第 49 卷 第 6 期
2022 年 6 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 6
June 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.6.19

A Rapid Analysis to Determine the Type of Meat using Fluorescence Spectrophotometry: Chicken Meat and Pork

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Abstract: A novel and rapid analysis of the type of meat using fluorescence spectrophotometry has been done. This method does not require complicated reagents and is environmentally friendly because it uses water as the solvent. The test sample was limited only to chicken meat and pork. Meat samples are taken from three chicken species, i.e., Broiler (Gallus domesticus), Domestic Chicken (Gallus gallus domesticus), Braekels Chicken (Gallus Turcicus), and Yorkshire pig (Sus scrofa Domesticus) species. Each sample was immersed in demineralized water. Then the extracted blood was analyzed using a fluorescence spectrophotometer. All types of samples exhibit a unique fluorescence spectra pattern. Furthermore, the mixed meat samples (pork and chicken) in various concentrations (0.5-30% of pork) were also analyzed. It was found that the excitation and emission peaks of mixed samples showed different spectra. This phenomenon can still be observed in a very low concentration of pork, 0.5%. The redshifted was observed, and the spectrum's intensity increased along with the concentration of pork. Scores of PCA (Principal Component Analysis) show four clusters of samples: Pork, Broiler, Domestic, and Braekels Chicken. This technique offers an efficient and accurate method to investigate meat contamination.

Keywords: meat contaminant, fluorescence spectroscopy, principal component analysis.

使用荧光分光光度法确定肉类类型的快速分析：鸡肉和猪肉

摘要：已经使用荧光分光光度法对肉类类型进行了新的快速分析。这种方法不需要复杂的试剂，而且因为它使用水作为溶剂，所以对环境友好。测试样品仅限于鸡肉和猪肉。肉样品取自三种鸡，即肉鸡 (家鸡)、家鸡、布雷克尔斯鸡 (鸡鸡) 和约克夏猪 (家畜)。将每个样品浸入软化水中。然后使用荧光分光光度计分析提取的血液。所有类型的样品都表现出独特的荧光光谱模式。此外，还分析了各种浓度（猪肉的 0.5-30%）的混合肉样品（猪肉和鸡肉）。发现混合样品的激发峰和发射峰呈现出不同的光谱。这种现象在非常低的猪肉浓度（0.5%

Received: March 24, 2022 / Revised: April 11, 2022 / Accepted: May 17, 2022 / Published: June 30, 2022

Fund Project: The Directorate General of KEMENRISTEKDIKTI (Ministry of Research, Technology and Higher Education of Republic of Indonesia) (The scheme *Penelitian Disertasi Doctor* with the scheme grant No. 1243/PKS/ITS/2020), *Beasiswa Pendidikan Pascasarjana Dalam Negeri* or BPPDN Scholarship (Grant No. 2693/E4/BP/2020), Institut Teknologi Sepuluh Nopember (Research scheme *Penelitian Kolaborasi Indonesia* with No. 1020/PKS/ITS/2019)

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）中仍然可以观察到。观察到红移，光谱强度随着猪肉浓度的增加而增加。主成分分析的分数显示了四组样本：猪肉、肉鸡、家养和布雷克尔鸡。该技术提供了一种有效且准确的方法来调查肉类污染。

关键词：肉类污染物，荧光光谱，主成分分析。

1. Introduction

Food labeling and authentication, as well as safety and quality, are greatly prioritized by industries because they play an important role in determining consumer choices[1]. They are also important to avoid unfair competition and assure consumer protection against the industry. Meat is one of the most commonly consumed foods worldwide and contains essential healthy nutrients, including proteins, compared to plants [2]. However, some individuals strictly avoid consuming certain types of meat for themselves. Hence, information related to traded products needs to be conveyed to consumers. So, labeling and authentication help protect consumers [3]. One of the meats that certain people avoid is pork widely traded. Pig is relatively easy to raise, has low maintenance costs, high yields, and high-fat content, giving a distinctive taste [3]. Pork consumption is forbidden for people who practice the Islamic and the Jewish faith [4]. 1.8 billion Muslims and Jews worldwide are certainly potential meat consumers. Additionally, certain groups feel pork is unhealthy due to its high-fat content [5].

There are two possibilities for someone buying an unwanted meat product: the first is meat contamination with other meat that occurs accidentally during production; the second is due to intentional product counterfeiting by the manufacturer [6]. This counterfeiting is detrimental to consumers and still occurs in the trade. The most common reason for counterfeiting is to downgrade the product quality so that the selling price is affordable and, in turn, can compete with other traders or get a more significant profit if one wants to sell at a regular price. The analysis of meat products has become an emerging challenge in overcoming this problem [7].

Methods for detecting the contamination of meat with pork are greatly needed. Currently, different techniques are used to determine pork contamination in various food products, specifically meat. The pork adulteration detection in cooked meatballs products using immunological strip tests has been reported [8]. Although this method is quick and simple, it needs laboratory sample preparation. Furthermore, ATR-FTIR spectroscopy coupled with chemometrics is a promising analytical technique for food quality studies [9]. It is quick, not destructive, and does not involve a complicated sample preparation process. This method is similar to the Fluorescence technique but costs a higher operational. LC combined with MS has also been reported to determine ingredients in livestock and poultry meat [10]. This method has high sample sensitivity and selectivity but needs specific reagents with a strenuous sample preparation procedure. Enzyme-linked immunosorbent assay (ELISA) to detect pork in meat products also has been reported, these method has sensitivity, specificity, and repeatability but requires expensive reagents and more time for sample treatment [11]. Real-time PCR fast detection assay for simultaneous pork, chicken, and beef has also been used for various products. Optical fiber and the electrochemical sensor were also developed for identifying pork contaminants in food [12,13]. The electrochemical sensor has good selectivity and sensitivity [14] but complicated sample preparation and expensive reagents [15,16]. Therefore, there is a need to develop a simple, fast, and relatively cheap method. In this paper, we reported a new method that is simple, fast, and inexpensive for distinguishing pork and chicken using spectrophotometry fluorescence. A similar technique has been reported in the biological and medical sciences [17–20]. The pork's fluorescence spectra characteristic can still be detected in mixed meat in low concentrations of pork as low as 0.5%. Before fluorescence measurement, the water extracted blood from meat and fresh whole blood from pork and chicken were directly taken and diluted by demineralized water. The methods were evaluated on sensitivity, repeatability, and PCA. The results show that the proposed methods can be used in the regular analysis.

2. Materials and Methods

2.1. Materials

The experimental samples of meat and blood from pork and chicken were taken from slaughterhouses at Sepanjang Market, Sidoarjo, East Java, Indonesia. Yorkshire Pig species was chosen for the Pork sample and labeled as “Pork”. Meanwhile, Broiler (Gallus Domesticus), Braekels (Gallus Turcicus), and Domestic (Gallus gallus Domesticus) were chosen for the chicken samples and labeled as “Broiler”, “Domestic”, and “Braekels “. These are the most common species available in the Indonesian market. The blood and meat samples are taken just after

slaughtering. A 120 µL of blood from each sample is mixed with 10 mL of demineralized water. It is kept in a vial, while 50 grams of meat from each sample is kept in a Ziplock bag. The meat is taken from pigs' and chickens' thighs. All the samples were kept in the icebox at 4°C and transferred to the laboratory. All samples were processed for analysis within 12 hours. The chemical reagents for fluorescence spectroscopy, including Sulfuric acid (H_2SO_4, 98%) and hydrogen peroxide (H_2O_2, 30%), were purchased from Merck, Germany, while Piranha solution (3 H_2SO_4: 1 H_2O_2) was used for all glass cleaning procedures.

2.2. Methods

2.2.1. Blood Sample Preparation

20 µL of each blood sample mentioned in section 1.1 was pipetted and then placed into a 100 mL volumetric flask and filled with demineralized water to the limit. This stock blood solution was diluted 100 times before measuring using spectrophotometer fluorescence.

2.2.2. Meat Samples Preparation

Ten grams of each meat sample mentioned in section 1.1 were cut into small pieces, placed in a beaker glass, and then immersed in 50 mL of demineralized water. The mixture was stirred using a magnetic stirrer for 5 minutes to extract the blood. Then, the mixture was filtered, and the filtrate was collected. Twenty microliters of the filtrate were pipetted and placed into a 100 mL volumetric flask, then added up with demineralized water to the limit. This stock meat extract solution was diluted 100 times before being measured using spectrophotometer fluorescence.

2.2.3. Preparation of Mixed Meat Sample

Mixed meat samples are made from pork and chicken meats (Broiler, Domestic, and Braekels Chicken) at various concentrations. The mixed meat samples were labeled "Mix". The percentage of pork was 0.5%, 1%, 10%, 20%, and 30% (w/w) with the total weight of 10 grams. This procedure was repeated for all three distinct types of chicken samples. The total weight of mixed meat was 10 grams.

2.3. Fluorescence Spectrophotometry Measurement

The blood and meat samples were analyzed using Perkin Elmer LS 55 fluorescence spectroscopy equipped with FL Winlab software [21]. The parameter of measurement was set as follows: Scan speed condition = 500 nm/min, $Slit_{Ex/Em}$ = 10nm/10nm. To determine the maximum excitation and emission parameters, the Prescan was performed at λ_{Ex} = 200-800 nm and λ_{Em} = 200-900 nm. This parameter was used for all measurements [22]. All measurement was repeated five times, and all preparation samples were repeated three times for each species. One-way Analysis of Variance (ANOVA, p=0.05) followed by Least Significant Difference (LSD) was performed to see the significant difference between the group. The Principal Component Analysis (PCA) method was also used to determine the grouping based on the sample species.

3. Results

3.1. Fluorescence Spectra of Blood and Extracted Blood Sample

The fluorescence spectra of blood (red line) and extracted blood (black line) from all samples are shown in Fig. 1. Fig. 1a shows fluorescence spectra for pork, whereas broiler, domestic, and braekels chicken samples are shown in Fig. 1b, 1c, and 1d, respectively. The fluorescence spectra from blood and extracted blood for the same sample type show the same excitation and emission wavelength. The exact values of emission and excitation peak wavelength are summarized in Table 1.

Table 1 Excitation and emission wavelength of blood and extracted blood

Sample	Blood Wavelength (nm)			Extracted blood Wavelength (nm)		
	Ex	Em_1	Em_2	Ex	Em_1	Em_2
Pork 1	309.2 ± 0.24	309.2 ± 0.24	620.1 ± 0.22	309.1 ± 0.22	309.3 ± 0.25	620.2 ± 0.24
Pork 2	309.2 ± 0.24	309.3 ± 0.25	620.1 ± 0.22	309.1 ± 0.23	309.1 ± 0.29	620.2 ± 0.26
Pork 3	309.2 ± 0.24	309.1 ± 0.22	620.1 ± 0.22	309.1 ± 0.22	309.1 ± 0.22	620.1 ± 0.22
Means	309.2 ± 0.24	309.1 ± 0.23	620.1 ± 0.22	309.1 ± 0.22	309.1 ± 0.25	620.2 ± 0.24
Broiler 1	330.2 ± 0.24	330.1 ± 0.22	678.1 ± 0.22	330.2 ± 0.24	330.1 ± 0.22	678.1 ± 0.22
Broiler 2	330.1 ± 0.22	330.2 ± 0.24	678.2 ± 0.24	330.1 ± 0.21	330.1 ± 0.23	678.2 ± 0.23
Broiler 3	330.2 ± 0.24	330.2 ± 0.24	678.2 ± 0.24	330.2 ± 0.24	330.2 ± 0.22	678.2 ± 0.22
Means	330.2 ± 0.23	330.2 ± 0.23	678.2 ± 0.23	330.2 ± 0.23	330.1 ± 0.22	678.2 ± 0.22
Domestic 1	334.1 ± 0.25	334.2 ± 0.25	663.2 ± 0.24	334.2 ± 0.25	334.2 ± 0.25	663.2 ± 0.24
Domestic 2	334.2 ± 0.23	334.3 ± 0.24	663.2 ± 0.24	334.1 ± 0.24	334.4 ± 0.26	663.2 ± 0.24
Domestic 3	334.2 ± 0.25	334.3 ± 0.25	663.1 ± 0.22	334.2 ± 0.25	334.3 ± 0.25	663.1 ± 0.25
Means	334.2 ± 0.24	334.3 ± 0.25	663.2 ± 0.24	334.2 ± 0.25	334.3 ± 0.25	663.2 ± 0.24
Braekels 1	332.2 ± 0.24	332.2 ± 0.24	658.4 ± 0.24	332.2 ± 0.24	332.1 ± 0.22	658.3 ± 0.24
Braekels 2	332.2 ± 0.24	332.2 ± 0.24	658.3 ± 0.23	332.2 ± 0.24	332.2 ± 0.24	658.1 ± 0.23
Braekels 3	332.3 ± 0.25	332.1 ± 0.22	658.2 ± 0.25	332.1 ± 0.22	332.1 ± 0.22	658.2 ± 0.22
Means	332.2 ± 0.24	332.2 ± 0.23	658.3 ± 0.24	332.2 ± 0.23	332.1 ± 0.23	658.2 ± 0.23

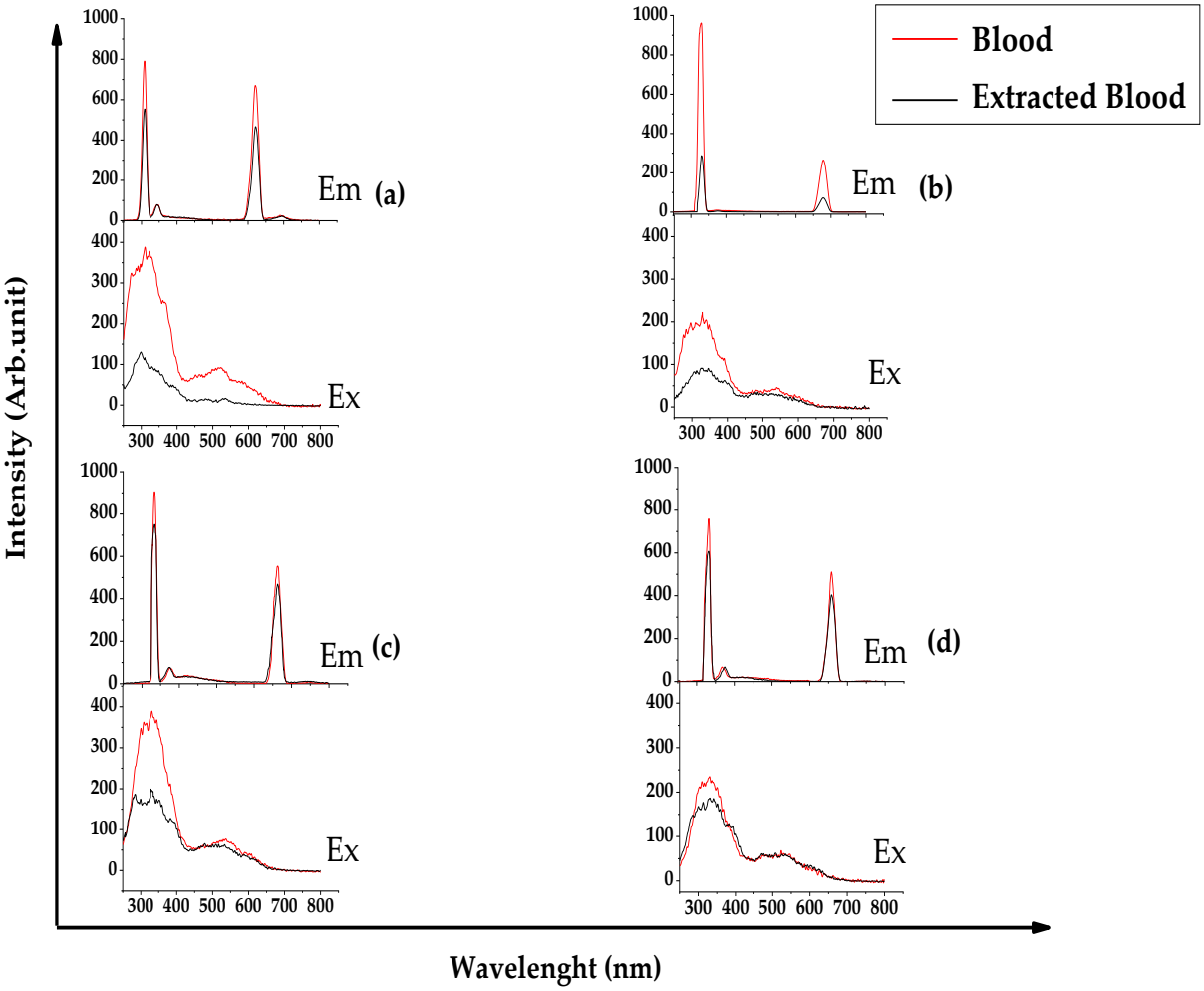


Fig. 1 The fluorescence spectra of pork (a), broiler (b), domestic (c), and braekels (d) with a red line and black line are for blood and extracted blood. Ex and Em are for excitation and emission wavelength, respectively

3.2. PCA Analysis Data for Blood and Extracted Blood Samples

Principal Component Analysis (PCA) data analysis reduces the number of variables in a matrix by finding the selected principal component with the highest variation in the data set. The second principal component is perpendicular to the first principal component, with the next highest variation [23]. The PCs are composed of scores, where the first represents the variance in sample direction, thus being used to assess similarities/dissimilarities among the samples. The latter represents the contribution of each variable for the model decomposition, thus being used to find critical spectral markers. This technique looks for inherent similarities/differences and provides a scores matrix representing each sample's overall "identity". The score information can be used for exploratory analysis providing classification between data classes.

PCA analysis data in this work were calculated from all sample's fluorescence spectra of blood and extracted blood (pork, broiler, domestic, and braekels). There are 15 repetitions for each sample. The results of the PCA score for excitation and emission spectra can be seen in Fig. 2 and 3, respectively; Part (a) is for blood, and part (b) is for extracted blood. The score on PC1 shows the bands responsible for separation wavelength between pork, broiler, domestic chicken, and braekels chicken. The score on PC2 shows the bands responsible for spectra intensity, representing component concentration from each sample. The pattern of grouping samples using PCA has distinguished each type of sample. Based on the plot obtained, pork (red dot), broiler (green dot), domestic (blue dot), and braekels (black dot) have different characteristics. The closer one point to another increases the similarity of the sample spectrum.

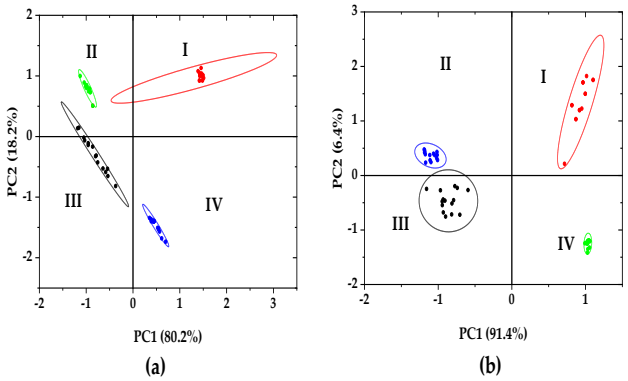


Fig. 2 PCA score for excitation spectra of blood (a) and extracted blood (b). (● Braekels) (● Pork) (● Broiler) (● Domestic)

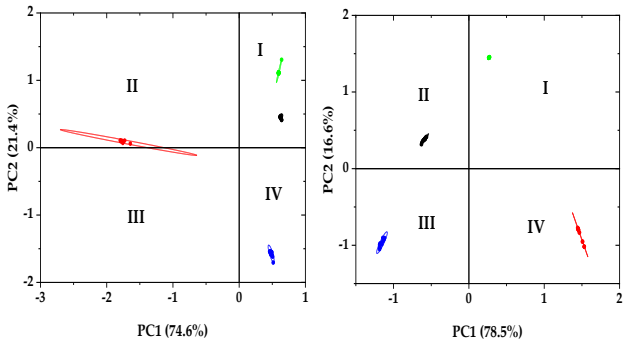


Fig. 3 PCA score for emission spectra of blood (a) and extracted blood (b). (● Braekels) (● Pork) (● Broiler) (● Domestic)

3.3. Fluorescence Spectra Mixed Meat Samples

The mixed samples from section 1.2.3 were measured using spectrophotometer fluorescence. The spectra obtained are overlaid for each type of mixed meat (for 0.5%; 1%; 10%; 20%; and 30% pork concentration) to analyze the changes in the fluorescence spectra. The influence of pork concentration on each type of chicken meat can be seen in Fig. 4, 5, and 6 for Broiler, Domestic, and Braekels chickens, respectively, which (a) is for excitation spectra, and (b) is for emission spectra. The detailed shift in wavelength can be seen in Table 2. Sample repetition to get the deviation standard uses the lowest concentration of pork (0.5%).

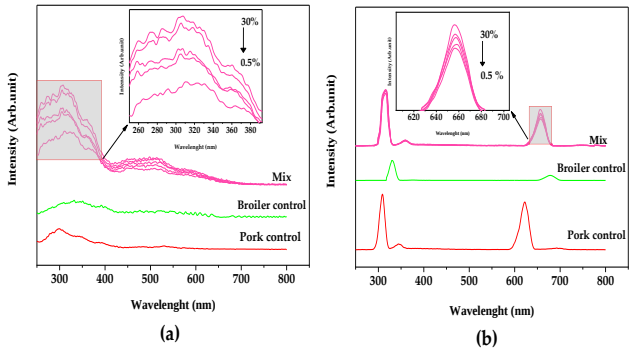


Fig. 4 The excitation (a) and emission (b) peaks of meat from pork control, broiler control, and mixed meat of broiler with pork concentrations of 0.5%; 1%; 10%; 20%; and 30%

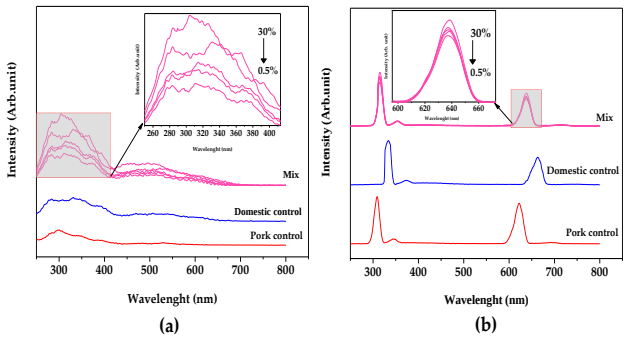


Fig. 5 The excitation (a) and emission (b) peaks of meat from pork control, domestic control, and mixed meat domestic with pork concentrations of 0.5%; 1%; 10%; 20%; and 30%

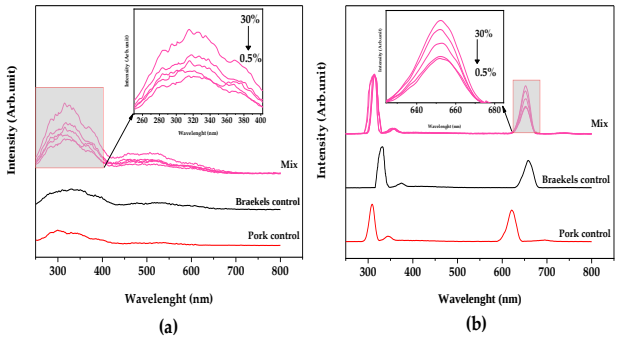


Fig. 6 The excitation (a) and emission (b) peaks of meat from pork control, braekels control, and mixed meat of braekels with pork concentrations of 0.5%; 1%; 10%; 20%; and 30%

Table 2 Excitation and emission wavelength mixed meat

Species	Control Meat Sample Wavelength (nm)	Chicken meat mixture with pork Wavelength (nm)	Description
Pork	309.0 ± 0.22	-	Excitation
	309.0 ± 0.26	-	Emission 1
	620.0 ± 0.24	-	Emission 2
Broiler	330 ± 0.23	316.2 ± 0.25	Excitation
	330 ± 0.23	316.2 ± 0.24	Emission 1
	678 ± 0.23	657.0 ± 0.25	Emission 2
Domestic	334 ± 0.25	315.0 ± 0.25	Excitation
	334 ± 0.25	315.2 ± 0.25	Emission 1
	663 ± 0.24	638.2 ± 0.25	Emission 2
Braekels	332 ± 0.23	314.2 ± 0.24	Excitation
	332 ± 0.23	314.2 ± 0.24	Emission 1
	658 ± 0.23	652.2 ± 0.25	Emission 2

3.4. PCA Data Analysis for Mixed Meat Samples

The PCA data analysis for each type of mixed meat (0.5%; 1%; 10%; 20%; and 30% of pork) from broiler, domestic, and braekels chickens are presented in Fig. 7, 8, and 9, respectively, in which (a) is for excitation spectra, and (b) is for emission spectra. The red dot is for the positive control (pork), while the negative control is green (chickens). Orange, purple, yellow, brown, and pink dots are 0.5, 1, 10, 20, and 30%. The score on PC1 shows the bands responsible for meat control and mixed meat concentration between pork and chicken type. The score on PC2 shows the bands' spectra intensity, representing component concentration from each sample.

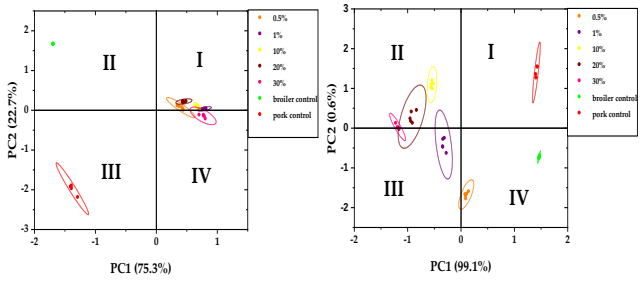
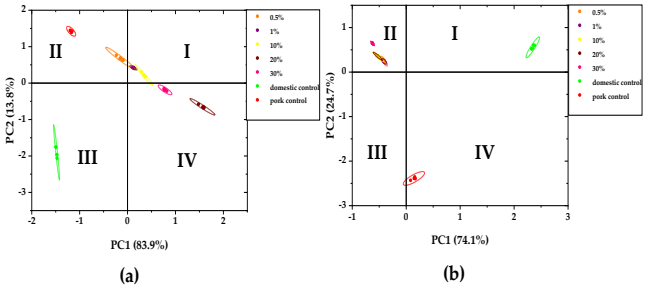
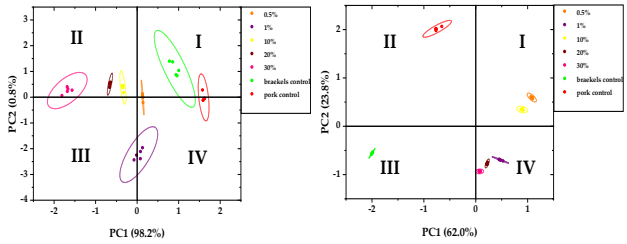


Fig. 7 PCA score for meat: excitation (a) and emission (b) for broiler control (●), pork control (●) and mixed meat between broiler with pork concentration of 0.5% (●); 1% (●); 10% (●); 20% (●); and 30% (●)



(a) (b)

Fig. 8 PCA score for meat: excitation (a) and emission (b) for domestic control (●), pork control (●) and mixed meat between domestic with pork concentration of 0.5% (●); 1% (●); 10% (●); 20% (●); and 30% (●)



(a) (b)

Fig. 9 PCA score for meat: excitation (a) and emission (b) for braekels control (●), pork control (●) and mixed meat between braekels with pork concentration of 0.5% (●); 1% (●); 10% (●); 20% (●); and 30% (●)

4. Discussion

4.1. Performance Fluorescence for Identifying Blood and Extracted Blood

Previous studies have been developed to identify meat from various species using the fluorescence method [24–26]. Consequently, this study aims to determine the characteristics of blood and meat sample. Demineralized water was used for the sample preparation because it is a universal solvent and does not pollute the environment. With a fluorescence spectrophotometer, our previous study used demineralized water as a solvent to characterize organic compounds in Andrographis paniculata L. [19]. A similar solvent is used to characterize the meat in this research since blood easily dissolves in water.

Table 3 Amino acid composition from pork and chicken blood in mg/g protein [27]

Parameter	Pork Bloods	Chicken Blood
Essential Amino Acid		
Leucine	116.30	94.75
Lysine	86.04	75.98
Histidine	57.82	48.07
Phenylalanine	57.49	56.00
Tyrosine	25.83	31.34
Non-Essential Amino Acid		
Aspartic Acid	111.25	79.00
Alanine	71.49	67.48
Glycine	40.64	35.57

The fluorophores group in the sample generates fluorescence spectra. The farther the kinship between species, the further the difference in content and blood conditions they have. The closer the kinship between species, the closer the content and condition of the blood. For this reason, we developed a simple method to distinguish between species. We try to compare the spectra obtained from the spectrophotometer's measurement with the help of PCA calculation. We proposed a method to differentiate materials based on their physicochemical properties. When applied light to the blood sample at 200-800 nm, some light will be absorbed, and then some energy will be used to do vibration relaxation (a non-radiative process). The next step is an emission process (radiative process) which has lower energy than the energy absorbed in the absorption process [28]. This fluorescent activity is unique for every material, including the blood of an animal. [29,30]. There was a slight difference in the energy absorbed and emitted by the *fluorophore* group of the blood from different species (Fig. 1). Before comparing blood from different species, we also prove that the blood (red line, Fig. 1) and extracted blood from the meat (black line, Fig. 1) have no significant difference in the peak fluorescence spectra. The significance test between blood and extracted blood for each species is summarized in Table S1. All null hypothesis was accepted. So, the analysis of blood and meat gives the same result. The actual application to get a meat sample is more convenient than a blood sample. More information from this data shows we have the potency to distinguish various chicken species, but it needs more samples for justification.

The PCA score supports the result, as shown in Fig. 2 (excitation) and 3 (emission). Each type of sample can be distinguished. The closer one point to another increases the similarity of the spectra. The exact value of the excitation and emission are presented in Table 2. Standard deviation, ANOVA with LSD, and significant test calculation are performed and summarized in Table S2. These results show a clear separation between each sample. We can distinguish not only pork from chicken meat but also can distinguish the origin of each type of chicken meat itself. The differences in the amino acids and other compounds that make up blood probably cause these different spectra between samples. In this analysis, the fluorescence spectra correspond to the fluorophore group contained in the blood. A higher number of fluorophores affects the spectra' intensity value absorbed and emitted from the sample. In this case, pork blood has more significant amounts of the essential and non-essential amino acids (Table 3), so the fluorophore group is also higher than Broiler, Domestic, and Braekels Chicken blood. On the other side, the type of fluorophore group determines the amount of energy absorbed and emitted, presented in the wavelength. Pork blood absorbs at a shorter wavelength compared to chicken blood.

4.2. Performance Fluorescence for Pork Contaminant Determination in Mixed Meat Samples

Fluorescence spectroscopy is successfully applied to distinguish every pure sample (i.e., pork, broiler, domestic, and braekels). This section would like to analyze if we mix pork with various chicken meat and then analyze using the same method. We see a decrease in wavelength when mixing pork with chicken meat (Table 2). The intensity increases successively as the concentration of pork increases in the mixing meat (Fig. 4, 5, and 6). So, we conclude that pork contamination in chicken meat mixtures can be identified by shifting the wavelength to the lower wavelength. The detection limit of this method achieves 0.5%, according to the smallest amount of pork added to the chicken sample. This result is also supported by the PCA score data shown in Fig. 7, 8, and 9. PCA data analysis separates pure meat (100% chicken meat) and mixed meat samples. There have been no reports of using this method to analyze adulteration or contamination of meat with other meats, while other methods are summarized in Table 4. Fluorescence analysis in distinguishing between meat is faster and simpler than other methods. Moreover, this study has another advantage as it eliminates reagent and complicated preparation steps, thereby shortening the measurement process's duration and cutting the operation cost. The whole process of analysis using this method is illustrated in Fig. 10.

Table 4 Comparison study of the fluorescence analysis with several methods

Method	Preparation step	Measurement time	Reference
Immunological strip test	• Need time to fabricate the device and preparation steps • Non-reusable	5 minutes	[8]
ATR-FTIR spectroscopy coupled with chemometrics	• Need Specific reagent for isolation sample • Need more time for sample preparation	30 minutes	[9]
LC method combined with MS	• Specific Reagen • Long time Preparation sample	25 minutes	[10]
Enzyme-linked immunosorbent assay ELISA	• Specific Reagen • Complicate preparation sample	1 hour 10 minutes	[11]
Real-time PCR	• Expensive Reagen • Complicate preparation sample	40 minutes	[16]
Fluorescence analysis	• Simple Preparation • No reagent	4 minutes	This research

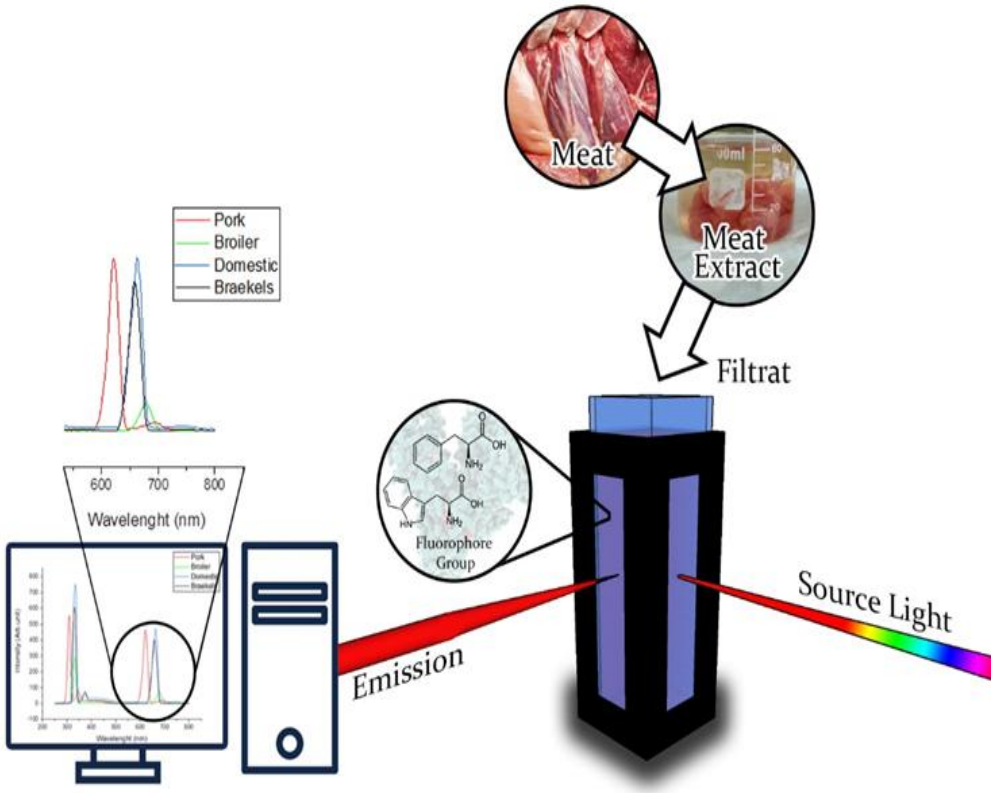


Fig. 10 Illustration of fluorescence process for meats determination

5. Conclusion

Spectroscopy fluorescence has been applied successfully to identify adulteration or contamination. This technique has not been used to be applied to identify adulteration meat. This method has the advantages of fast, simple, green, uncomplicated preparation, and low cost. The analysis utilizes the physicochemical characteristic of the blood sample. The blood sample can be obtained from the blood or extracted blood from the meat. The blood and extracted blood have been proved to have similar characteristics. This behavior is very useful to be used to differentiate between meat samples. We have successfully differentiated pork, Broiler, Domestic, and Braekels from their excitation and emission spectra.

Furthermore, we have successfully detected pork contamination to 0.5% in the meat adulteration case. PCA analysis also helps to show the clear mapping of data distribution between samples. In this study, we do research only on mixing chicken meat with pork. Other types of meat, such as beef or lamb, can give different mapping.

Acknowledgments

The authors thank the Directorate General of KEMENRISTEKDIKTI (Ministry of Research, Technology and Higher Education of Republic of Indonesia) for research funding under the scheme *Penelitian Disertasi Doctor* with the scheme grant number 1243/PKS/ITS/2020. This project was also supported by *Beasiswa Pendidikan Pascasarjana Dalam Negeri* or BPPDN scholarship (grant number 2693/E4/BP/2020) and Institut Teknologi Sepuluh Nopember under research scheme *Penelitian Kolaborasi Indonesia* with number 1020/PKS/ITS/2019.

References

[1] NG P. C., AHMAD RUSLAN N. A. S, CHIN L. X., AHMAD M., ABU HANIFAH S., ABDULLAH Z., and KHOR S. M. Recent advances in halal food authentication: Challenges and strategies. J*ournal of Food Science*, 2022, 87: 8–35. https://doi.org/10.1111/1750-3841.15998

[2] MABOOD F., BOQUÉ R., ALKINDI ABDULAZI Y., AL-HARRASI A., AL AMRI I. S., BOUKRA S., JABEEN F., HUSSAIN J., ABBAS G., NAUREEN Z., HAQ Q. M. I., SHAH H. H., KHAN A., KHALAF S. K., and KADIM I. Fast detection and quantification of pork meat in other meats by reflectance FT-NIR spectroscopy and multivariate analysis. *Meat Science*, 2020, 163: 108084. https://doi.org/10.1016/j.meatsci.2020.108084

[3] NUGRAHA W. S., CHEN D., and YANG S.-H. The effect of a Halal label and label size on purchasing intent for non-Muslim consumers. *Journal of Retailing and Consumer Services*, 2022, 65: 102873. https://doi.org/10.1016/j.jretconser.2021.102873

[4] YOUSAF S., & XIUCHENG F. Halal culinary and tourism marketing strategies on government websites: A preliminary analysis. *Tourism Management*, 2018, 68: 423–43. https://doi.org/10.1016/j.tourman.2018.04.006

[5] LIN-SCHILSTRA L., BACKUS G., SNOEK H., and MÖRLEIN D. Consumers' view on pork: Consumption motives and production preferences in ten European Union and four non-European Union countries. *Meat Science*, 2022, 187: 108736. https://doi.org/10.1016/j.meatsci.2022.108736

[6] MUHAMMAD A., AB TALIB M. S., HUSSEIN M. Z. S. M., and JAAFAR H. S. Motivations to Implement Halal Logistics Management Standards: A Review. In: MUHAMMAD HASHIM N., MD SHARIFF N. N., MAHAMOOD S. F., FATHULLAH HARUN H. M., SHAHRUDDIN M. S., and BHARI A. (eds.) *Proceedings of the 3rd International Halal Conference (INHAC 2016)*. Springer Singapore, Singapore, 2018: 333–342. https://doi.org/10.1007/978-981-10-7257-4_30

[7] LUBIS H. N., MOHD-NAIM N. F., ALIZUL N. N., and AHMED M. U. From market to food plate: Current trusted technology and innovations in halal food analysis. *Trends in Food Science & Technology*, 2016, 58: 55–68. https://doi.org/10.1016/j.tifs.2016.10.024

[8] KUSWANDI B., GANI A. A., and AHMAD M. Immuno strip test for detection of pork adulteration in cooked meatballs. *Food Bioscience*, 2017, 19: 1–6. https://doi.org/10.1016/j.fbio.2017.05.001

[9] BALAN B., DHAULANIYA A. S., JAMWAL R., AMIT, SODHI K. K., KELLY S., CANNAVAN A., and SINGH D. K. Application of Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy coupled with chemometrics for detection and quantification of formalin in cow milk. *Vibrational Spectroscopy*, 2020, 107: 103033. https://doi.org/10.1016/j.vibspec.2020.103033

[10] HE L., MA J., LI Q., WANG L., FAN S., and ZHANG Y. Determination of ingredients in livestock and poultry meat based on liquid chromatography-tandem mass spectrometry. *Journal of Future Foods*, 2022, 2: 53–60. https://doi.org/10.1016/j.jfutfo.2022.03.017

[11] THIENES C. P., MASIRI J., BENOIT L. A., BARRIOS-LOPEZ B., SAMUEL S. A., MESHGI M. A., COX D. P., DOBRITSA A. P., NADALA C., and SAMADPOUR M. Quantitative Detection of Chicken and Turkey Contamination in Cooked Meat Products by ELISA. *Journal of AOAC International*, 2019, 102: 557–563. https://doi.org/10.5740/jaoacint.18-0136

[12] PUSPITA I., IRAWATI N., MADURANI K. A., KURNIAWAN F., KOENTJORO S., and HATTA A. M. Graphene- and Multi-Walled Carbon Nanotubes-Coated Tapered Plastic Optical Fiber for Detection of Lard Adulteration in Olive Oil. *Photonic Sensors*, 2022, 12: 220411. https://doi.org/10.1007/s13320-022-0652-y

[13] KURNIAWAN F., NUGROHO A., BASKARA R. A., CANDLE L., PRADINI D., MADURANI K. A., SUGIARSO R. D., and JUWONO H. Rapid analysis to distinguish porcine and bovine gelatin using PANI/NiO nanoparticles modified Quartz Crystal Microbalance (QCM) sensor. *Heliyon*, 2022, 8: e09401. https://doi.org/10.1016/j.heliyon.2022.e09401

[14] ZULKARNAIN Z., SUPRAPTO S., ERSAM T., and KURNIAWAN F. A Novel Selective and Sensitive Electrochemical Sensor for Insulin Detection. *Indonesian Journal of Electrical Engineering and Computer Science*, 2016, 3: 496–502. http://doi.org/10.11591/ijeecs.v3.i3.pp496-502

[15] CAI Y., HE Y., LV R., CHEN H., WANG Q., and PAN L. Detection and quantification of beef and pork materials in

meat products by duplex droplet digital PCR. *PLoS One*, 2017, 12: e0181949. https://doi.org/10.1371/journal.pone.0181949
[16] KIM M.-J., & KIM H.-Y. A fast multiplex real-time PCR assay for simultaneous detection of pork, chicken, and beef in commercial processed meat products. *LWT*, 2019, 114: 108390. https://doi.org/10.1016/j.lwt.2019.108390
[17] MADURANI K. A., SUPRAPTO S., SYAHPUTRA M. Y., PUSPITA I., MASUDI A., RIZQI H. D., A. M. HATTA, J. JUNIASTUTI, M. I. LUSIDA, and KURNIAWAN F. Review—Recent Development of Detection Methods for Controlling COVID-19 Outbreak. *Journal of The Electrochemical Society*, 2021, 168: 037511. https://doi.org/10.1149/1945-7111/abe9cc
[18] SÁ M., BERTINETTO C. G., FERRER-LEDO N., JANSEN J. J., WIJFFELS R., CRESPO J.G., BARBOSA M., and GALINHA C. F. Fluorescence spectroscopy and chemometrics for simultaneous monitoring of cell concentration, chlorophyll and fatty acids in Nannochloropsis oceanica. *Scientific Reports*, 2020, 10: 7688. https://doi.org/10.1038/s41598-020-64628-7
[19] FARDIYAH Q., ERSAM T., SUYANTA, SLAMET A., SUPRAPTO, and KURNIAWAN F. New potential and characterization of Andrographis paniculata L. Ness plant extracts as photoprotective agent. *Arabian Journal of Chemistry*, 2020, 13: 8888–89897. https://doi.org/10.1016/j.arabjc.2020.10.015
[20] MADURANI K. A., SUPRAPTO S., YUDHA SYAHPUTRA M., PUSPITA I., HADI FURQONI A., PUSPASARI L., ROSYIDAH H., HATTA A. M., JUNIASTUTI, LUSIDA M. I., TOMINAGA M., and KURNIAWANA F. Fluorescence spectrophotometry for COVID-19 determination in clinical swab samples. *Arabian Journal of Chemistry*, 2022, 8(15): 104020. https://doi.org/10.1016/j.arabjc.2022.104020
[21] CASALE M., PASQUINI B., HOOSHYARI M., ORLANDINI S., MUSTORGI E., MALEGORI C., TURRINI F., ORTIZ M. C., SARABIA L. A., and FURLANETTO S. Combining excitation-emission matrix fluorescence spectroscopy, parallel factor analysis, cyclodextrin-modified micellar electrokinetic chromatography and partial least squares class-modelling for green tea characterization. *Journal of Pharmaceutical and Biomedical Analysis*, 2018, 159: 311–317. https://doi.org/10.1016/j.jpba.2018.07.001
[22] PRASAD S., MANDAL I., SINGH S., PAUL A., MANDAL B., VENKATRAMANI R., and SWAMINATHAN R. Near UV-Visible electronic absorption originating from charged amino acids in a monomeric protein. *Chemical Science*, 2017, 8: 5416–5433. https://doi.org/10.1039/C7SC00880E
[23] JOLLIFFE I. T., & CADIMA J. Principal component analysis: a review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 2016, 374: 20150202. https://doi.org/10.1098/rsta.2015.0202
[24] AMERICAN SOCIETY OF AGRICULTURAL AND BIOLOGICAL ENGINEERS. *Assessment of pork and poultry meat and bone meal using hyperspectral fluorescence imaging. 2016 ASABE International Meeting*, 2016. https://doi.org/10.13031/aim.20162462786
[25] WU B., DAHLBERG K., GAO X., SMITH J., and BAILIN J. A Rapid Method Based on Fluorescence Spectroscopy for Meat Spoilage Detection. *International Journal of High Speed Electronics and Systems*, 2018, 27: 1840025. https://doi.org/10.1142/S0129156418400256
[26] ISLAM K. Z., AHASAN M. A. A., HOSSAIN M. S., RAHMAN M. H., MOUSUMI U. S., and ASADUZZAMAN M. A Smart Fluorescent Light Spectroscope to Identify the Pork Adulteration for Halal Authentication. *FNS*, 2021, 12: 73–89. https://doi.org/10.4236/fns.2021.121007
[27] SORAPUKDEE S., & NARUNATSOPANON S. Comparative Study on Compositions and Functional Properties of Porcine, Chicken and Duck Blood. *Korean Journal for Food Science of Animal Resources*, 2017, 37: 228–241. https://doi.org/10.5851/kosfa.2017.37.2.228
[28] SKOOG D. A., HOLLER F. J., and CROUCH S. R. *Principles of Instrumental Analysis*. Brooks Cole, Belmont, 2017.
[29] SHRIRAO A. B., SCHLOSS R. S., FRITZ Z., SHRIRAO M. V., ROSEN R., and YARMUSH M. L. Autofluorescence of blood and its application in biomedical and clinical research. *Biotech & Bioengineering*, 2021, 118: 4550–4576. https://doi.org/10.1002/bit.27933
[30] LECRENIER M.-C., BAETEN V., TAIRA A., and ABBAS O. Synchronous fluorescence spectroscopy for detecting blood meal and blood products. *Talanta*, 2018, 189: 166–173. https://doi.org/10.1016/j.talanta.2018.06.076

参考文:

[1] NG P. C., AHMAD RUSLAN N. A. S, CHIN L. X., AHMAD M., ABU HANIFAH S., ABDULLAH Z., 和 KHOR S. M. 清真食品认证的最新进展：挑战和策略。食品科学杂志, 2022, 87: 8–35. https://doi.org/10.1111/1750-3841.15998
[2] MABOOD F., BOQUÉ R., ALKINDI ABDULAZI Y., AL-HARRASI A., AL AMRI I. S., BOUKRA S., JABEEN F., HUSSAIN J., ABBAS G., NAUREEN Z., HAQ Q. M. I., SHAH H. H., KHAN A., KHALAF S. K., 和 KADIM I. 通过反射傅里叶变换近红外光谱和多变量分析快速检测和量化其他肉类中的猪肉。 肉类科学, 2020, 163: 108084. https://doi.org/10.1016/j.meatsci.2020.108084
[3] NUGRAHA W. S., CHEN D., 和 YANG S.-H. 清真标签和标签尺寸对非穆斯林消费者购买意愿的影响。 零售与消费者服务杂志, 2022, 65: 102873. https://doi.org/10.1016/j.jretconser.2021.102873
[4] YOUSAF S., 和 XIUCHENG F. 政府网站上的清真烹饪和旅游营销策略：初步分析。旅游管理, 2018, 68: 423–43. https://doi.org/10.1016/j.tourman.2018.04.006
[5] LIN-SCHILSTRA L., BACKUS G., SNOEK H., 和 MÖRLEIN D. 消费者对猪肉的看法：十个欧盟国家和四个非欧盟国家的消费动机和生产偏好。 肉类科学, 2022, 187: 108736. https://doi.org/10.1016/j.meatsci.2022.108736
[6] MUHAMMAD A., AB TALIB M. S., HUSSEIN M. Z. S. M., 和 JAAFAR H. S. 实施清真物流管理标准的动机：回顾。在：MUHAMMAD HASHIM N., MD SHARIFF N.

N., MAHAMOOD S. F., FATHULLAH HARUN H. M., SHAHRUDDIN M. S., 和 BHARI A.（编辑）第三届国际清真会议论文集。施普林格新加坡，新加坡，2018: 333–342. https://doi.org/10.1007/978-981-10-7257-4_30

[7] LUBIS H. N., MOHD-NAIM N. F., ALIZUL N. N., 和 AHMED M. U. 从市场到食品盘：当前值得信赖的清真食品分析技术和创新。食品科技趋势，2016, 58: 55–68. https://doi.org/10.1016/j.tifs.2016.10.024

[8] KUSWANDI B., GANI A. A., 和 AHMAD M. 用于检测熟肉丸中猪肉掺假的免疫条测试。食品生物科学，2017, 19: 1–6. https://doi.org/10.1016/j.fbio.2017.05.001

[9] BALAN B., DHAULANIYA A. S., JAMWAL R., AMIT, SODHI K. K., KELLY S., CANNAVAN A., 和 SINGH D. K. 衰减全反射-傅里叶变换红外光谱结合化学计量学在牛奶中福尔马林检测和定量中的应用。振动光谱，2020, 107: 103033. https://doi.org/10.1016/j.vibspec.2020.103033

[10] HE L., MA J., LI Q., WANG L., FAN S., 和 ZHANG Y. 液相色谱-串联质谱法测定畜禽肉中的成分。未来食品杂志，2022, 2: 53–60. https://doi.org/10.1016/j.jfutfo.2022.03.017

[11] THIENES C. P., MASIRI J., BENOIT L. A., BARRIOS-LOPEZ B., SAMUEL S. A., MESHGI M. A., COX D. P., DOBRITSA A. P., NADALA C., 和 SAMADPOUR M. 酶联免疫吸附法定量检测熟肉制品中鸡肉和火鸡的污染。国际官方分析合作协会杂志，2019, 102: 557–563. https://doi.org/10.5740/jaoacint.18-0136

[12] PUSPITA I., IRAWATI N., MADURANI K. A., KURNIAWAN F., KOENTJORO S., 和 HATTA A. M. 石墨烯和多壁碳纳米管涂层锥形塑料光纤用于检测橄榄油中的猪油掺假。光子传感器，2022, 12: 220411. https://doi.org/10.1007/s13320-022-0652-y

[13] KURNIAWAN F., NUGROHO A., BASKARA R. A., CANDLE L., PRADINI D., MADURANI K. A., SUGIARSO R. D., 和 JUWONO H. 使用聚苯胺/氧化镍纳米粒子改性石英晶体微天平传感器进行快速分析以区分猪和牛明胶。赫利昂，2022, 8: e09401. https://doi.org/10.1016/j.heliyon.2022.e09401

[14] ZULKARNAIN Z., SUPRAPTO S., ERSAM T., 和 KURNIAWAN F. 一种用于胰岛素检测的新型选择性和灵敏电化学传感器。印度尼西亚电气工程与计算机科学杂志，2016, 3: 496–502. http://doi.org/10.11591/ijeecs.v3.i3.pp496-502

[15] CAI Y., HE Y., LV R., CHEN H., WANG Q., 和 PAN L. 双液滴数字聚合物链式反应检测和定量肉制品中的牛肉和猪肉材料。公共科学图书馆一，2017, 12: e0181949. https://doi.org/10.1371/journal.pone.0181949

[16] KIM M.-J., & KIM H.-Y. 一种快速多重实时聚合物链反应测定法，用于同时检测商业加工肉制品中的猪肉、鸡肉和牛肉。食品科技，2019, 114: 108390. https://doi.org/10.1016/j.lwt.2019.108390

[17] MADURANI K. A., SUPRAPTO S., SYAHPUTRA M. Y., PUSPITA I., MASUDI A., RIZQI H. D., A. M. HATTA, J. JUNIASTUTI, M. I. LUSIDA, 和 KURNIAWAN F. 回顾——控制新冠肺炎 爆发的检测方法的最新发展。电化学学会杂志，2021, 168: 037511. https://doi.org/10.1149/1945-7111/abe9cc

[18] SÁ M., BERTINETTO C. G., FERRER-LEDO N., JANSEN J. J., WIJFFELS R., CRESPO J.G., BARBOSA M., 和 GALINHA C. F. 荧光光谱和化学计量学用于同时监测海洋微拟球藻中的细胞浓度、叶绿素和脂肪酸。科学报告，2020, 10: 7688. https://doi.org/10.1038/s41598-020-64628-7

[19] FARDIYAH Q., ERSAM T., SUYANTA, SLAMET A., SUPRAPTO, 和 KURNIAWAN F. 穿心莲植物提取物作为光保护剂的新潜力和特性。阿拉伯化学杂志，2020, 13: 8888–89897. https://doi.org/10.1016/j.arabjc.2020.10.015

[20] MADURANI K. A., SUPRAPTO S., YUDHA SYAHPUTRA M., PUSPITA I., HADI FURQONI A., PUSPASARI L., ROSYIDAH H., HATTA A. M., JUNIASTUTI, LUSIDA M. I., TOMINAGA M., 和 KURNIAWANA F. 用于临床拭子样本中新冠肺炎测定的荧光分光光度法。阿拉伯化学杂志，2022, 8(15): 104020. https://doi.org/10.1016/j.arabjc.2022.104020

[21] CASALE M., PASQUINI B., HOOSHYARI M., ORLANDINI S., MUSTORGI E., MALEGORI C., TURRINI F., ORTIZ M. C., SARABIA L. A., 和 FURLANETTO S. 结合激发-发射矩阵荧光光谱、平行因子分析、环糊精改性胶束电动色谱和偏最小二乘类建模来表征绿茶。医药与生物医学分析杂志，2018, 159: 311–317. https://doi.org/10.1016/j.jpba.2018.07.001

[22] PRASAD S., MANDAL I., SINGH S., PAUL A., MANDAL B., VENKATRAMANI R., 和 SWAMINATHAN R. 源自单体蛋白质中带电氨基酸的近紫外-可见电子吸收。化学科学，2017, 8: 5416–5433. https://doi.org/10.1039/C7SC00880E

[23] JOLLIFFE I. T., 和 CADIMA J. 主成分分析：回顾和最新发展。英国皇家学会哲学汇刊 A：数学、物理和工程科学，2016, 374: 20150202. https://doi.org/10.1098/rsta.2015.0202

[24] 美国农业和农业协会生物工程师. 使用高光谱荧光成像评估猪肉和家禽肉和骨粉。2016 年美国社会农业和生物工程师国际会议，2016. https://doi.org/10.13031/aim.20162462786

[25] WU B., DAHLBERG K., GAO X., SMITH J., 和 BAILIN J. 一种基于荧光光谱的肉类腐败检测快速方法。国际高速电子与系统杂志，2018, 27: 1840025. https://doi.org/10.1142/S0129156418400256

[26] ISLAM K. Z., AHASAN M. A. A., HOSSAIN M. S.,

RAHMAN M. H., MOUSUMI U. S., 和 ASADUZZAMAN M. 用于识别猪肉掺假以进行清真认证的智能荧光光谱仪。食品与营养科学，2021, 12: 73–89. https://doi.org/10.4236/fns.2021.121007

[27] SORAPUKDEE S., & NARUNATSOPANON S. 猪、鸡、鸭血成分及功能特性比较研究。韩国动物资源食品科学杂志，2017, 37: 228–241. https://doi.org/10.5851/kosfa.2017.37.2.228

[28] SKOOG D. A., HOLLER F. J., 和 CROUCH S. R. 仪器分析原理。布鲁克斯科尔，贝尔蒙特, 2017.

[29] SHRIRAO A. B., SCHLOSS R. S., FRITZ Z., SHRIRAO M. V., ROSEN R., 和 YARMUSH M. L. 血液的自发荧光及其在生物医学和临床研究中的应用。生物技术与生物工程，2021, 118: 4550–4576. https://doi.org/10.1002/bit.27933

[30] LECRENIER M.-C., BAETEN V., TAIRA A., 和 ABBAS O. 用于检测血粉和血液制品的同步荧光光谱。塔兰塔，2018, 189: 166–173. https://doi.org/10.1016/j.talanta.2018.06.076

Supplementary Data

Table S1 Significant test

Sample		F-test	T-test	Conclusion
Pork	Blood Extracted Blood	Equal Variance	H_0 accepted	No Significant Difference
Broiler	Blood Extracted Blood	Equal Variance	H_0 accepted	No Significant Difference
Domestic	Blood Extracted Blood	Equal Variance	H_0 accepted	No Significant Difference
Braekels	Blood Extracted Blood	Equal Variance	H_0 accepted	No Significant Difference

Notes:
Peak Sample:
1. Pork: Ex = 309.1 nm, Em_1 = 309.1 nm, Em_2 = 620.2 nm
2. Broiler: Ex = 330.2 nm, Em_1 = 330.2 nm, Em_2 = 678.2 nm
3. Domestic: Ex = 334.2 nm, Em1 = 334.3 nm, Em2 = 663.2 nm
4. Braekels: Ex = 332.2 nm, Em1 = 332.1 nm, Em2 = 658.2 nm

Table S2 LSD test for each type of sample

Sample	Wavelength (nm)			LSD			Conclusion
	Ex	Em_1	Em_2	Ex	Em_1	Em_2	
Pork	309.1 ± 0.22^a	309.1 ± 0.25^a	620.2 ± 0.24^a	0.17	0.19	0.10	Significant difference
Broiler	330.2 ± 0.23^b	330.2 ± 0.22^b	678.2 ± 0.22^b				
Domestic	334.2 ± 0.25^c	334.3 ± 0.25^c	663.2 ± 0.24^c				
Braekels	332.2 ± 0.23^d	332.1 ± 0.23^d	658.2 ± 0.23^d				

Notes:
1. Values are given as means ± SD
2. Different superscripts in the same columns indicate significant differences