the mixture accurately until 5% pork contamination. The cooked meat can be analyzed through DNA testing that has been amplified by PCR with electrophoresis techniques. The amplified DNA of pigs and pork- beef mixture sample showed one band that has a size of about 2 kb, whereas cow's showed no clear bands on agarose gel (Boes, E., 2000).

There has also done research analysis of pork proteins using SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis) with discontinuous buffer system. From the results was found several protein bands which became distinctive protein bands. Raw pork found in distinguishing protein bands that are not found in beef in Rf 0.0885, 0.1435, 0.296 and 0.6825 with a molecular weight of 54.71 kD, 46.64 kD, 29.96 kD and 9.76 kD, respectively. While the cow found in distinguishing protein bands that are not found in pigs in Rf 0.0965 with a molecular weight of 53.46 kD and Rf 6.42 of 0.827 kD molecular weight. To a mixture of beef and pork with a ratio of 50:50% have not seen any difference in protein band appeared (Purwaningsih, A., 2005).

Efforts to identify pork meatball (bakso) through characterization of protein fractions by Using SDS PAGE was also carried out by research groups LKTI PIMNAS 2005 winner. The samples studied were: fresh pork and beef; stewed pork and beef in their respective temperature of 90 degrees Celsius for 15 minutes; meatballs with content 100% of beef; meatballs with 25% of pork + 75% of beef; meatballs with 50% of pork + 50% of beef, and last is meatballs with 100% of pork. The results showed that in fresh pork there are unknown proteins with molecular weight of 112.13 kDa that does not exist in samples

of fresh beef. Heating at a temperature of 90 degrees Celsius for 15 minutes caused a decrease in the thickness of the ribbons of protein in each sample. Boiled pork has specific characteristic of the presence of desmin protein which was not detected in boiled beef. The next difference is the absence of a protein tropomiosin on boiled pork, but these proteins were detected in beef. Furthermore, specific differences in beef meatballs is the presence of troponin T protein contained in large amounts, whereas at the level of mixing pork meatballs beef at 25%, 50% and 100% of these proteins were detected slightly. Thus the existence of mixing pork meatballs can be seen on the thickness of the level of troponin T protein bands decreased with the increase in the amount of pork added (Susanto, E. et al, 2005).

The presence of specific proteins on pork that does not exist in other animal flesh, giving the possibility that the presence of a typical molecular vibration properties of pork proteins which do not exist in other animal protein. This hypothesis brings us to the possibilities of pork protein identification using FTIR method. Delwiche, et al., (2007) have succeeded in measuring the amount of protein and β-conglicinin glicinin contained in soybean seeds using Near Infra Red Spectroscopy (NIR), to the boundary screening. Previously, these proteins usually separated by ultracentrifugation and electrophoresis methods. The new method for pork fat identification has been developed since 2003 using FTIR method, found the typical CH stretching vibration at samples of lard different to other animal fats (Jaswir, 2006). There has also developed a rapid quantitative method of measuring the new trans fatty acids through measuring the height of trans fatty acid absorption band at 966 cm-1 using the second derivative method (2D). This method succeeded in identifying and separating the interference bands at 962-956 cm-1 belongs to saturated fat trans fatty acids in the band at 966 cm-1. The success of the separation band interference can increase the sensitivity and accuracy of the determination of trans fatty acids at low concentrations ($\leq$ 0.5% of total fat) (Mossoba, et al, 2007). Therefore this research will study the typical protein molecular vibrations of beef and pork from FTIR spectral data with the help of data analysis using the Second Derivative (2D).

RESEARCH METHODOLOGY

In this study, the research object is a raw pork and beef, both in a pure state as a control and in a state of mixture of several variations of concentration. Pork contamination concentrations in beef consists of: 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1% (in weight percentages). Analysis of

FTIR spectra data obtained was done further by making the second derivative of the spectral curve, called Second Derivative method (2D). Variation of concentration of beef and pork mixture are made up on a small enough value to determine the detection limit of the method used.

a. Equipment and Materials

Material from this research is beef and fresh raw pork obtained on the market. Solid ingredients to make pellets for FTIR instrumentation is KBR. While the equipment used is a Varian 1000 FTIR Spectrometer. Tools optic include Michelson interferometer with a mirror covering the air motion, beam-splitter KBR, and deuterated triglycerin sulfate detector (DTGS). FTIR spectra were collected in the range of wave numbers 4000-700 cm^{-1}, at a resolution of 4 cm^{-1}, a scan done as much as 256 times which was then summed and averaged. As background reference material used ambient air.

b. Research Methods

All the samples used in the fresh raw state. The sample mixture of beef and pork made by homogenize the mixture using a blender at room temperature, to avoid protein denaturation. Plate (pellets) prepared by KBR to grind samples (0.1 to 2% by weight) with potassium bromide (KBR) in an agate mortar to reduce impurities that absorb IR radiation and then inserted into pellet sample maker and then in vacuum to remove water. The sample was pressed for about 10 minutes at a pressure of 80 torr (8 to 20 tons per unit area). Spectral data collection and making the second derivative curve using Fourier Differentiation techniques with the help of Resolution Pro software from Varian Inc.

RESULT AND DISCUSSION

Transmitan spectra of all outline sample has a pattern similar to one another, can not conclusively identified the difference. When spectra are transformed in the form of absorbance, they still provide almost the same pattern for all samples. But when the spectra derive twice so that the derivation is obtained the second derivatives, the peaks of the resulting spectra become progressively increase. It can be seen as shown in Figure 1. For example, in the wave number region between 1680-1690 cm-1, the absorbance spectra showed no peaks, the spectra only appear as a straight line, but on second derivative spectra, the straight-line curve is separated into more than one peak.
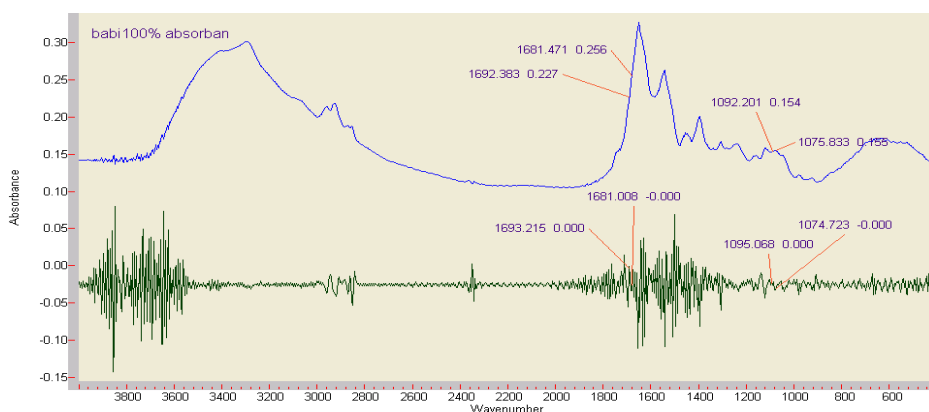
Figure 1. Transformation of spectra of pure samples of pork (a) absorbance spectra (b) 2nd derivative spectra.

In general all the infrared spectra (supplemented material) samples showed a similar pattern, typical peaks of functional groups can be observed in the region above the wave numbers 1000 cm^{-1}. At around 3400-3500 cm^{-1} there are two broad peaks, this is a typical form of basic stretching vibrations of NH of the peptide. Peak sharply at 1650 cm^{-1} is also typical for the vibration peak of C = O carbonyl sretching. Both these peaks are typical constituents for a peptide bond of proteins. Viewed from the generality of spectra patterns in the region 1000-4000 cm^{-1} and the presence of two peaks is the basic two functional groups, providing assurance that the spectra that are typical spectra of proteins.

a. The second derivative IR spectra

General description of the second derivative of the infrared absorbance spectra, all samples are given as in Figure 1. As a general description of transmittan spectra, second derivative spectra also show a similar trend. More detailed information will be extracted by dividing the discussion of the second derivative curve into three regions the wave numbers are: 2000-4000 cm^{-1}, 1000-2000 cm^{-1}, and 400-1000 cm^{-1}. This division into three regions based on the specificity of information that can be extracted from each region, where the region 2000-4000 cm^{-1} provides basic information about stretching vibration for a simple functional group, such as OH, NH, C = O and others. Wave number region 1000-2000 cm^{-1}, presumably represents the bending vibration region for functional group and functional group that interact with their environment. While the area under the wave numbers 1000 cm^{-1} is a finger print which provides for the breathing vibration spectra of molecules as a whole. Finger print region spectra tend to be very complicated but typical for each material.

Spectra of all samples generally show the same peak and are identical in most wave numbers, especially on the wave numbers of functional group on the basis of the group NH 3500 cm^{-1}, CH at 3000 cm^{-1}, and C = O carbonyl at 1700 cm^{-1}.
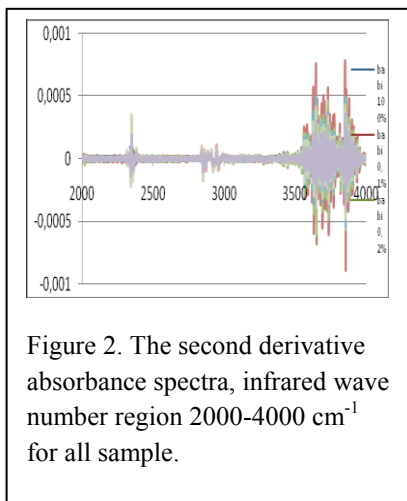

Figure 2. The second derivative absorbance spectra, infrared wave number region 2000-4000 cm^{-1} for all sample.
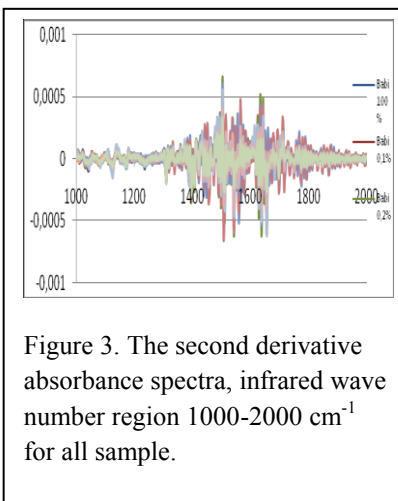

Figure 3. The second derivative absorbance spectra, infrared wave number region 1000-2000 cm^{-1} for all sample.

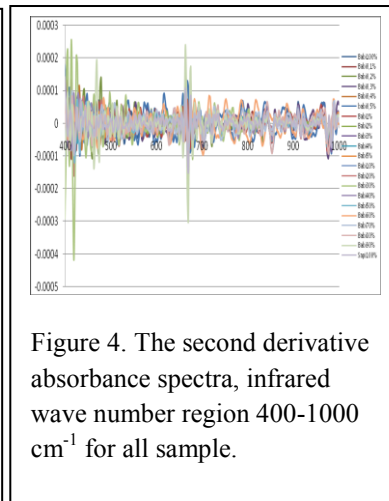Figure 4. The second derivative absorbance spectra, infrared wave number region 400-1000 cm^{-1} for all sample.

Wave number region 2000-4000 cm^{-1} the second derivative of the infrared absorbance spectra for all samples gave identical peaks, with the positions of all peaks are located at exactly the same wave numbers, there is no shift in wave numbers, although the intensity of each sample could be different. This indicates that the functional groups for all samples of raw meat, good meat beef, pork or a mixture of both is the same and have the same cluster environment. Noise section also provides a profile that is identical for all samples, whether beef, pork or pork and beef mixture. This shows that it can not be identified the specific vibrational protein of beef and pork at the level of group function. This phenomenon can be understood because all proteins are composed of bricks of amino acids all have the same functional groups include NH and C = O in the peptide bond, CH in the remaining portion of the molecule, and some of the carbon with hetero atoms other. The identification is then performed on the wave number region 1000-2000 cm^{-1}. Found that nearly all peaks are also identical, are on the same wave numbers, there is no peak shift. Typical peaks of functional groups can still be found in the 1700s the area belongs to the vibrations of C = O carbonyl. When traced in more detail turns out there are two distinct peak regions between beef samples with samples that contain pork or pork. This area is found at wave numbers 1680's and 1085's. Identification of specific protein spectra of pork and beef will be done in this area.

The second derivative absorbance spectra for the entire sample finger print area as shown in Figure 4. Although on the absorbance spectra transmitan and finger print region

is rather similar from one sample with another sample, it turns out after the derivation of the spectral absorbance twice, no one has any sample pattern and the position of the same peaks. Each sample has a pattern and a typical peak position. This shows that at the molecular level, in this case the protein is a macromolecule, each sample has the composition or structure and molecular environment that is not the same. But because there is no synchronized, there must, then the finger print region can not be used as a reference for identifying the typical infrared spectra of the pig protein.

b. Peak characteristic of the second derivative IR spectra at wave numbers 1680-1695 cm^{-1}

Identification of the characteristic peak of second derivative IR spectra of pork, beef and a mixture of both can be seen in Figure 5, 6, and 7. Description of second derivative spectra was divided into three groups to eliminate the hassle because the accumulation of spectra, that is pure beef and pork as a reference and a mixture of beef and pork with the concentration: (Figure 5.) 1%, 2%, 3%, 4%, and 5%, (Figure6.) 10%, 20%, 30%, 40% and 50%, (Figure 7.) 60%, 70%, 80% and 90%. At wave numbers 1680-1695 cm^{-1} there are two peaks for the samples containing pork, either pure or mixed with beef, but on pure beef on the wave number there is only one peak. Exceptions occur in pork-beef mixture with pork concentration 60% (w / w). This sample profile on the wave number 1680-1695 cm^{-1} has only one peak, the profile resembles the profile spectra of pure beef. Of the 20 samples there is a deviating sample, so the error can be estimated to have amounted to 5% of a typical accuracy of peak identification of pork infrared spectra at wave numbers 1680-1695 cm^{-1}. This allegedly caused inaccuracies of unhomogenity meat mixture sample, the possibility of sample drawn to measure infra-red spectra, coincidence of the meat that contain only beef.
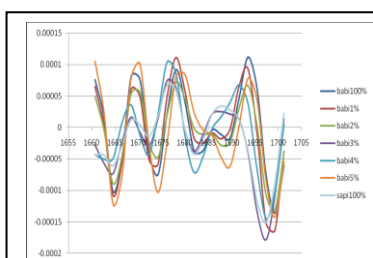
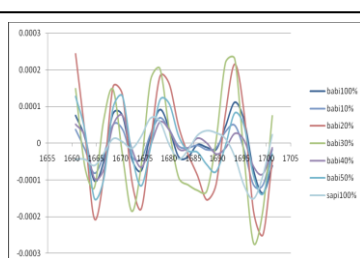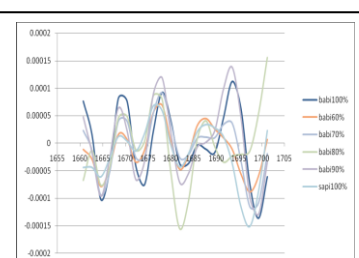|		
Figure 5. The second derivative infrared spectra in the 1680-1695 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 1%, 2%, 3%, 4%, 5% (w / b).	Figure 6. The second derivative infrared spectra in the 1680-1695 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 10%, 20%, 30%, 40%, 50% (w / b).	Figure 7. The second derivative infrared spectra in the 1680-1695 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 60%, 70%, 80%, 90% (w / b).

c. Peak characteristic of the second derivative IR spectra at wave numbers 1075-1090 cm^{-1}

At wave numbers 1075-1090 cm^{-1}, samples containing pork, either pure or mixed with beef containing two peaks, while pure beef has only one peak. Two peaks between 1075-1090 cm^{-1} on samples of pork-beef mixture with a concentration of 70% is not very clearly visible, making it less convincing. If the data is also considered not definitive, then the identification error rate of the infrared spectra typical peaks on wave number 1075-1090 cm^{-1} was also around 5%. Maybe in the future can be confirmed by using the method of derivation at a higher level.
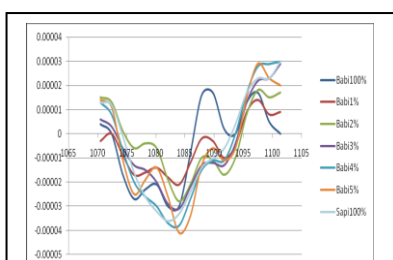

Figure 8. The second derivative infrared spectra in the 1075-1090 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 1%, 2%, 3%, 4%, 5% (w / b).
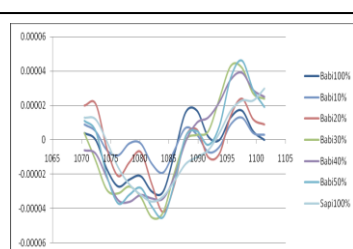

Figure 9. The second derivative infrared spectra in the 1075-1090 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 10%, 20%, 30%, 40%, 50% (w / b).
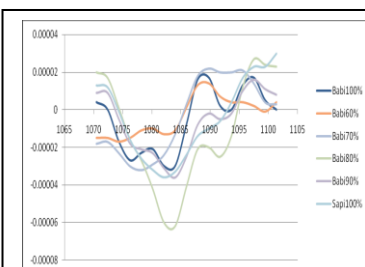

Figure 10. The second derivative infrared spectra in the 1075-1090 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 60%, 70%, 80%, 90% (w / w).

d. The concentration of protein contamination on beef smallest pigs can still be detected by FTIR methods.
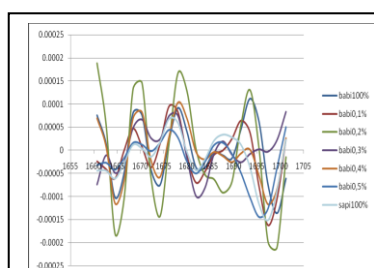

Figure 11. The second derivative infrared spectra in the 1680-1695 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 0.1%, 0.2%, 0.3%, 0, 4%, 0.5% (w / w).
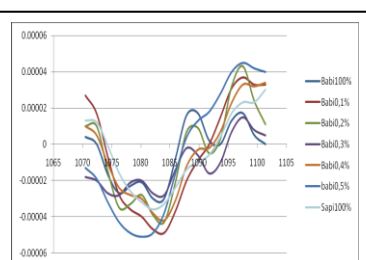

Figure 12. The second derivative infrared spectra in the 1075-1090 cm^{-1} for samples of pure pork, pure beef, and pork and beef mixed with pork concentration: 0.1%, 0.2%, 0.3%, 0, 4%, 0.5% (w / w).

In the concentration range of 0.1 to 0.5% (w / w) contamination on beef pork, the second derivative IR spectra were showed the typical peak at both wave numbers 1680-1695 cm^{-1} and the wave number 1075-1090 cm $^{-1}$. Deviations occur in samples of beef-pork mixture with pork concentration of 0.5% (w / w). Spectral profile of these samples have only one peak in the range of wave numbers 1075-1090 cm^{-1}. This profile resembles the profile of the spectra of pure beef. Possible reasons are the same as the previous case because the unhomogenity sample, so that samples drawn only contain elements of beef.

The intensity of the second derivative spectra for the entire sample has a uniform height, so the intensity of the peak area of spectra at two typical spectra of pork is not linear to the concentration of pork. This indicates that the spectral peak intensity at both peaks typical of this region can not be used for quantitative identification of pork contamination concentration in the mixture. But same intensity spectra typical peaks in both regions is precisely to guarantee the accuracy of the qualitative identification of the presence of contamination of pork in the mixture until the concentration of trace.

Two types of special molecular vibration of pork protein in the wave number 1680-1695 cm^{-1} is derived from general amide CO stretching vibration (1670-1690 cm^{-1}) and cluster RS-CO-N (1700-1680 cm^{-1}) in bonds peptides. Two types of special molecular vibration of pork protein in the wave number 1075-1090 cm^{-1} originating from the CN stretching aliphatic amines to primary amines with alpha carbon atom of the secondary (-CH$_2$-NH$_2$) in the 1090-1065 cm^{-1} and the tertiary (-CH- NH$_2$) in the 1140-1035 cm^{-1}).

CONCLUSION

The results of this research can be concluded that: special molecular vibration of raw pork protein appears on the second derivative IR spectra in the wave number 1680-1695 cm^{-1} and 1075-1090 cm^{-1}. Two types of special molecular vibration of pork protein in the wave number 1680-1695 cm^{-1} is derived from general amide CO stretching vibration (1670-1690 cm^{-1}) and cluster RS-CO-N (1700-1680 cm^{-1}) in bonds peptides. Two types of special molecular vibration of pork protein in the wave number 1075-1090 cm^{-1} originating from the CN stretching aliphatic amines to primary amines with secondary forms of the alpha carbon atom (1090-1065 cm^{-1}) and tertiary (1140-1035 cm^{-1}). The smallest

concentration of pork protein contamination in the beef meat that is still detected through this second derivative method of IR spectra is up to 0.1% (b/b). Necessary to repeat the same research sample meat mixture sapid an raw pork from a more diverse population, both in terms of source material and a different time frame, so that they can know the consistency of both transcription.

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