Simple Thermal Analysis as a Green Method for the Detection of Meat Adulteration

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Abstract: *Differential scanning calorimetry (DSC) is one of the most widely developed thermal analysis methods for meat samples for halal authentication of food or processed products. Research on adulteration detection for various types of meat and its derivatives has been developed before and still requires organic solvents. Therefore, the concept of the "green method" is being tried to develop in this research. DSC analyses are performed in the same experimental conditions for all sample powder: sample mass 2 mg, temperature range 30–400 °C, and heating rate 20 °C min^{-1}. The results showed there is a characteristic minor endothermic peak for each meat. Chemometric analysis was carried out using the principal component analysis (PCA) method to ensure that the thermal characteristics of each meat were utterly different in both pure and mixed meat. The results of this analysis indicate that each pure meat has a different score plot. Therefore, the developed thermal analysis method is quite reliable in determining the different types of meat based on the characteristic minor endothermic peak in the thermogram and the score plot from PCA analysis.*

Keywords: *DSC; chemometric analysis; method development; minor endothermic peak; pork*

■ INTRODUCTION

Adulterating beef with pork or other meats is still common in many countries, such as China and Indonesia. That was done for economic concerns or to obtain greater profits [1]. Adulterating meat has not been a significant problem for a long time if consumed fresh or because it is available in small quantities. However, with improved technology and modern storage facilities, meat adulteration has become a significant problem. In this case, a food product that does not list or mention the ingredients used on the final product label is considered a food adulteration. Food fraud or adulteration has huge economic potential, believed to be worth several billion dollars annually [2]. For example, it was reported in the Guardian newspaper that 900 people were imprisoned in China for meat fraud involving 20,000 tons of unsuitable meat, including mink, rat, and fox. In addition, 4% of lamb is sold for takeaway dishes containing other types of meat [1].

Research on adulteration detection for various types of meat and its derivatives has been carried out using various methods such as high-pressure liquid chromatography (HPLC), gas chromatography (GC), electronic nose (EN), polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), nuclear magnetic resonance (NMR), near-infrared (NIR) spectroscopy, laser-induced breakdown spectroscopy (LIBS), fluorescent light spectroscopy, Fourier transform infrared (FTIR) spectroscopy, mass spectrometry (MS), Raman spectroscopy (RS), and thermal analysis such as differential scanning calorimetry (DSC) for the detection of fat in meat at low temperatures [2]. Naturally, the analytical methods that have been developed have advantages and disadvantages, including the complexity of sample

preparation, measurement, and data processing. In addition, the detection method that has been developed still requires organic solvents.

Real-time PCR is one standard method for analyzing non-halal meat [3]. In recent years PCR has been widely developed for DNA analysis of non-halal meat in processed products containing halal meat [4-5]. DNA-based identification is a reliable technique with high speed and sensitivity [6]. However, one of the drawbacks of this method is that it requires sophisticated instruments and trained operators, making it unsuitable for field-based analysis [7]. In addition, the use of organic solvents used during sample preparation and analysis induces that this method can pollute the environment if the waste is not handled correctly, which can cause a reduction in the number of wild animals, a decrease in ecosystem function, and threaten human health [8]. Thus, the development of environmentally friendly analytical methods can be an option to prevent negative impacts on the environment and living things. One of the solid analysis methods, such as thermal analysis, can be a prospective environmentally friendly method for the future [9]. Several modern thermal analysis methods, such as DSC, DTA, and TGA, have been used to determine the ingredients' characteristics to see the safety and quality of these foods. DSC is one of the most widely developed methods, especially for food samples, namely meat or its derivatives, for halal authentication of food or processed products. DSC makes analysis can be simple and fast. In addition, the small sample requirement for analysis is one of the advantages of this method [2].

Therefore, the development of an analytical method with the concept of the "green method" [9], using a combination of thermal analysis with DSC as a confirmation tool and electrothermal for the routine analysis carried out at high temperatures by looking at the decomposition temperature of the sample makes this method more efficient because it does not use organic solvents and the steps of measuring and processing data are shorter and easier. This study aims to develop a reliable and environmentally friendly thermal analysis method, especially for determining various types and assessing the halal-ness of meat products using DSC instruments and electrothermal semi-manual tools.

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were beef, pork, rabbit, and chicken obtained from the Yogya Junction 8 Toserba (Bandung, Indonesia) and traditional markets in the Bandung area. In addition, Indium (Merck, Bandung, Indonesia) was used for DSC analysis.

Instrumentation

The tools used were a 5" kitchen knife (Qian Jin, Singapore), 245 mL small jar (Yoshikawa SW TX07, Japan) vial, spatula, tweezers, capillary tube, watch glass, alcohol thermometer, analytical glass scale (Fujitsu FSR-A220, Japan), 9 L toaster oven (KLAZ, Ace Hardware, Indonesia), blender & chopper (Homu, China) electrothermal (Electrothermal AZ 9003 IA9000, UK), DSC (Rigaku Thermo Plus EVO2 DSC8231, Japan), aluminum pans & plate, and hydraulic presses for DSC analysis obtained from Rigaku (Rigaku, Tokyo, Japan).

Procedure

Meat powder manufacturing

The manufacture of meat powder begins with collecting each meat by choosing the same part, namely the breast of meat. Furthermore, meat powder was made by a convective drying method using an oven at 40 °C [10-11]. The meat is first separated from the fat that looks white using a kitchen knife, then sliced thinly 1–2 mm thick, and then placed in a container lined with aluminum foil. Drying was carried out using an oven for 22 h, then the dry weight of the meat was weighed. If it has been constant for weighing several times, the drying is considered complete [11]. An alcohol thermometer inserted into the oven at the same time as the meat drying is used to control the temperature stable at ±40 °C. The following process is smoothing dried meat using a blender and filtering using a 200-mesh sieve to produce a fine powder with uniform particle size. Determination of drying shrinkage can be done by looking at the difference between wet weight and dry

weight, which is expressed as a percentage of drying shrinkage in the meat [10-12].

Sample preparation

The sample for electrothermal observation was prepared by weighing 2 mg of meat powder with an analytical balance. The sample was inserted into the mouth of the capillary tube and gently tapped until it reached the bottom of the tube. In addition, for the sample analysis of DSC, into an aluminum pan that had been previously tare, as much as 2 mg of meat powder was added and then weighed with an analytical balance. Furthermore, the sample is evenly spread as thin as possible to cover the container's bottom. Then the pan is covered with a cover plate and compressed until it is tightly closed. Pans that have been closed can be directly analyzed by DSC [13].

Method validation

The specificity of a method can be determined by analyzing the thermogram of an empty aluminum pan and ensuring that there is no pan interference in the qualifying sample. In this case, the ability to choose between compounds of closely related structures must be demonstrated. This ability should be confirmed by obtaining a positive result by comparing the characteristic thermogram profile of pork with a negative result of a sample that does not contain analytes, i.e., beef powder. Furthermore, the results were confirmed by ensuring that a positive response was not obtained from beef powder. In this study, the resulting thermogram must show that the procedure is not affected by impurities or adding other substances to the sample. It can be done by spiking or adding the beef powder to pork powder in a ratio (1:1), or 1 mg each is mixed into a mortar, homogenized, put into a pan, and weighed with an analytical balance. A good thermogram from the spiking results will show that the test results are not affected by the presence of foreign materials [14-15].

Accuracy and precision are not needed in the validation of qualitative analytical methods, but to ensure good repeatability in measurement and recovery of analytical results that are close to the actual value. In this study, accuracy and precision tests are still carried out [14-15]. The accuracy and precision tests were carried out on one sample concentration with three measurements on each meat sample. The accuracy test that is carried out to assess the measure of accuracy or proximity of the analysis results to the average can use the recovery parameter by the difference between the obtained analysis results and the average measurement results, which is expressed as a percentage of the recovery. The precision test is determined by the Relative Standard Deviation (RSD) parameter by the difference between the calculation result of the standard deviation and the average measurement result [14].

Sample analysis

Electrothermal observations were carried out by entering a capillary tube filled with samples into a semi-manual electrothermal furnace, then heated in the range of 30–350 °C with a heating rate of 10 °C min^{-1}. Observations were assisted with a smartphone camera, and the reading was carried out three times. In addition, the DSC analysis was carried out to study the thermal profiles of various types of meat. The meat powder sample that has been put in an aluminum pan and tightly closed is heated at a temperature of 30–400 °C with a heating rate of 20 °C min^{-1} [16]. An empty aluminum pan is used as a reference.

Data analysis

DSC thermogram data is analyzed with the help of software related to various available programming. Thermogram data analysis was performed using the ThermoPlus software. The resulting data is stored in Excel, then processed by making an overlay of the sample thermogram profile to see the difference in thermal characteristics of both pure and mixed samples. Chemometric analysis for multivariate data classification using the PCA technique was performed using Minitab software version 19 (Minitab, LLC).

■ RESULTS AND DISCUSSION

Meat Drying

Meat drying is the first step to obtaining meat powder samples that will be used to analyze various types of meat using the thermal analysis method (Table

1). The most abundant component in meat is water, followed by protein and fat, while carbohydrates, minerals, and vitamins are contained in much smaller amounts [17]. The great water content makes the meat unable to last long at room temperature, so it must be stored in a cold refrigerator to extend its shelf life of the meat.

In this study, the meat was made into powder by conventional drying methods using an oven at 40 °C to prevent protein denaturation, which began at around 40 °C [11,18]. Drying time was carried out for 22 h because it is the optimal time for drying meat so it can be mashed into powder [11]. Meat that has been in powder form, as shown in Table 1, can be stored at room temperature for an extended period so it could be used for the thermal analysis method which is one of the solids analysis methods to measure the heat profile of a sample [9].

From the results of weighing the meat before and after drying, the drying shrinkage from the largest to beef, followed by pork, chicken, and rabbit. The drying shrinkage plays an essential role in water transport during heating [19]. In this case, the drying process can cause a loss of water content in the meat. Based on the research by Bampi et al. [11], showing that the maximum temperature reached inside the samples while using the convective drying method was 40 °C, and 22 h were necessary to reduce the meat moisture from 2.50 to 1.25 g g^{-1} (dry basis), it means that during drying there is a drying shrinkage of 50%. However, in this research that has been done on meat that also uses an oven at a temperature of 40 °C, the percentage of drying shrinkage shows almost the same results, even exceeding the average water content of meat in general. That means the meat's water content can evaporate completely at that temperature. Naturally, the water content of meat is approximately 75% and 20% protein, with the remaining 5% representing a combination of fat, carbohydrate, and minerals. Still, the percentage of water can vary depending on the type of meat [20]. Based on the research from Li et al. [21], fresh beef has 65–80% water content, which is highly perishable, while dehydrated beef is more suitable for transport and storage due to its longer shelf life and lower mass and volume. Furthermore, Table 2 shows that the drying residue data for each meat can be representative to explain that the

Table 1. Pictures of meat before and after dried to powder

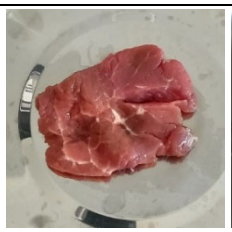
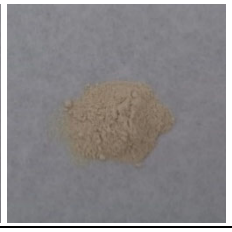
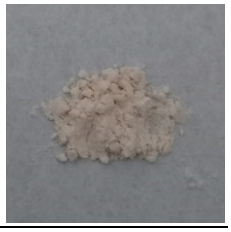
Meat Types	Beef	Pork	Rabbit	Chicken
Raw meat				
Dry meat				
Meat powder				

Table 2. Drying shrinkage of meat

Meat types	Wet weight (g)	Dry weight (g)	Drying shrinkage (%)	Drying residue (%)
Beef	20.255	3.950	$\frac{20.255-3.950}{20.255}\times100=80.5$	19.5
Pork	33.885	8.018	$\frac{33.885-8.018}{33.885}\times100=76.3$	23.7
Rabbit	48.481	13.249	$\frac{48.481-13.249}{48.481}\times100=72.7$	27.3
Chicken	28.476	7.514	$\frac{28.476-7.514}{28.476}\times100=73.6$	26.4

drying carried out has wholly evaporated the water and left only other content of the meat such as protein.

Specificity Test

Method validation is carried out to provide sufficient evidence that the analytical method can fulfill its objectives [15]. In this case, the validation process is determined through laboratory testing, which shows that the performance characteristics of the procedure have met the requirements following its intended use [14]. The development of the analytical method carried out in this study is qualitative analysis or identification so that the specificity test is selected as a characteristic of analytical performance in method validation [14].

The results of the pork specificity test showed that at a temperature of 186.9 °C, the pork sample obtained an actual positive result (True Positive), and the beef obtained an actual negative result (True Negative). That can be seen in Fig. 1, where a minor endothermic peak appears in the pork sample's thermogram profile with a peak temperature of 186.9 °C, while in the beef sample, there is no peak at that temperature. To ensure that the analytical procedure is not affected by the presence of impurities or the addition of other substances to the sample, spiking or addition of beef to pork is carried out in a ratio (1:1). The test results on the mixed sample showed that the minor endothermic peak which was the characteristic thermal characteristic of the pork sample

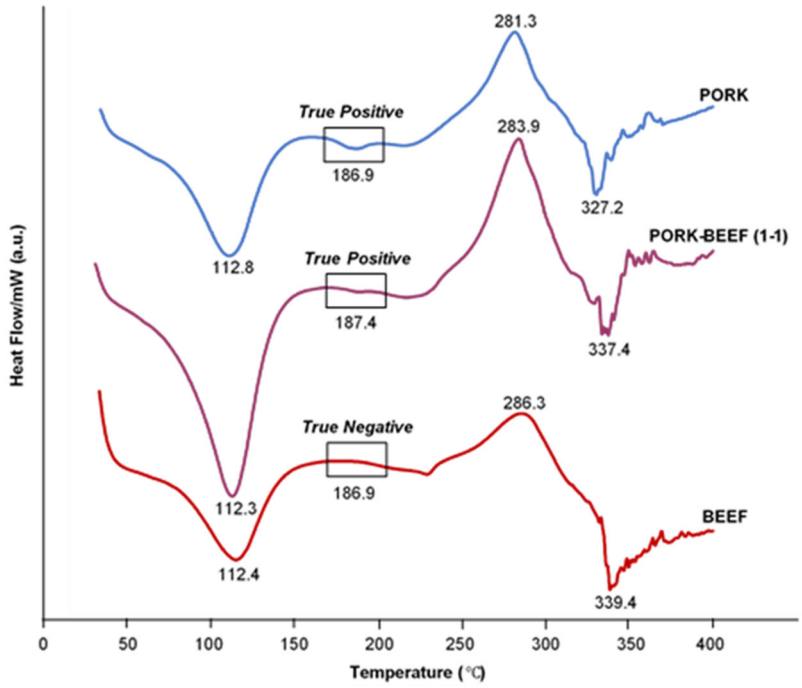


Fig 1. DSC thermogram of pork specificity test results

still appeared at the peak temperature of 187.4 °C. In this case, a peak temperature shift of 0.5 °C and a reduction in enthalpy occurred in the pork sample after adding beef. A previous study by Talik et al. [22] showed a significant effect on the composition of the mixture or analyte concentration on enthalpy, where the analyte concentration was directly proportional to the enthalpy. The smaller the analyte concentration, the smaller the enthalpy. In another study, a sample adulterated showed a peak temperature shift from the initial position [23].

Accuracy and Precision Test

The method has good precision, determined for one concentration level of 2 mg of meat sample. The results were expressed as the RSD with the condition that the value is < 1.3% [24]. Based on Table 3, the RSD value for various types of meat has met the requirements, and the smaller the RSD value from a series of measurements, the more precise the method used. In addition, the recovery of various types of meat is in the range of 99.99–100.02%. These results are still included in the recovery requirements, namely 98–102%, according to the level of analyte concentration [24].

Meat Powder Analysis

In its development, the DSC method was used to observe the behavior of the denaturation process in the muscle tissue of halal and non-halal animals without being dried into a powder. The thermogram results clearly show each sample's thermal characteristics of the denaturation process [25]. The development of the DSC method in this study was carried out for the halal authentication of food ingredients in the form of meat. This method was developed for the identification of non-halal meat or qualitative analysis. In Islamic law, if a food has been contaminated with ingredients that are forbidden, then the food becomes haram [26]. In this case, there is no tolerance for haram ingredients in a food, so regardless of the concentration of haram ingredients detected, the food will still have haram status. Therefore, this research focuses on developing an environmentally friendly qualitative analysis method for identifying non-halal meat as an effort for halal authentication.

The DSC signal is presented in the form of a thermogram, with the x-axis representing temperature and the y-axis representing heat flow. To carefully compare the results of the thermograms of each meat sample before and after mixing, the unit of heat flow on the y-axis is made an arbitrary unit while the temperature on the x-axis is fixed with the unit °C. This is done to see in detail the differences in thermal characteristics, especially the characteristic minor endothermic peaks of each sample, through an overlay of a thermogram profile made using Excel. To create the overlay, the raw data from the ThermoPlus software integrated with DSC is exported in the form of an Excel file. Furthermore, temperature (°C) and heat flow (mW)

Table 3. Validation of the DSC method based on the characteristic minor endothermic peak of meat

Sample	Repeatability of measurements (n=3) (°C)	Average minor endothermic peak (°C)	Mean recovery (%)	% RSD
Beef	228.1 228.9 230.3	229.1	99.99	0.48
Pork	186.9 186.9 186.7	186.9	99.99	0.09
Rabbit	193.4 193.1 191.0	192.5	99.99	0.68
Chicken	177.9 178.7 180.2	178.9	100.02	0.65

data from each sample are collected to be overlayed by changing the heat flow value either by adding or subtracting values so that the resulting thermogram profile can be seen clearly when you want to compare differences between samples.

The DSC thermogram profile from Fig. 2 contains major endothermic peaks that appear in the temperature range of 103–113 °C in the beef, pork, rabbit, and chicken samples, with peak temperatures being at 112.4, 112.8, 109.5, and 103.4 °C. In addition, minor endothermic peaks that are characteristic of both beef, pork, rabbit, and chicken and only appear in samples are at peak temperatures of 229.1, 186.9, 192.5, and 178.9 °C, respectively. Another endothermic peak in the temperature range of 320–340°C was only found in the beef, pork, and rabbit samples with peak temperatures at 339.4, 327.2, and 324.7 °C, respectively. Meanwhile, the DSC thermogram profile did not show any endothermic peaks in that temperature range in the chicken sample. The DSC thermogram profile also indicates the presence of exothermic peaks in the beef, pork, rabbit, and chicken samples in the temperature range of 280–300 °C with peak temperatures being at 286.3, 281.3, 284.5, and 297.9 °C.

Furthermore, Table 4 shows that the first major endothermic peak in the temperature range of 103–113 °C in the DSC thermogram profile Fig. 2 in each sample is the release temperature of water molecules. That can be observed by electrothermal, where the sample looks drier than the initial before heating. In addition, the top of the capillary tube looks dewy after the heating is complete. This is caused by the sample releasing water molecules in the form of gas at that temperature range so that the sample looks dry and there is dew on the top of the capillary tube.

The other endothermic peak in the temperature range of 170–230 °C is a characteristic minor peak of every meat because it appears consistently in three measurements using DSC. On direct electrothermal observation and from the results of video recording with a smartphone camera, it can be seen that a small portion of the sample undergoes a physical transformation, and a big portion does not undergo physical transformation.

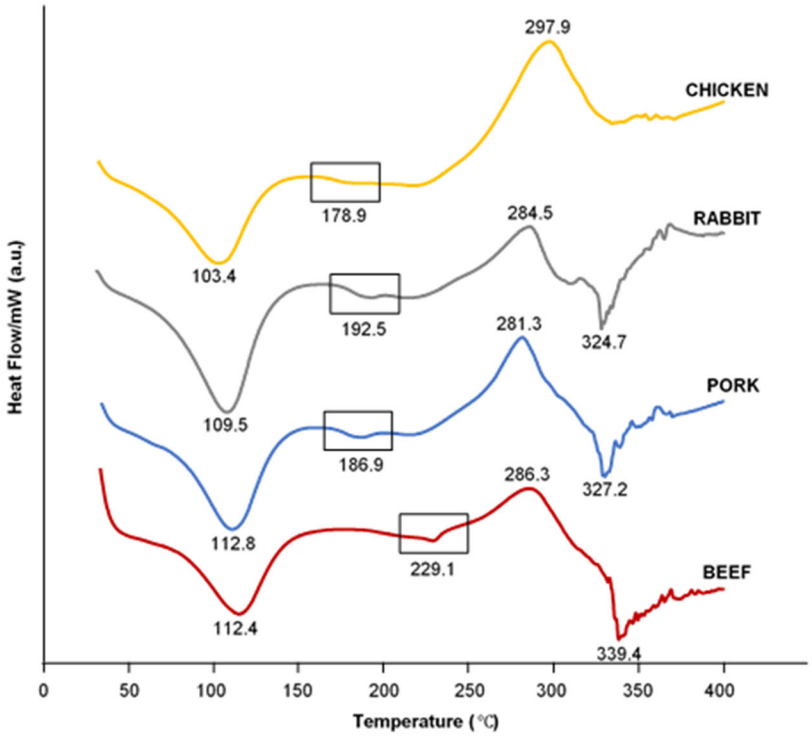
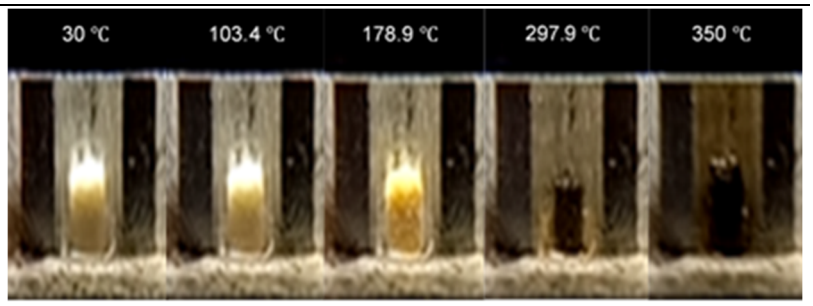
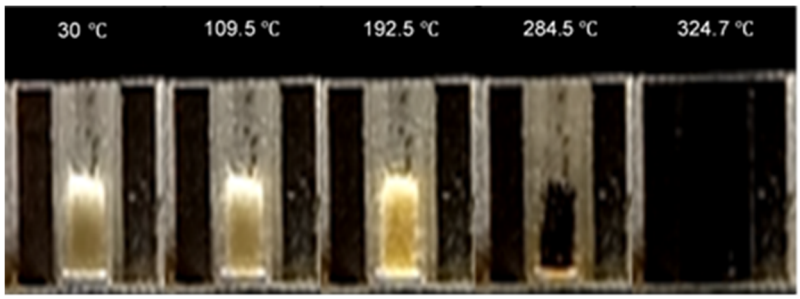
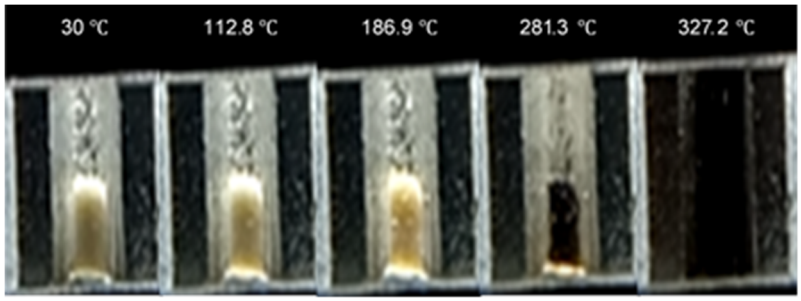
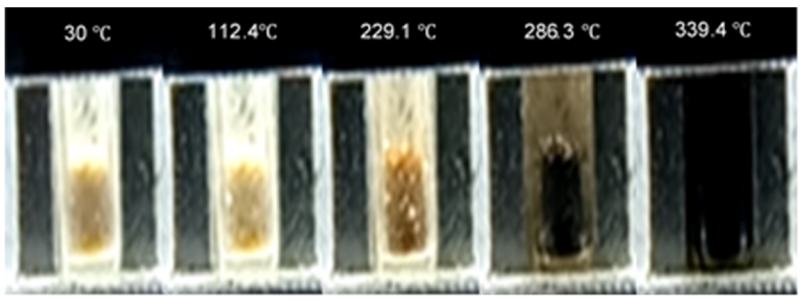


Fig 2. DSC thermogram profile of various types of meat

Table 4. Results of observing various types of meat with electrothermal

Sample	Results of observing
Chicken	
Rabbit	
Pork	
Beef	

That can be caused by the small concentration of components in the sample and still in the multi-component sample, not in pure form, so it is more difficult to observe.

In addition, the exothermic peak that appears on the DSC thermogram profile of each sample in the temperature range of 280–300 °C is the decomposition temperature where, if observed by electrothermal, each sample undergoes a carbonation process or only leaves carbon as a residue. In the temperature range of 320–340 °C, it can be seen in the DSC thermogram profile that there is an endothermic peak when observed by electrothermal. Each sample undergoes a melting process or phase change from solid to liquid. However, in the chicken sample in this temperature range, there is no endothermic peak in the DSC thermogram profile, and observations made by electrothermal up to 350 °C in the sample do not show that there is no melting process.

Based on research conducted by Weiss et al. [27] regarding the thermal stability tested on eight standard

amino acids, including glycine, cysteine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, and histidine, shows that several processes can occur on heating, such as chemical decomposition or sublimation without decomposition.

This is in line with research that has been carried out on samples of meat powder which is known to contain amino acids where information on thermal characteristics starts from the temperature of the release of water molecules, which is indicated by the sample appearing dry, then the decomposition temperature which is indicated by a change in color to black in the sample, and the point at which the sample turns black. Melting can be observed by electrothermal so that the purpose of determining the type of meat using the thermal analysis method can be achieved.

Mixing pork and beef with several comparisons was carried out to study the thermal characteristics of pork before and after being mixed. In addition, the research design based on cases that occurred in the field related to contaminated meat or adulteration of beef with pork is expected to make an analytical method developed that reliably detects the presence of pork in beef which can be seen with the DSC thermogram, profile.

Several previous studies related to the detection of the presence of pork in food products have been successfully carried out using DSC with the research design of mixing pork content in food products intentionally to study the thermogram profile before and after mixing [23,28-30]. However, the research focuses on detecting lard instead of protein or muscular tissue from pork [2]. Azir et al. [23] conducted a study on fatty acid composition, triacylglycerol profile, and thermal properties of lard in cocoa butter showed a real difference between lard and cocoa butter in their thermal characteristics. In another study, lard was intentionally mixed into butter with various concentrations; qualitatively, the thermogram profile analysis showed subtle differences between butter, lard, and their mixtures [28].

Based on the experiments, the characteristic minor endothermic peak at pork up to a concentration of 33.3% in the mixed sample can still be detected with a very small enthalpy. In addition, there is a shift in the peak temperature at a concentration ratio of 1:2, which seems to decrease by 0.6 °C (Fig. 3). Meanwhile, at a concentration ratio of 1:1, the peak shift was seen to increase by 0.5 °C. A shift in peak temperature due to the adulteration of pork content in food samples has been reported by Azir et al. [23]. In this study, a sample of butter adulterated with lard showed a peak temperature shift after adding lard.

In the experimental results, the mixed sample not only produces a shift in peak temperature, but the enthalpy also affects the ratio of the pork concentration in the mixed sample. The enthalpy of the unmixed pork sample at its characteristic endothermic peak is 1.275 J/g. In contrast, the mixed pork-beef sample with a concentration ratio of 2:1, 1:1, and 1:2 each has an enthalpy of 0.705, 0.445, and 0.114 J/g. In this case, the enthalpy at pork's characteristic minor endothermic peak is directly proportional to the concentration. This result is in line with the research of Nurrulhidayah et al. [28], where the enthalpy at the characteristic endothermic peak of the butter sample appears to decrease along with the decrease in sample concentration due to adulteration of lard added intentionally.

Mixing beef with other meat powders, such as chicken and rabbit, was carried out to ensure that each meat powder's characteristic minor endothermic peak would still appear in the peak temperature region after being mixed. The ratio of 2:1 was chosen so that the characteristic peak of each meat powder still appears with a peak that can still be seen so that it can be considered in distinguishing the beef mixture from each meat powder. In addition, the design of this study was also carried out to see the purity of beef, which can be observed from the DSC thermogram profile. Fig. 4 shows that the characteristic endothermic peak in beef at 229.1 °C is not visible in the DSC thermogram profile when mixed with other meat powders, even at the largest beef concentration (66.67%) in the beef-pork mixture (2:1) seen in Fig. 3 still does not show the characteristic endothermic peak of beef. This thing can be a marker of the presence of a mixture in the beef.

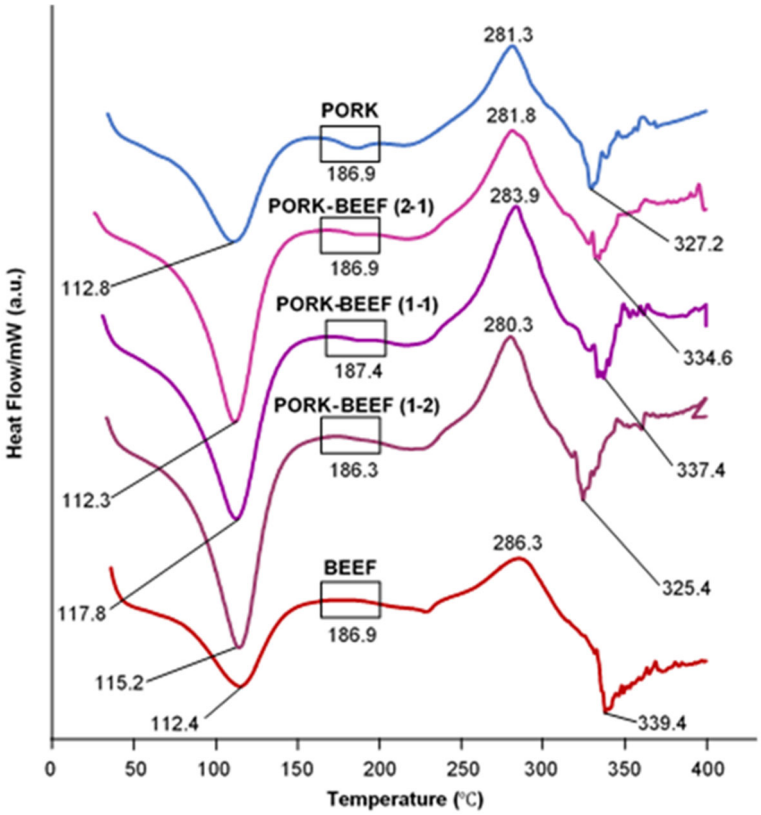


Fig 3. DSC thermogram profile of beef and pork mixing with comparison of 2:1, 1:1, and 1:2

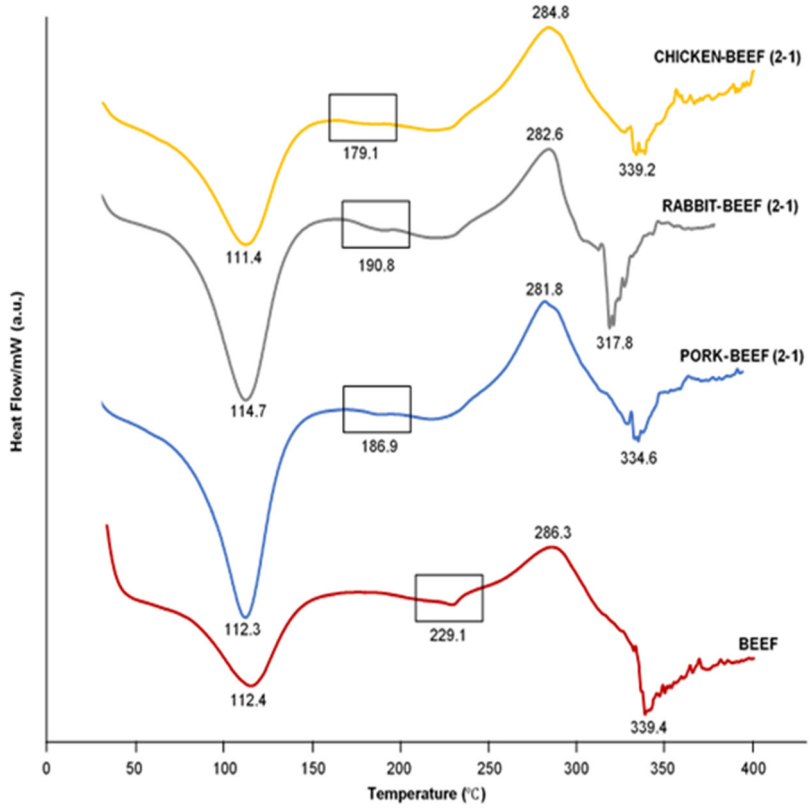


Fig 4. DSC thermogram profile of beef mixing with various meat in 2:1 comparison

From the experimental results, the characteristic minor endothermic peak in the peak temperature area of each meat powder still appears after being mixed. However, there is a shift in peak temperature and a reduction in enthalpy in each mixed sample. Fig. 4 shows that the characteristic peak temperature of chicken in the mixed sample is 179.1 °C, while the rabbit and pork are at 190.8 and 186.9 °C, respectively. The peak shift in characteristic endothermic peaks significantly occurred in the mixed sample containing rabbits, which experienced a peak decrease of 1.7 °C. In contrast, in the mixed sample containing chicken, the peak shift only occurred by 0.2 °C. However, the peak temperature does not change in the sample mixture of pork. The characteristic peak enthalpies of the pork, rabbit, and chicken samples before mixing were 1.275, 2.189, and 1.044 J/g, but after mixing, the enthalpies of pork, rabbit, and chicken were reduced to each of 0.705, 1.510, and 0.590 J/g.

Peak shifts and a decrease in enthalpy at the characteristic endothermic peak of a food sample due to adulteration of pork content have also been reported by Azir et al. [23]. In this study, a sample of butter added intentionally with lard showed a decrease in enthalpy along with a reduction in the concentration of butter due to the addition of concentration. Adulteration of lard, in addition to impurity carried out, can cause a shift in butter's characteristic minor endothermic peak [23].

DSC parameter data starting from Ton, Tp, Tof, and enthalpy were obtained from the processing of the thermogram profile of each sample using the ThermoPlus software integrated with DSC (Table 5). At the same time, the data range is the distance between Ton and Tof. Three peaks that appeared below 300 °C were chosen for chemometric analysis because they were peaks that could still be observed when electrothermal observations were carried out.

Table 5. DSC parameters obtained from thermograms of pure and mixed samples

Sample	Peak	DSC Parameters				
		Ton (°C)	Tp (°C)	Tof (°C)	Enthalpy (J g^{-1})	Range (°C)
Beef	1	83.1	112.4	141.1	−126.047	58.0
	2	223.5	229.1	234.4	−1.575	10.9
	3	231.0	286.3	312.7	76.548	81.7
Pork	1	81.1	112.8	136.5	−143.363	55.4
	2	170.9	186.9	195.3	−1.275	24.4
	3	248.2	281.3	299.2	145.267	51.0
Rabbit	1	79.5	109.5	133.2	−185.652	53.7
	2	175.1	192.5	199.6	−2.189	24.5
	3	244.5	284.5	298.7	77.295	54.2
Chicken	1	70.4	103.4	130.2	−129.579	59.8
	2	166.2	178.9	191.1	−1.044	24.9
	3	245.8	297.9	324.7	162.412	78.9
Pork-Beef (1:2)	1	92.5	115.2	136.4	−196.271	43.9
	2	180.3	186.3	193.4	−0.114	13.1
	3	247.9	280.3	321.4	116.892	73.5
Pork-Beef (1:1)	1	94.4	117.8	137.5	−229.405	43.2
	2	177.7	187.4	193.6	-0.445	13.9
	3	250.9	283.9	324.8	148.625	73.9
Pork-Beef (2:1)	1	86.5	112.3	134.7	−185.647	48.3
	2	176.7	186.9	194.8	−0.705	19.1
	3	245.7	281.8	325.4	139.132	79.7
Rabbit-Beef (2:1)	1	84.9	114.7	137.6	−182.283	52.7
	2	172.3	190.8	196.7	−1.510	24.4
	3	242.7	282.6	313.8	93.281	71.1

Sample	Peak	DSC Parameters				
		Ton (°C)	Tp (°C)	Tof (°C)	Enthalpy (J g^{-1})	Range (°C)
Chicken-Beef (2:1)	1	83.0	111.4	136.5	−129.711	53.5
	2	168.7	179.1	190.3	−0.590	21.6
	3	245.7	284.8	314.1	136.162	68.4

Ton: Temperature onset; Tp: Temperature peak; Tof: Temperature offset

Nine samples of pure and mixed meat powder were subjected to chemometric analysis using the PCA technique to see the differences in each sample based on the closeness of the score plots and the similarity of physicochemical properties. PCA is a technique for reducing the amount of data when there is a correlation present, and it is not helpful if the variables are uncorrelated [31].

To construct a validation model in adulteration studies, pork and beef mixtures were used as adulteration in the 0–100% w/w range. All DSC parameters in the thermogram, including onset, peak, offset, enthalpy, and range, are subjected to Partial Least Square (PLS) regression (Fig. 5). Table 6 shows the R^2, RMSE, and SE values obtained during cross-validation using the leave-one technique for validating chemometric models. The R^2 value for the actual value of pork is 0.9982, while the DSC prediction value (y-axis) is 0.9967; the results showing > 0.99 on both values illustrate the match between the predicted value and the actual value [29]. Furthermore, the RMSE and SE values for the calibration sample were lower than the validation sample, but the difference in values for the two samples was not too significant. The smaller the RMSE and SE values in the calibration and validation samples, the lower the error model will be developed [29].

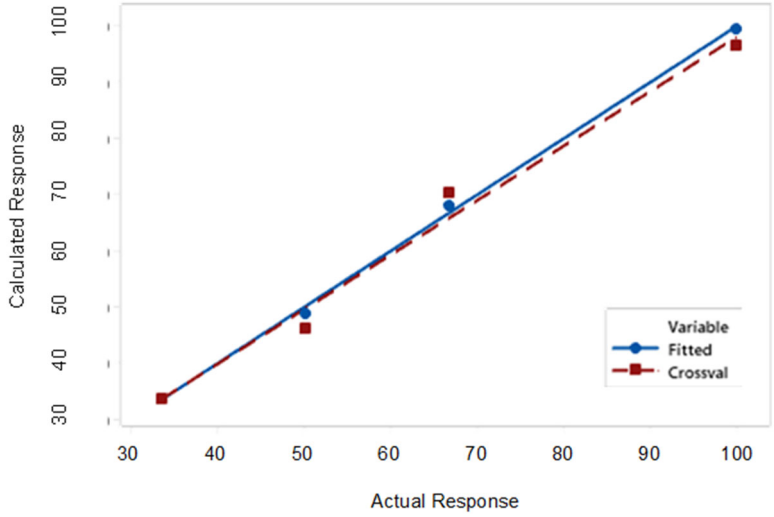


Fig 5. Scatterplot of actual vs predicted values of pork powder as an adulterant in beef powder using Partial Least Square Regression (PLSR)

Table 6. Multivariate statistical summary from DSC-PLSR calibration for characteristic minor endothermic peak thermograms of pork, and their admixtures with beef

Calibration models	Factor	Calibration			Validation		
		R^2	RMSEC	SEC	R^2	RMSEP	SEP
Characteristic minor endothermic peak PLS	2	0.9982	1.0248	4.2012	0.9967	3.0948	7.8516

RMSEC, root mean square error for calibration; RMSEP, root mean square error for prediction; SEC, standard error for calibration; SEP, standard error for prediction

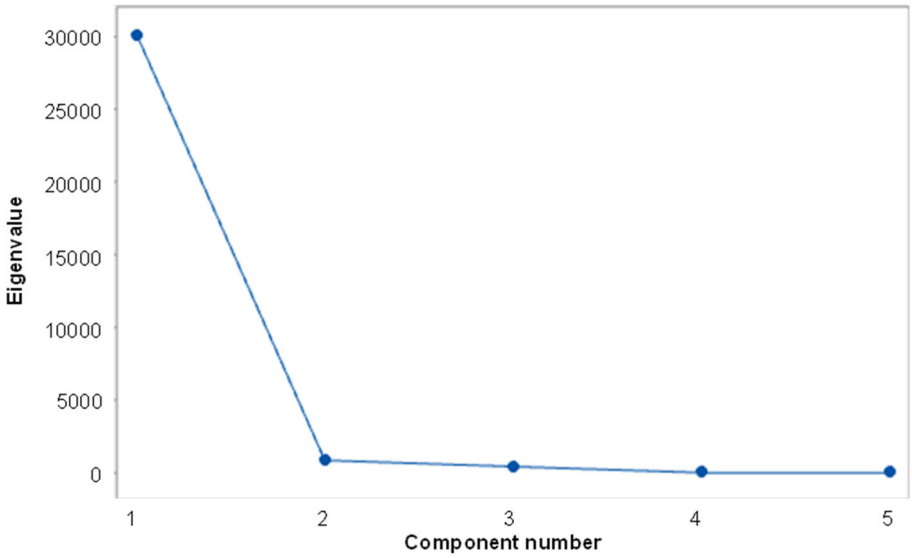


Fig 6. The results of the scree plot sample using PCA analysis

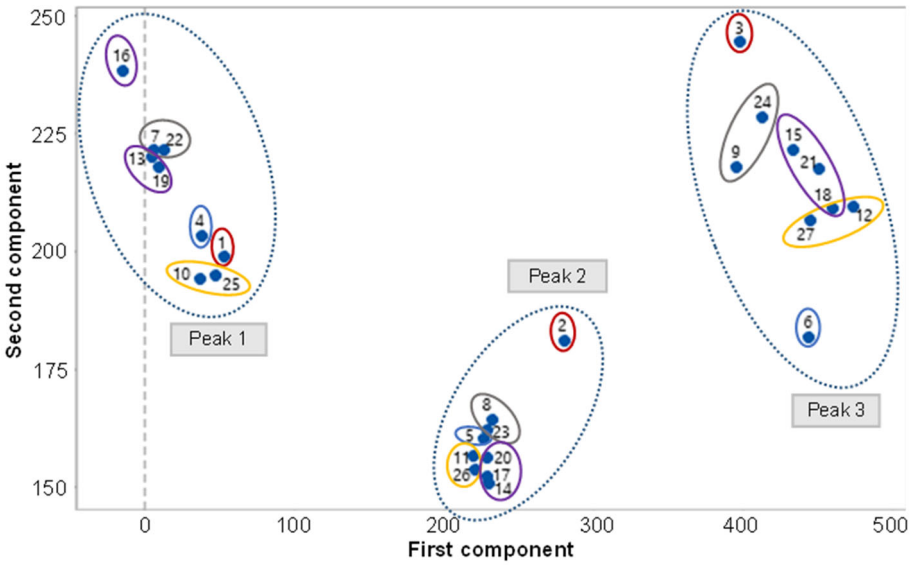


Fig 7. Score plot of pure and mixed samples. A peak of pure beef (red); A peak of pure pork (blue); A peak of beef and pork mix (violet); A peak of pure and mixed rabbit (grey); A peak of pure and mixed chicken (yellow)

The PCA score plot of the sample was described by the first and second principal components (PC1 and PC2). PC1 and PC2 explain the maximum variance of the entire sample with a value of 98.5% consisting of 95.8% PC1 and 2.7% PC2.

The result of PCA is referred to as PC and two or more samples with the same PC may be considered similar. The closest score plot between PC1 and PC2 shows the similarity of characteristics between the samples [32]. Fig. 7 shows three peaks of samples observed based on the closeness of the score plots and the similarity of the thermal characteristics. In pure samples of meat powder the score plot between PC1 and PC2 for each meat showed different results or there were differences in the thermal characteristics of each sample. This classification explains that the samples are divided into four different groups a fact that is not easy to see from the original data [31]. PCA is not only used for the classification of pure samples being analyzed but also to detect sample adulteration which in this study was

intentionally mixed between samples of meat powder to see differences in the thermal characteristics of pure and mixed meat. The shift in the score plot of the pure sample for each meat powder after being mixed with other meat (beef) can be a consideration in determining the purity of a sample, meaning that there are differences in the thermal characteristics of the pure sample and the mixed sample.

Previously the potential use of DSC in combination with multivariate calibration was reported to verify boar meat adulteration in processed food products, namely meatballs; these data support the effectiveness of DSC in analyzing and detecting wild boar meat adulteration. Which was successfully classified in meatball samples using the chemometric method of PCA [29]. In another study, the DSC and PCA methods successfully detected differences in samples of lard forgery into beef and chicken fat up to a concentration of 0.5%, as seen in the heating thermogram profile [30].

Based on the results of the research that has been carried out. It is hoped that the analytical method that has been developed can be used routinely for testing the halal-ness of food products, especially those made from meat. Suppose the sample has been appropriately classified. Halal authentication should only be sufficient until qualitative analysis. Still, to see the quantitative relationship between the sample and the emerging thermal characteristics. Further research is needed to see the sensitivity of the thermal analysis method that has been developed.

■ CONCLUSION

Developing a thermal analysis method with an environmentally friendly concept has succeeded in identifying differences in beef, pork, rabbit, and chicken. This method can determine the type of meat that is analyzed through the DSC thermogram profile, which is a thermal characteristic of each meat. The PCA technique has successfully classified meat samples based on their thermal characteristics. The difference in thermal characteristics between pure and mixed meat samples can also be seen clearly after chemometric analysis.

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■ AUTHOR CONTRIBUTIONS

Conception and design, I.N.; acquisition of data, A.; analysis and interpretation of data, I.N. and A.; took part in drafting the article or revising it critically for important intellectual content, I.N. and A.; agreed to submit to the current journal, A.; gave final approval of the version to be published, I.N. and A.; and agree to be accountable for all aspects of the work, I.N. and A.

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