Study on fluorescence spectra: characteristics of broiler and pig blood

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Abstract. The fluorescence method has been used for identification of blood disease and blood
type of human because of specific characteristics. In this research, we observed the
fluorescence characteristics of broiler and pig blood. The blood of broiler and pig were
obtained from the local slaughterhouse in Surabaya. Each blood was measured directly using
fluorescence spectrophotometer. All experiments were conducted at wavelength of 200 nm to
800 nm with scan speed of 500 nm/min and SlitEx/Em of 10nm/10nm. Fluorescence
characteristic of the blood samples was shown from these excitation and emission peaks. From
investigation, the broiler bloods have fluorescence spectra at 350.5 nm for excitation and 349.7
and 698.0 nm for emission. Meanwhile, the fluorescence characteristic of pig blood was
appeared at 311.0 nm and 309.0; 622.0 nm for excitation and emission, respectively. The
results showed that between broiler and pig blood have significant different fluorescence
characteristic.

1. Introduction
Blood is a bodily fluid that functions as a conduit of nutrition and oxygen into every living cells and
also as a carrier of the excess of metabolism from the same cell. Blood contains 55% of blood plasma
and 45% of cellular part. Blood plasma consists of 92% water which functions as a travel medium for
blood cells. Furthermore, blood plasma also contains albumin, fibrinogen, and globulin, whereas the
cellular part contains red blood cells, white blood cells and platelets [1]. Blood compounds contain
proteins in the form of DNA and RNA strands. The constituent components of nucleotides consist of
three types of molecules, namely pentose sugar (deoxyribose in DNA or ribose in RNA), nitrogenous
bases, and phosphate groups. The order of sequences and the number of nucleotide bases carries
genetic information in cells. Each type of microorganism has a sequence and number of nucleotide
bases which can be very different [2]. Fluorophore molecules could be either utilized alone, or serve as a fluorescent motif of a functional system. Based on molecular complexity and synthetic methods, fluorophore molecules could be generally classified into four categories: proteins and peptides, small organic compounds, synthetic oligomers and polymers, and multi-component systems [3].

The characteristics of fluorophore molecules in blood can be used as indicators to determine the halal processed of meat products. Where the blood component contained in the meat is not much different from the content in the blood. The development of analytical methods in the field of spectrophotometry is growing, especially in the fields of biology and health. Methods that are widely used as blood tests on living things include the PCR method. In previous study, pork protein in gelatin capsule can be identified using the electrophoresis technique Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) which is a long time and has a low accuracy [4]. Detection of pork contamination in imported meat using DNA extraction and PCR was reported by several scientists [5–8]. PCR method has a high degree of accuracy in identifying DNA molecules found in living things. Unfortunately, the PCR method requires a fairly long sample preparation and complex DNA isolation method and uses expensive reagents. Therefore it is necessary to develop a method that is faster and more efficient.

The use of fluorimeter methods in the determination of blood characteristics is increasing. Few studies have been reported on the application of native fluorescence spectroscopy of bio fluids in the diagnosis of tumoral diseases. Recently, the fluorescence emission characteristic of blood plasma has been exploited to discriminate patients with oral malignancy from the healthy ones [9,10]. In the previous work, fluorescent spectrophotometers are used to identify blood groups in humans [11,12]. The fluorescence method is a simple method without the need for complicated sample preparation. Blood has specific fluorescence characteristics in different conditions and environments [13]. Because of that, blood is widely used as an indicator in determining the nature and characteristics of living organisms. In this study, animal blood was used from two different species, i.e. broilers and pig blood, to test their characteristics using the fluorescence spectrophotometer method. Fluorescence is the emission of light by a fluorophore compound that can absorb light or other electromagnetic radiation. Light emitted by a compound has longer waves and lower energy than absorbed radiation.

2. Methods

2.1. Materials

Fresh bloods from broilers and pig were collected from slaughterhouses at Sepanjang Market, Sidoarjo, and East Java, Indonesia. Demineralized water was used for cleaning and chemical preparation. H₂SO₄ and H₂O₂ was purchased from Merck.

2.2. Preparation and characterization of samples

Blood samples were taken from each of broilers and pig. Each blood was taken 20 µL and put in a different vial bottle. Blood solution was prepared by diluting of each blood sample in a 100 mL volumetric flask using demineralized water. The fluorescence characteristic of blood solution from boilers and pig was measured using fluorescence spectrometer (Perkin Elmer LS 55). All measurements were carried out at wavelength of 200-800 nm with scan condition speed of 500 nm/min and SλHEx/Em of 10nm/10nm [14,15].

3. Results and discussion

The fluorescence characteristic of boiler and pig blood was successfully distinguished by fluorescence spectroscopy. The fluorescence characteristic of the samples was revealed from the excitation and emission peakas shown in Figure 1. The maximum peak of emission was longer than the maximum peak of excitation. This was due to the difference of energy from the excited state and ground state when the process of absorbing energy was greater than the emission process [16]. The phenomenon of excitation and emission in fluorescence can be explained as in Figure 2. Peak excitation occurs because of the electron transition from ground state (S₀) to the level of the singlet by vibrational
excitation ($S_2$). After the molecule undergoes a transition, the molecule will emit its energy that has been absorbed during the transition from the basic level ($S_0$) to the level ($S_2$). The scattering (emission) will occur by radiating photons whose energy is in accordance with the difference in the level of excitation ($S_2$) and level ($S_0$). When a molecule is excited from a solution, it will quickly relax to the lowest electronic vibration level $S_1$. Vibration relaxation to the lowest vibration level $S_1$ immediately deactivates the molecule. After reaching this level, the molecule can return to the baseline, for example by emission radiation. This release of energy by radiation is called fluorescence ($S_1$ to $S_0$). The fluorescence wavelength is greater than the absorbance wavelength [17].

According to Figure 1, the broilers blood have maximum excitation wavelength at 350.5 nm and emission wavelengths at 349.7 and 698.0 nm. On the other hand, the fluorescence characteristic of pig blood was appeared as excitation and emission peaks at 311.0 nm and 309.0; 622.0 nm, respectively. The different fluorescence characteristic between broilers and pig blood was very significant so that it should be studied further, especially to distinguish samples that contain broilers and pig bloods. The difference in wavelengths that occur due to the content of fluorophore compounds found in each animal's blood has different characteristics. Fluorophore is a substance capable of emitting light after having absorbed energy. Most cells contain endogenous fluorophores in their mitochondria and lysosomes [13]. Previous study on identification of pork and broiler meat, done using the PCR method, was able to distinguish various types of fresh meat from various species with RNA isolation technique [18]. PCR method has various weaknesses, including requiring complicated sample preparation, expensive reagents and requires a long time (40-90 minutes) process for each sample [8]. The blood composition is not much different than fresh meat so the blood can be used for identification of species. The fluorescence method did not require complicated sample preparation and
a relatively faster processing time (about 20 minutes each sample).

![Diagram](image)

**Figure 2.** Diagram illustrating the main primary deactivation processes following excitation from the ground electronic singlet state ($S_0$) to excited electronic singlet states ($S_1$ and $S_2$) [17].

4. Conclusion

The fluorescence characteristic of broilers and pig bloods was studied using fluorescence spectroscopy method. The results showed that the fluorescence characteristic of broilers and pig blood were significantly different. The boilers blood has excitation peak at 350.5 nm and emission peak at 349.7 and 698.0 nm. For pig blood, the excitation and emission peaks were appeared at 311.0 nm and 309.0; 622.0 nm, respectively. From this study, the difference characteristics of both of samples can be used and investigated further for halal purpose.

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