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Application of a Photoacoustic Tomography System: A Case Study on the Monitoring of Pig Tissue Decomposition

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Abstract

Pig carcasses have been used in forensic research because they share several similarities to human cadaver, including decomposition. In several studies, the decomposition of pig's cadaver for a certain time can be used as a model to determine the time of death of a human. This study was conducted to determine the relationship between the days of spoilage of pig tissue and the level of average sound intensity produced by the sample. Then, in this study, pig skeletal muscles were allowed to decay with a variation of 1–5 days. Afterward, these muscles were imaged using diode laser-based photoacoustic tomography. Results of the experiment show that the average acoustic intensity level from the first day until the fourth day has increased (78–92 a.u for young pig and 76–86 a.u for old pig) but decreased on the fifth day (88 a.u for young and 84 a.u for old pig). These results can improve forensic imaging because such results can be applied to determine the time of death of human by plotting the ratio of the average sound intensity level of the sample to the number of days of decomposition.

Keywords: condenser microphone, decomposition, diode laser, forensics, photoacoustic tomography, photoacoustic imaging

Introduction

In forensic entomology, most experiments use the corpses of non-humans or animals, and in this domain, pig carcasses have been the most widely used model [1]. Pig carcasses are often used in forensic research because they can be easily replicated in large numbers at low cost, whereas access to human corpses is limited to taphonomy or medical ethics facilities with many associated difficulties. In addition, pig carcasses are similar to human corpses with regard to body mass range, anatomy, body composition, fat-to-muscle ratio, skin hair coverage, gut microbiota, and decomposition. The profile of volatile organic compounds (VOC) released by decomposing pigs is also known to be comparable to human decomposition residues [2].

For example, Payne used domesticated pig carcasses to monitor decomposition because the time of death of pigs could be identified. The carcass of domesticated pigs could be obtained in large quantities with uniform age and mass, whereas the skin of pigs was relatively hairless; consequently, the sampling of insects over pigs had been easier in comparison with other alternative carcasses [3]. In addition, Payne used cages with different mesh sizes to provide open and restricted access for insects to document the daily changes in the decay and decomposition of the carcass. The results showed that the carcasses, which had been protected from insects, remained intact for months, whereas the carcasses that had been exposed to the insects initially lost 90% of the mass in just 6 days. The access of the insect to the carcass has been a major determinant of carcass decay.

With regard to the remains of pig decomposition, which shares similarities to human decomposition, Rosier *et al*. made a demonstration by placing several human and animal organs (pigs, mice, birds, and others) into jars and leaving these organs to rot [2]. Then, they used a thermal desorber in combination with gas chromatograph and mass spectrometry to identify the VOCs that had been released in the laboratory environment during decomposition over a 6-month period. The results obtained a combination of eight compounds (ethyl propionate, propyl propionate, propyl butyrate, ethyl pentanoate, pyridine, diethyl disulfide, methyl [methylation] ethyl disulfide, and 3-methylthio-1-propanol) found in the remains of human and pigs' decay, which differs from the remains of other animal decay. However, some compounds that are only found in humans are identified, which can be used as markers in training human carcass sniffers.

The use of pigs as a human analogy in forensic science, in addition to providing information about the pattern of decay, can also provide information about the time of death. Similarly, Niederegger *et al*. used pigs to solve the case of the discovery of a half-buried human corpse, which time of death had been unknown, using only shorts and socks [4]. Pigs with the same mass weight were buried with the same environmental conditions from which the human corpse had been found. The results of the experiment showed post mortem interval, which had been approximately 2–3 weeks. These results were later confirmed by the witnesses who confirmed the estimated time of death of the human corpse or the victim.

At present, the use of imaging such as computed tomography (CT) and magnetic resonance imaging has been widely used in forensic radiology [5]. The first postmortem CT was reported in the late 1970s in a case of a fatal cranial bullet wound. CT has been widely used in detecting the cause of death and in gathering insight into the postmortem state, instead of the time of death, of the deceased.

With regard to object imaging, another technique known as photoacoustic tomography (PAT) has also been widely used. Using PAT, the object is irradiated by a short-pulsed laser beam [6] (Figure 1). Some of the light is absorbed by the object, while the remaining light is converted into heat. Then, the heat becomes a pressure rise through thermoelastic expansion. The pressure rise is propagated as an ultrasonic wave, which is referred to as a photoacoustic wave. Thus, the photoacoustic waves are detected by an ultrasonic transducer and used by a computer to form an image.

A photoacoustic imaging device based on a diode laser with an open-cell model was created and developed by Silalahi (2017) for laboratory purposes [7]. This prototype system has utilized a commercial microphone that works on audio frequencies and photoacoustic data processing using Fourier transformation-based software. The system can image the same biological tissue as the original sample with good image contrast, such as measuring blood concentrations, distinguishing formalin and non-formalin tissues, and analyzing the addition of formalin levels in flesh.

The ability of the PAT tool to properly image biological tissue can be used for forensic research purposes. With regard to the statement, in this study, PAT has been used to take images of pork carcasses that had been left to rot from 1–5 days after being decapitated. The study has been conducted by taking and decomposing the muscles of two pigs with different weights and ages, namely, young pigs aged 4–5 months weighing 30–40 kg and old pigs 3–4 years old weighing 70–80 kg. The results of the study are presented in the form of image and average sound intensity level that have been gathered from the sample. Based on the shared characteristics between pig carcass and human corpse, the results of the study can be used as an overview or an analogy for estimating the time of human death because the studies on the time of death of human corpses have always relied on analysis in the fields of entomology, biology, and chemistry.

Methods

Sample preparation. The researchers gathered the samples of the study by taking tissues (leg/ham) of pig carcass shortly after the pigs had been decapitated at the abattoir. The muscles were gathered from pig carcasses because their muscles had a homogeneous structure. Then, the thigh muscles were collected in five pieces with the same dimensions and mass. These pieces should be exposed for 5-day variation, starting from Day 1 until Day 5. These five pieces served as a weight control for the sample mass and its initial shape prior to decomposition. Afterward, each piece had been placed in a plastic jar, which had been perforated around the side with a diameter ±5 mm to provide access to the insects to start the spoilage.

A sample was collected from one of the jars and was imaged using the PAT tool every day. The size for sample imaging was 8 mm × 8 mm with a thickness of 0.5 cm through an acrylic box, which design could be found in Figure 2. The acrylic box feature helped the samples to attain the same size and thickness. In addition, it assisted the flattening of the sample surface. A black stained-glass plate was used as the sample base whenever the samples were imaged using a PAT tool to attain the contrast media of the sample.

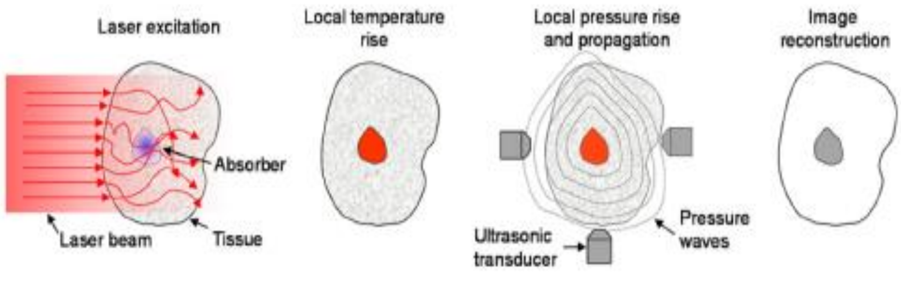

Figure 1. PAT Imaging Principle [6]

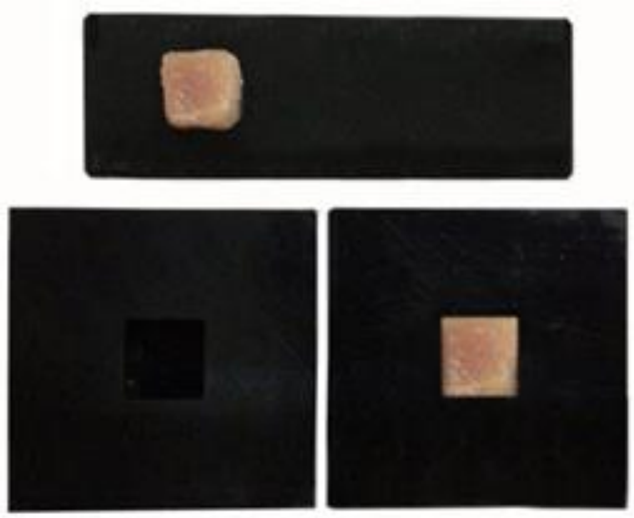

Figure 2. Acrylic Box (Below) as the Control of Sample Dimensions and Thickness and Black Glass (Above) as the Sample base in Imaging

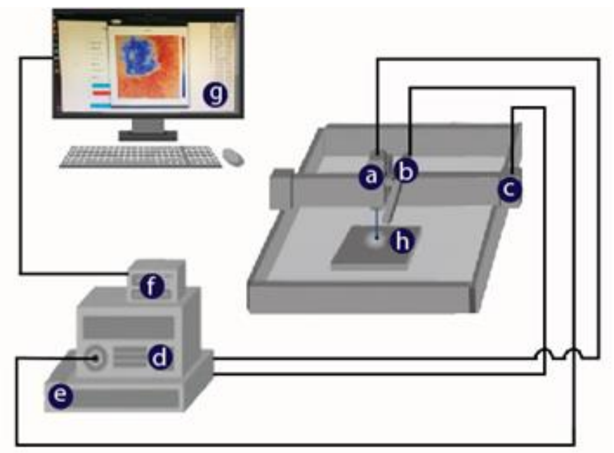

Figure 3. Schematic of the Photoacoustic Tomography Imaging System based on (a) Diode Laser, (b) Electret Condenser Microphones, (c) Motor Stepper, (d) Soundcard, (e) Microcontroller, (f) Raspberry Pi, (g) Interface Screen, and (h) Sample/Object [9]

Photoacoustic tomography imaging tool. The photoacoustic imaging device that had been used was based on a diode laser with an open-cell model (Figure 3).

In this system, the laser that had been used was a blue diode laser with a wavelength of 450 nm and an output power of 1000 mV to generate photoacoustic waves. The resulting photoacoustic waves were then detected by a sound detector in the form of a Behringer 8000 condenser microphone that worked at audio frequencies. Afterward, the detected sound was amplified by the Behringer soundcard. The detector and mechanical system were integrated and controlled by computer software. Meanwhile, for photoacoustic data processing, Fourier-transformation-based software was used. Thus, this system could image the network and reduce the use of hardware and required costs.

Before taking images of decaying pig tissue samples, characterizing the frequency and duty cycle on the PAT tool was necessary for researchers. The characterization aimed to determine the frequency of laser modulation and duty cycle and produce quality object images. Optimal frequency and duty cycle were fixed and used to image pig tissue samples with decomposition variations of 1–5 days. The scan results showed the image and average acoustic intensity level of the object, which could provide the ratio of decay days to the average acoustic intensity level of the sample.

Results

Monitoring the decomposition of pig tissue. The results of monitoring the decomposition of pig tissue are shown in Figure 4 and Table 1.

On the sixth day, almost all of the sample mass was eaten by maggots, and it was difficult to collect because it had changed to liquid (Table 1). Thus, this study was limited to the fifth day. For further research, liquid decomposition must be characterized.

Optimum frequency and duty cycle. The frequency and duty cycle should be determined to confirm whether or not the PAT tools can image the tissue well. Each material (tissue, fluid, etc.) has different characteristics, including its optimum frequency and DC. For each frequency and DC setting in imaging pig muscle in the research environment, different maximum intensity levels are produced (Figure 5).

From Figure 5, found that the frequency and optimum DC are 20,000 Hz and DC 40%, respectively. The intensity profile of the pig muscle sample at 20,000 Hz has the least noise (Figure 6).

Figure 7 shows the results of sample imaging at 20,000 Hz and 40% DC with a good contrast. The background (sample base) is shown in red with high intensity level, whereas the pork muscle sample is shown in blue with a low intensity level. The resulting intensity level is related to the sound absorption ability of the sample.

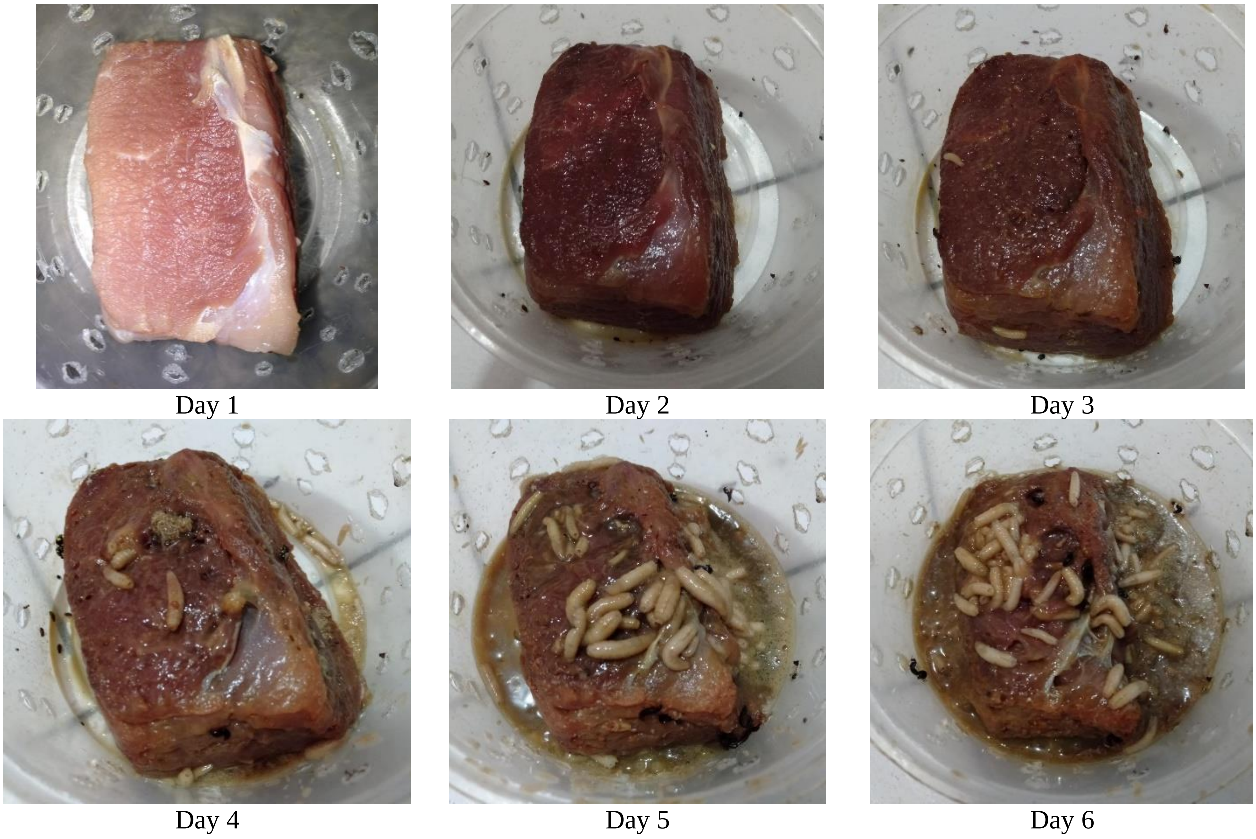

Figure 4. Decomposition with Control Variation in 1 to 6 Days

Table 1. Physical Characteristics of Samples during Decomposition (Figure 4)

Day	**Characteristic**
1	Fresh meat, red color, smells fishy
2	The meat is red, starting to emit a foul smell and a little liquid; at the same time, there are small flies flying around the jar
3	The meat has decreased in color, the stench has increased, the liquid has increased, and the small maggots have been found around the sample
4	The red color of the meat has decreased in comparison to the previous day, the smell is very strong, the decomposition liquid is increasing, and the maggots are getting bigger
5	The color of the meat has decreased, the pungent odor has begun to decrease, the decomposition fluid has increased dramatically, and the maggots has increased in size; on the contrary, some of the mass from the meat has decreased due to being eaten by the maggots
6	The color of the meat is similar to the fifth day, the pungent smell is still present, the decomposition liquid is increasing, the maggots are getting bigger, and eventually the mass of the meat has been very little

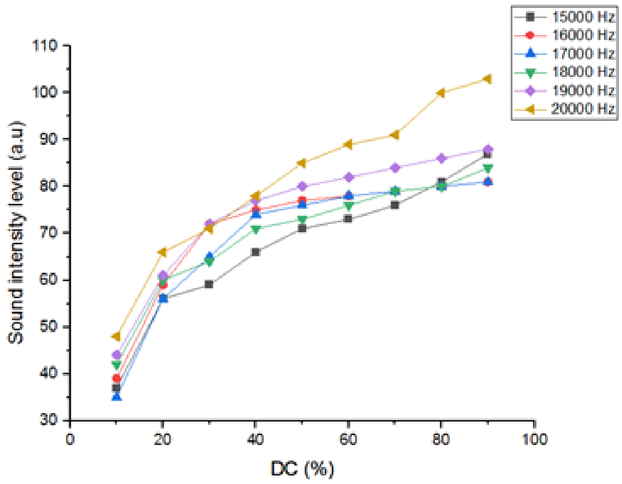

Figure 5. Plot of Frequency and Duty Cycle Data against the Level of Sound Intensity Produced

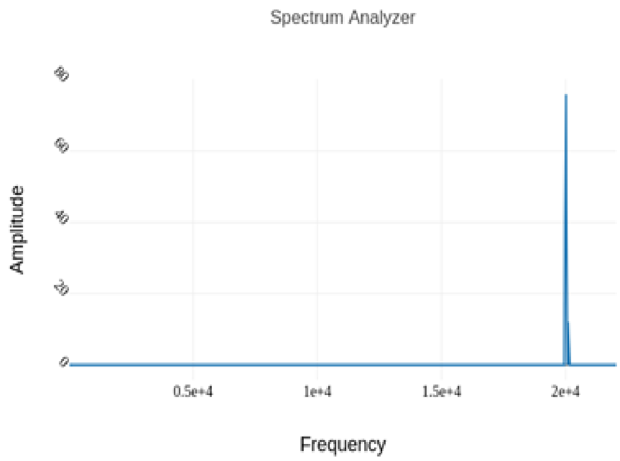

Figure 6. Spectrum at 20,000 Hz

Sample thickness. The PAT tools that have been used can image tissue with a small size from 0.75 mm, with a penetration depth of 0.5 mm [7]. Thus, an experiment can be conducted to determine the size and minimum thickness of the sample and make sampling more effective and efficient. The study was conducted by performing experiments with several thickness variations, starting from 0.5 cm to 3 cm with a dimension of 8 mm × 8 mm. A thickness of 0.5 cm has been selected for ease when cutting the sample, particularly when the sample has been watery. If the sample is too small, then it is easily broken and uneven. Figure 8 shows the results of determining the average sound intensity level for each thickness of the sample.

Differences in sampling locations. With regard to the location of decay sampling, the sample is divided into three areas: the surface area that interacts directly with air, the middle area with a certain depth from the surface, and the bottom area that is submerged in the decomposition liquid. The sample distribution is illustrated in Figure 9. The division of this area is important to see the relationship between the sampling area and the average sound intensity level produced.

The average sound intensity level for the first to fifth day of decay and for the upper, middle, and lower regions is shown in Table 2 and Figure 10.

Relationship between the average sound intensity level and day of decay. This study aims to determine the ratio of the average sound intensity level of the sample to the number of days of decomposition. The results of PAT images for the young and old pig samples collected from old pigs and the samples collected from the young pigs are shown in Figure 11.

The resulting image in Figure 11 was plotted using origin software to obtain a range of sample intensity values, and then the average was calculated. The average sound intensity level for the samples of old and young pigs is shown in Figure 12.

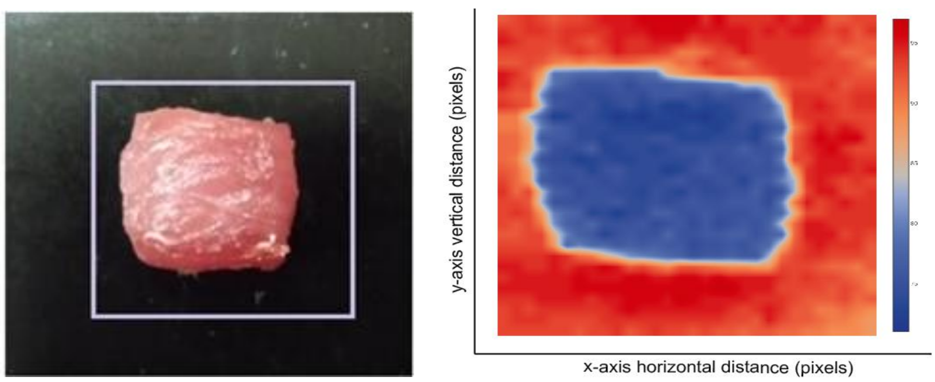

Figure 7. Sample and Scanned Image Results with a Frequency of 20,000 Hz and a Duty Cycle of 40%

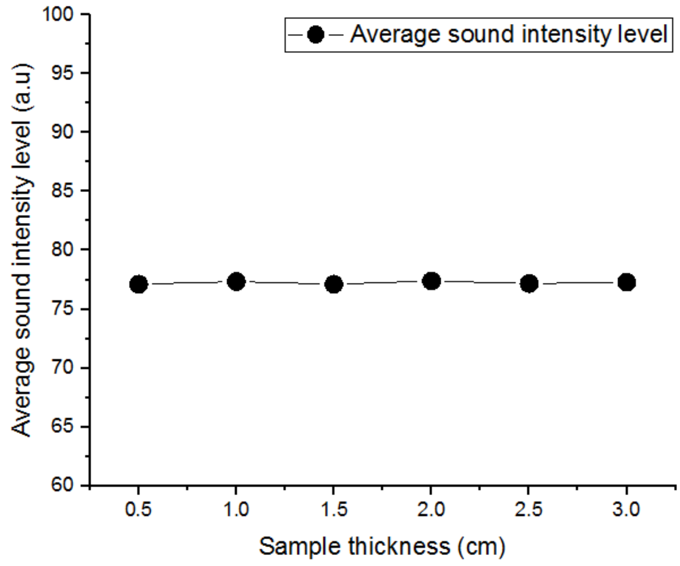

Figure 8. Ratio of the Thickness of the Sample to the Level of the Average Sound Intensity Produced

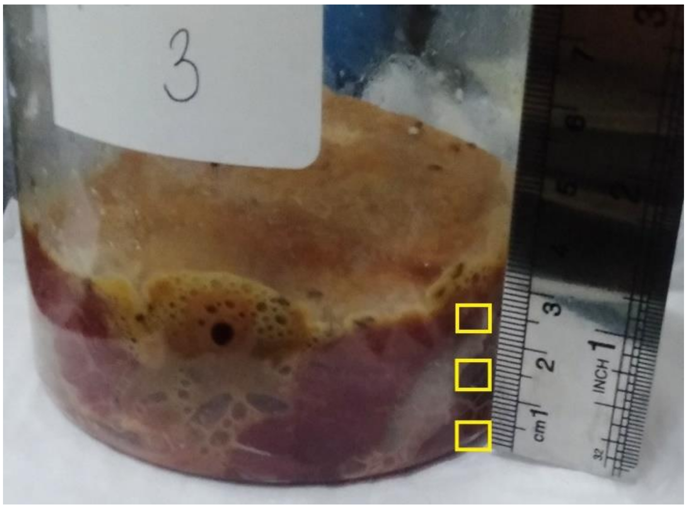

Figure 9. Categorization of Sampling Locations: Surface, Middle, and Bottom Areas

Table 2. Results of the Average Intensity Level for Each Sampling Location on Each Day of Decay

Location	1	2	3	4	5
Upper	77 a.u	79 a.u	84 a.u	86 a.u	85 a.u
Middle	77 a.u	80 a.u	83 a.u	87 a.u	84 a.u
Lower	77 a.u	80 a.u	83 a.u	86 a.u	84 a.u
Standard deviation	0.00	0.51	0.50	0.45	0.53

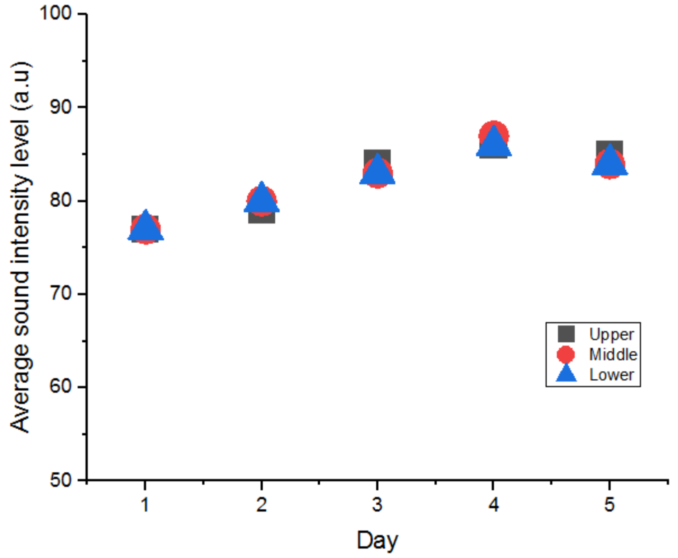

Figure 10. Sample Locations against the Average Intensity Level Shown in Table 2

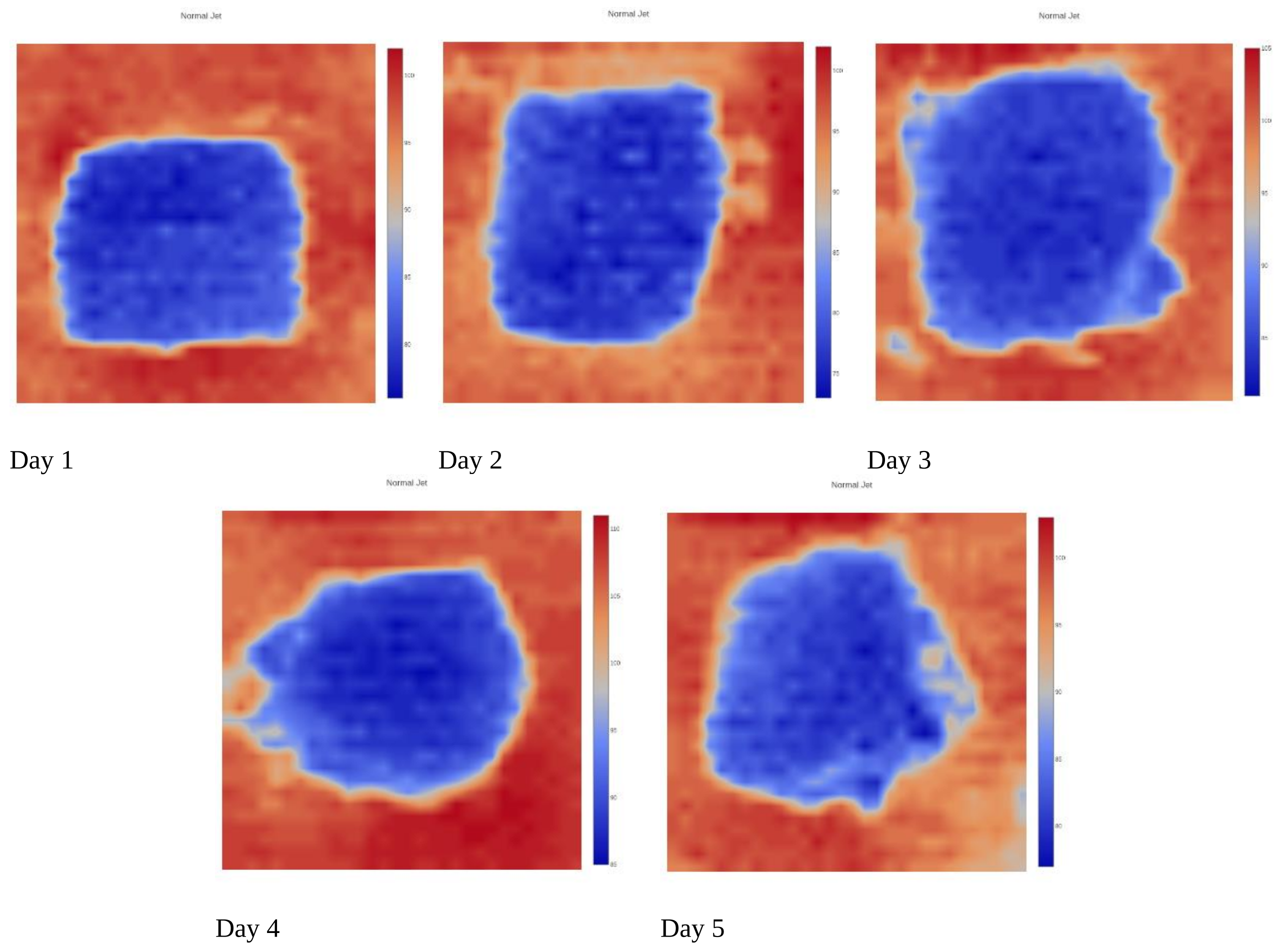

Figure 11. Young Pig Tissue Image with Variations in the Number of Days of Decomposition from the First to the Fifth Day

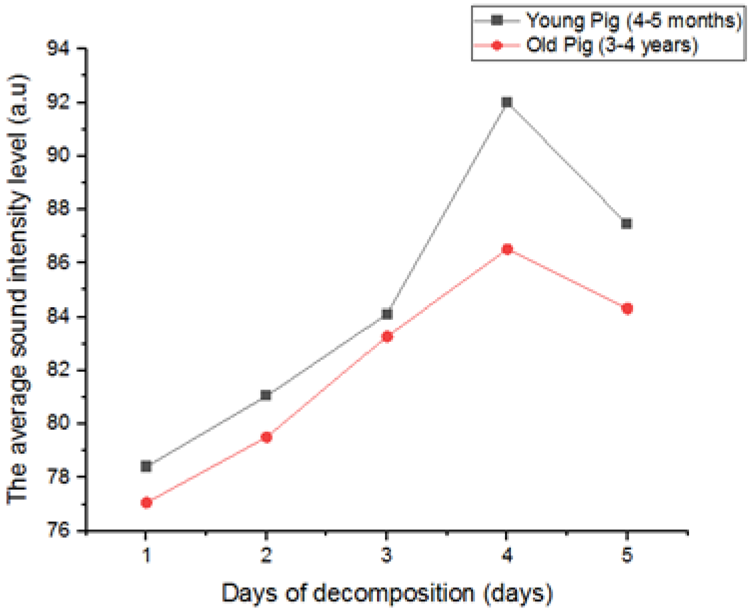

Figure 12. Ratio of the Number of Days of Decay to the Mean Sound Intensity Level for Samples of Old Pigs (Red Plot) and Young Pigs (Blue Plot)

Discussion

After the time of death, autolysis will be conducted, in which digestive enzymes break down body tissues [8]. Autolysis first begins in the cells that are more metabolically active and have more hydrolytic enzymes. Although autolysis has been found to begin as soon as 4 min after death, the time at which it begins varies by tissue and organ type. The first organs to decay are the intestines, stomach, pancreas, liver, heart, and blood, followed by the lungs, kidneys, bladder, and brain. Afterward, the skeletal muscle is the next tissue to decay, followed by more durable connective tissue. In addition, this study has used the leg of the pork that has a homogenous structure as a control variable for the decay because this part has higher level of durability than any other organ.

During autolysis, the cells show two main types of microscopic and macroscopic changes [8]. The first type is coagulative necrosis, which has been characterized by the preservation of the original structure of the tissue for several days after cell death. Then, the second type is liquefactive or colliquative necrosis, which has been defined as the partial or complete disintegration of dead tissue into a fluid, viscous mass. Thus, the presence of fluid around the corpse are also found during decomposition.

In addition to monitoring autolysis, interesting information can be found from the size of the maggots on each day of decomposition (Figure 4). Since the presence of the flies around the sample, the maggots that have appeared are visible to the eye on the third day, and this situation continuous every day with the increase of the size of the maggots. Autolysis is also characterized by the appearance of gas from protein metabolism. Gases resulting from decomposition in pigs is similar to that produced during the decomposition of human corpses. In this study, the pungent smell of gas had started on the second day. The maximum gas odor has been found on the fourth day, whereas on the fifth day, the odor has slowly decreased because of the sample mass. Furthermore, the sample mass has begun to shrink because they are being eaten by the insects.

The information on the physical changes of the muscles during decomposition can be related to the use of PAT imaging tools. Notably, the PAT tools can image and differentiate networks. In this case, these tools can provide information in the form of images and average sound intensity level from the decaying sample throughout daily variation.

Figure 5 shows that the greater the value of the optimization frequency and the duty cycle, the higher the maximum intensity level has been produced. The optimum frequency is determined by the highest peak intensity value generated for each frequency variation with the minimum noise. In this research environment, the noise remains high at frequencies of 15,000 Hz and 16,000 Hz, which are marked as background frequencies. At high temperature, the value of the duty cycle (light intensity) or the laser power emitted will burn the sample. Pork muscle samples will dry and change to dark red at DC 50% and burn at DC 90%. Therefore, the frequency and optimum DC are 20,000 Hz and DC 40%, respectively. The intensity profile of the pig muscle sample at 20,000 Hz has the least noise (Figure 6) and good contrast (Figure 7).

Figure 8 shows the results of determining the average sound intensity level for each thickness of the sample, which has the same value for each thickness and small standard error (0,11). Different samples produce the same intensity level value because several image results of the sample can display an image of meat fibers that accidentally stick out of the sample with the same intensity level. The minimum thickness of the sample is important for sampling if human flesh is used in the future. Thus, by taking a small part of a human corpse, the time of death particularly for corpses that may suffer from an accident, mutilation, and so on may be determined.

Figure 9 show the sample distribution, then the results of the average intensity level for each sampling location on each day of decay shown by Figure 10. Figure 10 shows that the value of the average intensity level generated in each sampling area has almost the same value. The difference in the existing values is due to the noise that breaks through the imaging process, resulting in different ranges of sound intensity values and average values. However, the difference or standard deviation (Table 2) is small, which can be ignored. Thus, sampling can still be performed either in the surface, middle, or bottom areas that are submerged in the decomposition liquid.

As shown in Figure 11, the PAT tool can distinguish the rotten tissue from the background. The selection of the right background can increase the contrast of the image between the background (red) and decaying tissue (blue), starting from the first day to the fifth day. The difference in the image (Figure 11) is due to the shape of the sample, in which the sample can be easily formed into a box through the mold on the first day (Figure 2). In addition, from the second day onwards, the sample becomes difficult to form because the tissue is getting softer and juicier. The same occurrence in Figure 11 also applies to the old pig sample.

The tissue of the old pig is darker red in comparison with that of the young pig. In addition, the old pig has denser muscle structure than the young pig. During decomposition, the tissue of the old pig tissue lasts longer than that of the young pig, whereas the change in color within the tissue of the young pigs is faster than that of old pigs. The young pig has shown more active rotting characterization based on its strong odor, and the maggots grow at a fast rate. The maggots have preferred the tissue of the young pork.

Acoustic impedance is also affected by the density of the medium and the color difference among the soft tissues [7]. Differences in density and color among soft tissues indicate that biological tissues have different mechanical, optical, and thermal properties, thereby resulting in different acoustic impedances. As shown in Figure 12, old and young pigs have the same pattern, including an increasing level of acoustic intensity from the first day until fourth day and the decreasing level of acoustic intensity on the fifth day. From the first day to the third day, the average acoustic intensity level has linearly increased. Then, the fourth day seems to be the peak for the increase of the acoustic intensity within the sample because of the pungent odor and softer tissues. On the fifth day, the sample has suffered from a decrease in the level of acoustic intensity. Physically, the pungent odor has begun to decrease because of more decomposition liquid. Consequently, on the sixth day, almost all of the tissue mass has been eaten away by the maggots; therefore, the sample has been difficult to image because it has turned into liquid.

The difference among the samples collected from the old pig and those collected from the young pig lies in the range of acoustic intensity levels. Young pig has a higher acoustic intensity range than the old pig because the tissue of the young pig is still fresh from the first until the fifth day of decomposition. This result is due to differences in tissue density and color and other biological factors that influence decomposition. Thus, the age and weight of the tissue have the characteristics of their respective acoustic intensity levels. If further studies will be conducted on human cadavers, then these studies should focus on several factors such as sex, weight, and age. Furthermore, the plot of the average daily intensity level can be used as a reference to determine the time of human death in the span of days and or until all parts of the human body have decomposed.

Conclusion

This study demonstrates the ability of a PAT imaging system to monitor the decomposition of pig tissue. During decomposition, pig tissue undergoes various physical changes, which have been indicated by different average sound intensity levels for each day of decomposition. The results of the experiment show that the average of the acoustic intensity level from the first day until the fourth day for young and old pigs has increased from 78 a.u. to 92 a.u. and from 76 a.u. to 86 a.u., respectively, but decreased on the fifth day (88 a.u. for young pig and 84 a.u. for old pig). This information can be useful for forensic studies, where PAI can be used in the future to determine the time of death by plotting the ratio of the average sound intensity level of the sample to the number of days of decomposition. If further research will be conducted on human cadaver, then it should consider the human age, weight, and sex.

Acknowledgements

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