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Review

Potential technical parameters for the authentication of carrion meat (tiren): A review

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Abstract

Assuring halal status of animal-based foods is an ongoing challenge in Indonesia. An adequate halal food supply will make it easier for the people to obtain halal-certified foods that contain animal products. The government must reach an agreement on quality infrastructure for its supply chain, considering that basic foodstuffs are the essential ingredients for all edible derivatives. One major obstacle in implementing halal assurance for the consumer is the presence of carrion meat (raw or processed) in the market. The testing standards for the authentication of carrion meat are currently not a priority for either cattle or poultry. Therefore, the aim of this review was to identify important procedures that some experts have carried out for the detection or testing of carrion meat. The information and data collected and analysed may provide potential technical parameters for detecting carrion meat. This review used a descriptive exploratory method and the forum group discussion. As a preliminary result, 14 potential technical parameters were obtained and tested with further verification and evaluation. The technical parameters studied included malachite green-H_2O_2, correlated protein with meat texture, peroxiredoxin-6, blood biochemistry, blood pH, capacitance value, meat colour, Warner-Bratzler shear force, blood loss variation, meat quality, water holding capacity (WHC), resistance value, *E. coli* load, and coliform load. The proposed parameters will be discussed by the technical committee by consensus when submitted to the national standard draft.

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Introduction

The challenge of halal certification is not only for products containing pork and alcohol (*al-khamr*), but also for meat products from animals that died before being slaughtered (tiren), otherwise known as carrion. In 2019, it was found that the processing of tiren chickens in Balok Village, Mojokerto (Ramadani, 2019) led to the circulation in the market of 50 tiren chickens in the city of Banjarmasin per day (Edinayanti, 2019). In August 2012, a joint team including the Department of Agriculture, Department of Health, veterinarians, and Central Java Police discovered the circulation of tiren chicken meat in Solo traditional markets (Wismabrata, 2012). The domestic authority prohibits the circulation of beef and chicken for consumption, and can also damage the market price by selling meat of around IDR 55,000/kg while the price of beef reaches IDR 80,000/kg (Wismabrata, 2012). The emergence of the tiren meat phenomenon is incidental, and implies that the government has not taken the problem seriously. Moreover, tiren meat is included in the government policy challenge in implementing meat certification at the slaughterhouse (*rumah potong hewan*; RPH) and poultry slaughterhouse (*rumah potong unggas*; RPU) levels.

The research related to carrion meat detection conducted from 2000 - 2019 has increased, along with the problems that have arisen in the community. In 2000, carrion meat could be detected with the malachite green (MG)-hydrogen peroxide (H_2O_2)

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testing method, as well as physical, chemical, and microbiological observations. The variables used in the detection protocol were colour, tenderness, pH, water holding capacity, and microbial load (Bintoro, 2006). In 2013, a method of detecting carrion meat with the capacitance value method using the ligase chain reaction (LCR) tool was also carried out. The results showed that carrion meat's capacitance value was greater than that of fresh meat (Rakhmadi *et al.*, 2013). In 2015, a research was carried out to detect chicken carrion meat using a colour sensor and artificial neural networks. This method can detect samples of meat from the chest and thigh of chickens (Abrori *et al.*, 2015). In 2018, another method for carrion meat detection made use of the malachite green and pH test (Swari *et al.*, 2019). In 2019, research on the detection of carrion meat using the meat resistance method with the ATMega8 microcontroller was carried out. The results showed a 96% success rate in detecting tiren chicken (Dwiatmaja and Rakhmadi, 2020).

Testing halal products as a requirement for halal product certification consists of three different types: porcine testing, detection of the alcohol content, and the detection of carrion meat (tiren). Several techniques for detecting carrion meat have been developed; however, there is currently no nationally agreed standard for the detection of carrion meat. The use of national standards would facilitate the authentication of carrion meat for market surveillance, improving the adequacy of audit evidence for halal certification of meat, and minimising the potential for inadequate results leading to unfairness in the meat trade, as well as the neglect of consumer protections from unhealthy and non-certified halal meat products (Sood, 2013).

Based on these facts, Badan Standardisasi Nasional (BSN, 2018), which facilitates the development of standards, should conduct studies to promote agreement regarding the importance of formulating National Indonesian Standards (SNI) for carrion meat detection from all stakeholders and provide recommendations for appropriate and scientifically appropriate testing methods and according to the needs of laboratories in Indonesia.

Theoretical framework

According to Islamic law, it is forbidden to consume carrion meat, as described in the Qur'an, Surah al-Baqarah verse 173, and Surah al-Maidah verse 3. Sharia law provides wisdom about the potential for consuming haram food in the form of carrion meat.

The existence of carrion meat (tiren) in the commercial food industry could be due to five factors namely (1) few sellers know about carrion meat; (2) the lack of consumer awareness on the potential of carrion meat's existence in the marketplace; (3) lack of government supervision regarding carrion meat; (4) non-uniform policies in the central and regional governments related to the mandatory slaughter of animals in halal-certified slaughterhouses; and (5) the absence of standards for carrion meat (tiren) detection methods, including processes that are designed to monitor the circulation of consumer goods and to function as the final determining procedure of the halal meat certification audit system in Indonesia (Table 1, Figure 1).

Carrion meat can be categorised into four types based on its source namely (1) carrion meat from dead animals with no slaughtering process; (2) carrion meat from slaughtered dead animals; (3) carrion meat from halal slaughtered animals, but is either no longer fresh or rotten (*i.e.*, stored too long or contaminated with bacteria); and (4) carrion meat sourced from animals slaughtered for idols or idolatry.

Research in meat production has identified a number of recognised post-mortem indicators after livestock has been slaughtered. Post-mortem indicators of the status of meat can be used to develop methods for detecting whether the meat available in the market is fresh, halal, or carrion meat. Such methods of detection would be beneficial for the government and local businesses to monitor the circulation of meat products in the market.

Several post-mortem indicators have been converted into technical parameters that can be measured and analysed. Technical parameters that can determine which animals were not properly slaughtered are called post-mortem parameters (A). Technical parameters that can determine if an animal was slaughtered after being stunned are called post-mortem parameters (B). Standard meat sold in the market that is neither fresh nor spoiled or kept for a long period in the freezer is designated as post-mortem parameter (C) (Figure 2).

Based on several studies, meat from slaughtered and non-slaughtered animals has different physical and chemical properties that affect the meat's quality. Slaughtered meat has a higher

Table 1. Indonesian and international view on carrion meat/animal carrion.

Difference	Regulation						
	Halal law (Sharia Islam)	**Act - Indonesia**	**Ministry Regulation - Indonesia**	**USDA - AS**	**WHO**	**FAO**	**ISO**
Position of carrion meat	Carrion is a living creature that dies without being slaughtered (Arabic definition). Carrion is an animal that dies without being slaughtered under Sharia, by dying alone without the cause of human intervention (Sharia definition) or an act of slaughter that is not following the method of slaughtering of Islam.[1]	Animal carrion is something that is categorised as waste that cannot be used for human purposes, let alone eaten.[2]	Animal carrion is something that has the potential to spread the disease to the environment and humans, so it must be regulated.[3]	The provision of animal meat is regulated by the process of cutting it or how to slaughter it which is fulfilled with health and humanitarian requirements.	The use of animal meat must be done through a hygienic slaughter / slaughtering process.	Carrion is animal that is served as food but not through a humane process of slaughter.	There is an international standard that describes the standardisation of the slaughter of livestock.

Source: Results of research data processing 2020. [1]Al Ath'imah Wa Ahkaam Al Shoid Wal DZabaa'ih, Dr. Sholeh Bin Abdillah Al Fauzan, second edition 1419H, Maktabah Al Ma'arif, Riyadh. [2]Law No. 32 of 2009 on Environmental Protection and Management: "Prohibits anyone from committing an act that causes environmental pollution and / or damage". [3]Ministry of Agriculture Decree (MoA) No.114 of 2014 concerning "Slaughter of Sacrificial

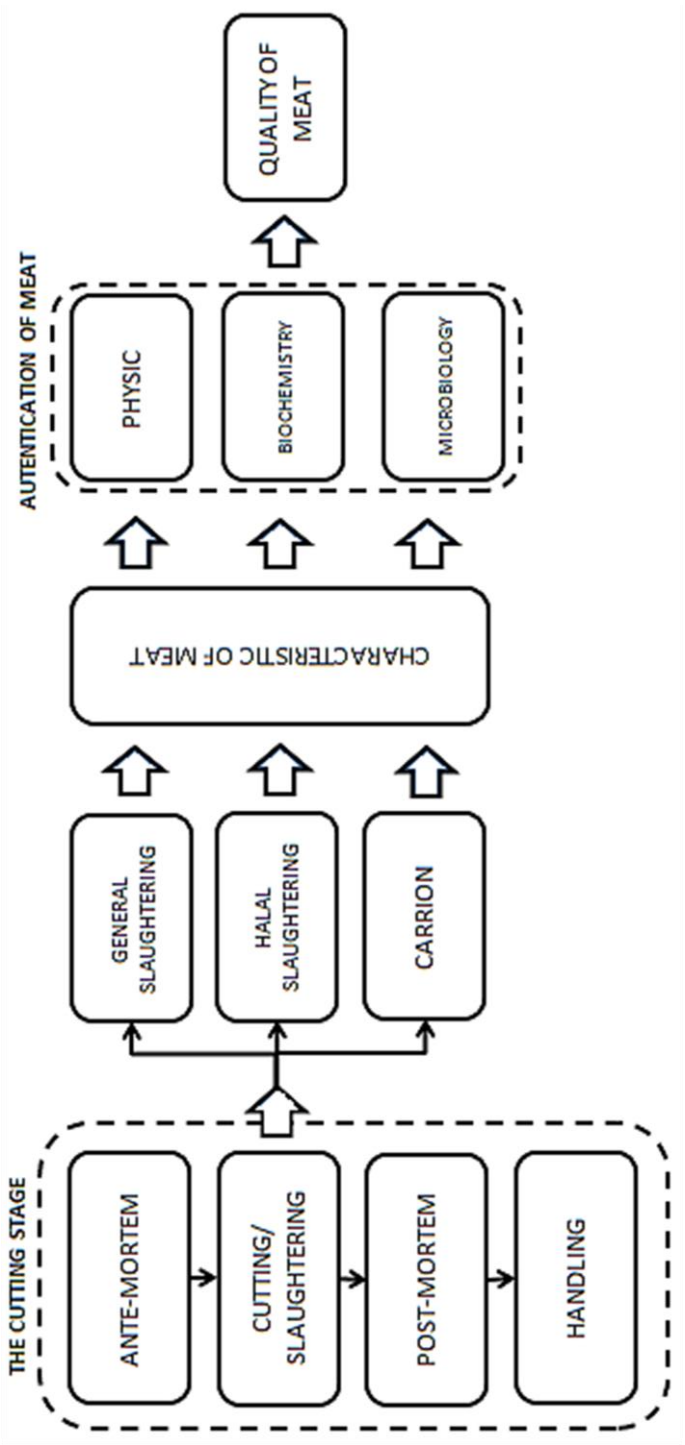

Figure 1. Relationship between type of slaughter and meat quality parameters. (Source: Results of research data processing 2020)

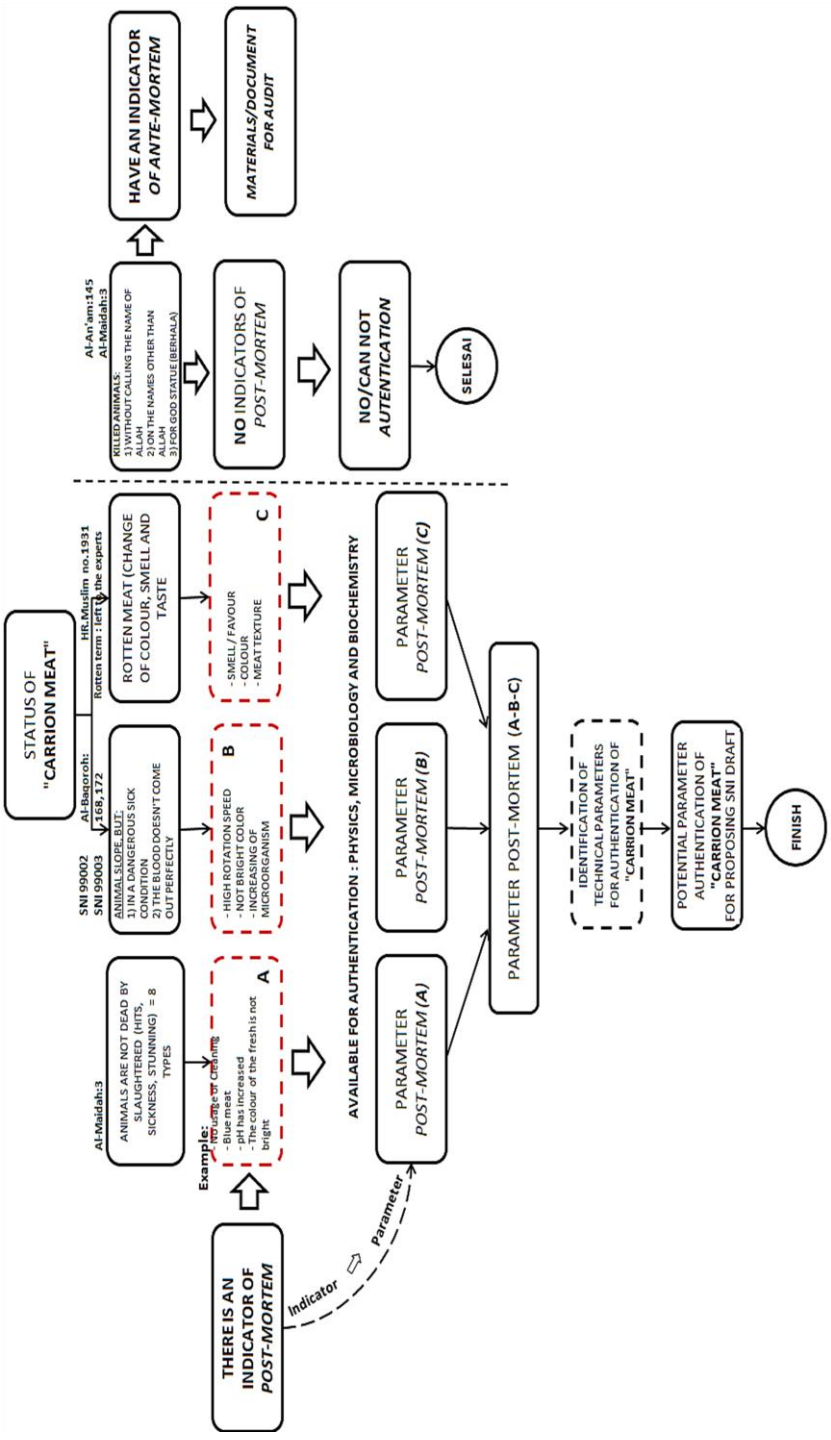

Figure 2. Concept outline as to why the detection of carrion meat could be a part from the halal product authentication process. (Source: Results of research data processing 2020).

quality and hygiene grade than non-slaughtered meat (carrion) because it has undergone controlled (regulated and known) ante-mortem and post-mortem procedures. Carrion meat cannot be considered as having the same condition as that from slaughtered animals because carrion meat has no known ante-mortem data or information about post-mortem contamination. Moreover, meat slaughtered according to halal specifications has a higher level of freshness and stability than conventionally slaughtered meat due to factors number 5, 6, and 9 (Table 2). In general, the conventional slaughter of meat does not require that the animal is still alive after being stunned (number 5), nor does it pay attention to the welfare of the animal at the time of slaughter (number 4). According to Pisestyani *et al*. (2015), stunning causes a decrease in blood flow due to a subsequent reduction in blood pressure. As a result, the quality of the meat rapidly deteriorates. Therefore, points 4, 5, and 9 in Table 2 are critical factors for the increased growth of microorganisms in the flesh of these animals.

Furthermore, halal meat has a higher quality assurance than conventional (non-halal) meat. Meat from animals slaughtered under halal norms must meet at least nine meat quality criteria, whereas conventionally slaughtered meat only meets three, and carrion meat lacks any qualification criteria. These observations indicate that slaughtering procedures have a strong correlation with meat quality (Table 3).

Malachite green and hydrogen peroxide testing

Malachite green (MG) is a chemical reagent made from organic compounds, and can be used as a chemical dye or as an antimicrobial agent in cultivation. Malachite green is classified in the dyestuff industry as a triaryl methane dye, and used as a pigment. Formally, MG can refer to either the chloride or oxalate salt, with the respective chemical formulas: $C_{23}H_{25}ClN_2$ and $C_{23}H_{25}N_2 \cdot C_2HO_4 \cdot 0.5C_2H_2O_4$ (Bhernama, 2017).

From a technical perspective, MG competes with haemoglobin (Hb) to increase oxygen levels because Hb has a higher affinity than MG, so that Hb will bind to oxygen first. Blood loss is affected before and after the animal is slaughtered, either due to improper stunning processes, slaughter without stunning, bruising and bleeding under the skin and in muscle, or handling animals that are not hung after slaughtering (Ulfa, 2019). Disturbances in blood circulation cause Hb to be oxidised in the meat, and increases the process of decay or results in a decrease in the quality of the meat. Haemoglobin status can be determined by testing with MG and H_2O_2 in meat samples. If there is Hb in the meat, the Hb will bind to O_2 from H_2O_2, MG will not be oxidised, and therefore will remain green (Lawrie, 1995).

The so called 'imperfect bleeding' can cause a decrease in meat quality, including rapid decay which occurs because blood is an excellent medium for the growth and development of microorganisms. In other procedures, MG test has been used to determine whether or not a phlebotomy has been correctly carried out (Ulfa, 2019).

Hydrogen peroxide (H_2O_2) is a chemical compound, that in its pure form is a pale blue clear liquid (Housecroft, 2005) that is slightly thicker than water. H_2O_2 is used as an oxidising agent, whitening agent, and antiseptic. Concentrated H_2O_2, or "high test peroxide", is a reactive oxygen species that has been used as a rocket propellant (Hill, 2001). The instability of its peroxide bonds is a dominant feature of its chemical properties.

The appropriate concentrations of MG and H_2O_2 need to be adjusted for testing tiren meat. The colour change in the MG test of rotten carrion meat may also be influenced by the presence of hydrogen sulphide (H_2S), which reacts with myoglobin to form sulfmyoglobin and a green colour (Lawrie, 1995). Therefore, H_2O_2 is a supporting component of the MG test.

The testing method is conducted as follows: the meat sample is extracted using a stomacher, then placed in 14 mL of distilled water in an Erlenmeyer flask, and allowed to stand for 15 min. The meat extract is filtered, and 0.7 mL of filtrate is placed into a test tube. One drop of MG and one drop of 3% H_2O_2 are added to the filtrate, and the solution is kept at room temperature for 20 min. A positive reaction results in a green colour that shows imperfect blood loss. A negative reaction occurs if a clear blue colour is formed, meaning that there is a complete loss of blood at the time of slaughter (Ulfa, 2019).

More recent technology makes use of an innovative reagent for the rapid detection of carrion meat, with an accuracy rate of 95%. This reagent is called durante, which works by using a liquid blood extract from meat containing Hb which reacts chemically with the reagent solution (Drastini, 2000).

Table 2. Correlation of criteria for general and halal slaughter meat on meat quality.

No.	Slaughter criteria	Critical points for:	Carrion meat	General slaughtered meat	Halal slaughtered meat	Reference
1	Ante-mortem animal condition must be healthy	Meat quality	No (-)	Yes (+)	Yes (+)	Adeyemi and Sazili (2014) Manalo and Gabriel (2020)
2	Animals are guarded from fear or stress	Meat quality	No (-)	Yes (+)	Yes (+)	Ningrum (2017) Wilujeng (2017)
3	Make sure the animal is alive	Carrion / halal	No (-)	Yes (+)	Yes (+)	Farouk *et al.* (2016)
4	Pay attention to the welfare of animals when being slaughtered, with the method of stunning	Meat quality	-	Yes (-)	No (+)	Grandin (2014) Verhoeven (2014)
5	Ensure that the animal is alive after stunning	Carrion / halal	No (-)	Yes (+) / No (-)	Yes (+)	Fuseini *et al.* (2016) Nakyinsige *et al.* (2013) Khalid *et al.* (2015)
6	Allows stunning with strikes for ruminants, and electric shocks for poultry	Meat quality	-	Yes (-)	No[1] (+)	Salamano (2013) Aksu and Matur (2006) Aghwan *et al.* (2016)
7	The slaughter must be done by Muslim or non-idolater[2], and Christian and Jewish slaughter is permissible	Carrion / halal	No (-)	No (-)	Yes (+)	Fuseini *et al.* (2016) Riaz and Chaudry (2003) Armanios and Ergene (2018)
8	Ensure the three veins are cut: *vena jugularis*[3], *arteri carotid*[4], oesophagus (respiratory tract and food tract)	Meat quality	-	No (+)	Yes (+)	Khalid *et al.* (2015) Nakyinsige *et al.* (2013) Abdullah *et al.* (2019)
9	Ensure the blood is gushing and flowing out.	Meat quality	No (-)	Yes (+) / No (-)	Yes (+)	Velarde *et al.* (2003) Bakhsh *et al.* (2018) McNeal *et al.* (2003) Mulley *et al.* (2010)

Source: Results of research data processing 2020. Description: (+) = quality increased, (-) = quality decreased. [1]The non-penetrative bolt gun is the only permitted method. [2]Based on the interpretation that is meant by "slaughtered meat which is called a name other than Allah: in Surah al-Baqarah: 172, and interpreted by Surah al-Maidah: 3 as "a slaughter that is slaughtered for idols". [3]*Vena jugularis* is vein blood vessel. [4]*Arteri carotid* is blood vessel that leaves the heart.

Table 3. Relationship between fulfilment of slaughter criteria and meat quality.

No.	Type of meat	Fulfilment of the related (+) criteria "Deterioration of meat quality"	Number of criteria
1	Halal slaughter	1, 2, 3, 4, 5, 6, 7, 8, 9	9
2	General slaughter	1, 2, 3	3
3	Non-slaughter / Carrion	-	0

Source: Results of research data processing 2020.

Protein content correlates with meat texture

Meat protein plays a role in binding the water content of meat; therefore, a high protein content increases the ability to retain moisture in meat, which reduces the free water content, and *vice versa* (Lawrie, 2003). The ability to hold high moisture in meat results in a tender meat texture. Feed and temperature are among the factors that affect the protein content in meat. According to Fernandez (2009), livestock fed with concentrated types of feed have a higher protein content as compared to livestock that only consumes plants. An increase in storage temperature also affects the protein content in meat. An increase in temperature in cuts of meat can result in various degrees of denaturation of myofibril protein and connective tissue. A high protein content in meat determines the delicate texture of the meat.

Meat protein is classified into three major groups namely myofibrils, stroma, and sarcoplasm. Essential components of myofibril protein in the structure of muscle fibres are actin and myosin. Myofibril protein is a protein that is abundant in muscles, and vital for muscle contraction (spasm) and relaxation (rest). The conditions under which the animal is slaughtered and handled after slaughtering are essential factors for controlling muscle contraction (spasm) that will determine meat tenderness.

Peroxiredoxin-6

Peroxiredoxin-6 is an enzymatic antioxidant. Peroxiredoxin 6 (1-Cys peroxiredoxin) is a member of the peroxiredoxin family, and has unique properties that differ from other mammalian peroxiredoxins. It lacks cysteine, a deficiency that is resolved with the use of glutathione and π glutathione S-transferase to complete the catalytic cycle. Peroxiredoxin-6 is also the only peroxiredoxin capable of reducing phospholipid hydroperoxide through glutathione peroxidase (Gpx) activity (Arevalo and Vazquez-Medina, 2018). Peroxiredoxin-6 has been identified as a potential marker of tenderness in beef, as well as a potential marker in NST (non-stunning) meat that was positively correlated with WBSF (Warner-Bratzler) results, likely due to the troponin-T content, which showed a strong positive correlation with shear forces and softness (Ouali, 2013).

Blood biochemistry

Blood biochemistry includes the following parameters: lactate, glucose, urea, total protein, creatinine, creatine kinase (CK), calcium, ferrous, and lactate dehydrogenase (LDH). Some also include levels of iron, poly-unsaturated fatty acids (PUFAs), glucose, and Hb (Imlan *et al*., 2020). The halal or Islamic slaughter process is carried out for the production of halal chicken. Slaughtering must be carried out by cutting the chicken's throat so that the animal dies quickly without suffering. Cutting the throat results in more bleeding, and a rapid rate of blood flow in the blood vessels before clotting can occur. The method of slaughter is correlated with the composition and quality of the post-mortem meat, mediated by a variation in coagulated blood. The chemical composition and post-mortem quality of broiler chicken breast meat obtained from various slaughtering methods has been compared (Addeen *et al*., 2014).

Chicken breast meat from Islamic slaughter, beheading, and conventional neck-cutting methods resulted in bled samples that contained haem iron levels of 2.41, 2.35, 2.56, and 3.41 mg/100 g, and non-haem iron levels of 10.09, 12.47, 14.21, and 18.10 mg/kg, respectively. Similar haem and non-haem contents were found among the bled samples. During storage at 4°C for eight days, chicken meat from Islamic slaughter showed lower values for peroxide and thiobarbituric acid reactive substance in the first four days of storage as compared to other methods ($p < 0.05$). The protein content of chicken meat did not show any differences among the various

slaughtering methods. The PUFA levels of chicken meat in the Islamic slaughter method were higher than the bled samples with other methods after eight days of storage (Addeen *et al*., 2014).

A higher number of mesophilic bacteria, total viable count, and some psychrophilic bacteria were observed in the unbled samples, as compared to slaughtered samples ($p < 0.05$). Samples without blood had higher a*, ΔE*, and ΔC* values than samples with blood, and the L* and a* values decreased after eight days of storage. Islamic slaughtering methods can reduce iron or haem in muscles, and reduce lipid oxidation in post-mortem chicken (Addeen *et al*., 2014).

Blood pH

Blood pH is an indicator of bacterial growth. The pH value of chicken meat is correlated with the meat's physical quality (Swari *et al.*, 2019). Chicken meat with a pH value above 6.0 is alkaline, while a pH value below 6.0 indicates that the meat is acidic. Alkaline chicken meat indicates that the meat has undergone autolysis, in which cell destruction processes are carried out by enzymes from within the cell itself, thus resulting in cell death. Meat in this condition shows the onset of spoilage. Meat with a pH below 6.0 indicates that the meat is still fresh because the meat has a pH in the optimal (*i.e.*, lower) pH category. Meat with an acidic pH will inhibit microbial growth.

The normal pH of broiler chickens ranges from 5.96 to 6.07 (Van-Laack, 2000). The pH of meat is very important in inhibiting spoilage. The pH of tiren chicken meat is typically higher than that of healthy chickens. The condition of the animal affects the high pH value of tiren chicken meat while it is still alive. Tiren chicken meat comes from chickens that have died before being slaughtered. Stress, lack of rest, and transportation can cause these deaths, which result in low glycogen levels and a subsequent inhibition in the formation of lactic acid. After the enzyme is no longer active and the glycogen supply runs out, bacteria will grow. The presence of putrefactive bacteria results in the formation of ammonia (NH_3), one of the products of bacterial metabolism; thus, pH increases due to the alkaline nature of ammonia (Mutiasari, 2014).

A pH value above 6.00 in tiren chicken meat is caused by the animal experiencing stress due to heat, travel (fatigue and stress), lack of oxygen, and lack of food and water (Lawrie, 2003). According to Prayitno *et al.* (2010), the use of feed that has a high energy content can affect the glycogen levels in broiler muscle, with a subsequent effect on the pH value of the meat (Swari *et al.*, 2019).

The carrion's pH value was measured by crushing and mixing carrion in distilled water (neutral pH content), followed by measurement with a pH meter (AOAC, 2005). A sample of 5 g of carrion was added to 5 mL of distilled water, crushed using a mortar, then put into a glass beaker, and measured using a pH meter that had been calibrated first using buffer liquids of pH 7 and 4. The pH was measured three times, and the average value was calculated. Bacterial growth was dependent on several factors including nutrients, time, temperature, water, pH, and the availability of oxygen. Blood that had left the body (blood vessels) still contained a significant amount of oxygen and other chemical substances needed for bacterial growth. Blood agar media, for example, is often used as a bacterial culture medium in medical research.

Capacitance value

The capacitance value can identify tiren chicken meat based on the measurement of physical characteristics that are not in direct contact with the chicken meat to be measured. Meat samples weighing 25 g each were taken from various parts of the chicken. Small pieces of tiren chicken and conventional meat were measured for their capacitance using an LCR meter. Measurements were carried out by inserting the meat in the middle of two parallel aluminium plates connected to the LCR meter. The capacitance value of the samples was shown on the LCR meter screen (Rakhmadi *et al.*, 2013). The analytical parameter in this method is that the capacitance value of tiren chicken is higher than that of fresh chicken. The greater capacitance of tiren chicken meat as compared to fresh chicken meat indicated that the size of the storage capacity of tiren chicken meat was greater than the size of the load storage capacity of fresh chicken meat (Rakhmadi *et al.*, 2013).

Furthermore, tiren chicken meat has greater permittivity than fresh chicken meat. Permittivity is a physical quantity of meat that describes how an electric field affects, and is influenced by a dielectric medium, and its value is determined by the material's ability to polarise the medium in response to the electric field (Bottcher, 1973). As compared to fresh chicken meat, the amount of permittivity of tiren chicken meat is due to the decomposition process of

chemical substances in tiren chicken meat, which is faster than that of fresh chicken meat (Sulastri, 2006), and correlates with the total microbial content of tiren chicken meat (Bintoro, 2006).

An existing technology related to this concept is a meat detector for tiren chicken (not slaughtered) that uses a conductance sensor based on the ATMEGA 16 microcontroller designed by Ghozali (2016). In addition, there are capacitance-based (non-slaughter)-based chicken detection gloves to detect healthy and halal chicken meat, an innovation developed by Rakhmadi *et al.* (2013).

Meat colour

The meat colour test is included in the organoleptic test that is influenced by the meat protein content. The main difference between carrion and fresh chicken is in the blood content of the meat. Carrion chicken meat comes from chickens whose blood did not drain from the carcass. Therefore, the Hb content is very high, and results in a potentially darker colour in the meat. According to Asmara (2006), the red colour of non-slaughtered chicken is inconsistent. The occurrence of darker flesh colour and bacterial activity is also influenced by oxygen and temperature (Pearson, 1994). Treating the carrion meat with higher temperature will result in a darker colour which is easier to identify.

Yulistiani's research showed that the colour of fresh chicken meat with 0- and 1-h carrion meat were the same (and therefore difficult to distinguish), namely yellowish-white, while 2-h carrion chicken meat was pale white and slightly reddish (Yulistiani, 2010). Three-hour carrion chicken meat was reddish-white, and 4-h carrion chicken meat was dark red. Meanwhile, Asmara's research showed that meat from non-slaughtered chickens has a reddish colour (Asmara, 2006).

The main components (80 - 90%) of meat pigment are Hb and myoglobin. Meat colour is determined by Fe status (ferrous and ferric). When cut, the colour of the flesh is violet. After half an hour in an atmosphere of sufficient O_2, the meat becomes bright red. If the meat is kept in a tightly closed container without O_2, oxymyoglobin will form, and the colour will remain bright in the form of ferric iron (Fe^{3+}). If exposed to some O_2, metmyoglobin will form, which is brownish red due to the presence of ferrous iron (Fe^{2+}) (BBPP Batu, 2012).

The colour of fresh chicken meat is yellowish-white, which is consistent with the conclusion by Cross (1988) that the colour of chicken meat is caused by provitamin A which is found in meat fat and the pigment oxymyoglobin. Lawrie (2003) found that the oxymyoglobin pigment is important in fresh meat; this pigment is only present on the surface and creates a meat colour in accordance with consumer expectations. The pigment from Hb results in a darker chicken meat colour due to imperfect blood loss (at the time of slaughter).

It has been found that stress before slaughter leads to an increase in catecholamines and creatinine kinase. Increased catecholamines, a group of hormones that have catechol groups secreted by the adrenal glands in response to stress (Roades, 2008), and creatinine kinase causes rapid glycolysis, thus resulting in a build-up of lactic acid in meat. Stress before slaughter also causes a decrease in glycogen levels which leads to a high meat pH and water holding capacity; in addition, the resulting meat is more rigid, and has a darker colour (Chulayo, 2012).

The observation of meat colour refers to the standard of meat colour (SNI 3932:2008), which has a score of 1 - 9, namely: quality value 1 - 5, bright red; quality value 6 - 7, dark red; and quality value 8 - 9, red old. The colour score value is determined based on the colour score corresponding to the meat colour; the standard meat colour starts from bright red, red, and dark red. According to Nurani (2010) and Gunawan (2013), factors causing the colour change in meat from bright red to brown or pink will occur if the meat is in contact with the air for too long.

The colour of high-quality beef is bright red as compared to lower-quality dark red meat. Good beef must be fresh, red, shiny, pale, smooth, not sour, and not rotten (Lawrie, 2003). According to SNI 01-3947-1995, the optimal pH of meat ranges from 5.3 - 5.8, 6 h post-mortem, and the colour of the meat will be bright red. Complete SNI can be trusted when healthy beef has the distinctive red colour of fresh meat, as well as the distinctive aroma of fresh meat, dry appearance, high level of chewiness, and maximum microbial load of 0.5 million microorganism per gram of meat (SNI 01-3947-1995).

The quality of chicken is determined according to SNI 3924: 2009 which includes information on carrion and chicken meat.

An important factor after slaughtering that affects the quality of the meat is withering. Meat withering will affect tenderness, flavour, and water-binding. Furthermore, according to Aberle (2001), cattle that are not rested will produce dark-coloured,

hard textured, dry meat, and have a high pH value and water-binding capacity.

Recent technology for carcass meat detection systems uses colour sensors and artificial neural networks; and are recent innovations by Abrori *et al.* (2015). In addition, a beef quality detection tool is based on odour, using the ATMEGA32 Microcontroller and Fuzzy Logic. Furthermore, there are also innovations from Kusworo Hadi, a Diponegoro University lecturer, who can assist consumers with image technology based on smartphone cameras.

Warner-Bratzler shear force

Warner-Bratzler is a standard method for measuring meat tenderness that works on the shear force standard test principle. This tool can determine the best quality of meat based on test methods and analysis of meat texture. The Warner-Bratzler, a meat tenderiser, can test the quality of any type of meat including beef, lamb, or mutton. The compression machine on this tool needs to be adjusted based on the type of meat tested.

Based on the results observed by Kiran *et al.* (2019), a significantly higher value of Warner-Bratzler shear force (WBSF) ($p < 0.01$) was observed in meat from stunning (ST) animals as compared to non-stunning (NST) animals. In that study, a 24-h post-mortem pH, a covariate in the WBSF analysis, was carried out to understand whether the higher WBSF values observed in the ST samples were the effect of pH or not. The observations showed that the difference in WBSF values between the stunned and non-stunned samples was due to the significant number of Type I squares for WBSF. However, the number of Type III squares adjusted for pH as a covariate was not significant ($p = 0.08$), and this suggested that the observed variation in WBSF between ST and NST samples was mainly due to pH.

Likewise, Hopkins (2015) determined that the relationship between shear forces and the parameter of lowering the pH of sheep carrion using linear and spline estimation models, and found a relationship between a higher pH of 24 for carrion sheep with a high value of shear forces. The association between stunning and tenderness of the meat was reported to be inconsistent. Previous researchers have asserted that meat tenderness of non-stunned animals relative to the ST group at 24 h post-mortem was due to variations in pH and calpain activity.

Significantly higher shear force values were evidenced in gaseous-stunned rabbit meat relative to rabbits slaughtered under halal specifications (Nakyinsige, 2014), and no difference ($p > 0.05$) in the cooking loss was observed between meat from ST and NST animals. Previous studies have also shown that stunning did not significantly affect ripening loss in sheep (Vergara, 2005).

Electrical stunning increased L* and decreased a* values which showed that the ST sample was light in colour, and no differences were observed for b* values between ST and NST meat samples. These results are consistent with previous findings that showed that the meat of non-stunned lamb was darker (lower L* value) than that of stunned lamb (Linares *et al*., 2008). Higher L* and lower a* values were also observed in meat from sheep that was electrocuted at 24 h post-mortem (Vergara, 2000). Reduction in redness in ST samples was also associated with a higher pH as shown by Hopkins (1998) who observed reduced redness in fresh sheep at 24 h post-mortem with high pH (Kiran *et al.*, 2019).

Variations in blood loss

Analysis of freshly drawn blood from slaughtered animals provides information about the type and level of stress the animal experienced during stunning and bleeding. Changes in blood volume, percent bleeding efficiency, and biochemical parameters in electrically stunned (ST) versus non-stunned (NST) animals are as follows. Much higher blood loss ($p < 0.05$) was observed in NST animals relative to ST, but percent bleeding efficiency did not differ ($p > 0.05$) between ST and NST sheep. Likewise, no difference in post-exsanguination blood loss was found between traditionally slaughtered and stunned sheep (Khalid *et al.*, 2015).

However, studies have also reported contrasting results on the effect of slaughter methods on blood loss (Sabow, 2015). Cardiac arrest due to stunning is believed to reduce the amount of blood lost during slaughter (Warris, 1984). Post-haemorrhagic blood loss in sheep was expressed as approximately 4% of their live weight (Warris, 1984), and in that study, the percentage of blood loss ranged from 3.2 to 3.39%. An insignificant variation ($p > 0.05$) was observed for serum total protein levels between ST and NST sheep. Similar results were also observed in rabbits that were stunned with gas as compared to animals slaughtered under halal

conditions (Nakyinsige, 2014).

Pre-slaughter stress causes an increase in circulating lactate levels, especially in pigs (Lametsch *et al*., 2002). In that study, they did not observe any difference ($p > 0.05$) in lactate levels between the ST and NST samples. Similar results were also reported in rabbits which were stunned with gas (Nakyinsige, 2014). An estimation of glucose is a good indicator of stress in animals because of its involvement in energy metabolism during stressful situations, in which liver glycogenolysis increases glucose levels (Nakyinsige, 2014). In that study, ST caused an increase ($p < 0.05$) in glucose levels in sheep as compared to NST in animals.

However, several other intrinsic and extrinsic factors appear to have an effect on blood glucose levels. In a previous study by Choe *et al*. (2009), considerable variation in blood glucose levels was observed in animals under the same slaughtering conditions. Nakyinsige (2014) observed significantly higher glucose levels in gas-stunned rabbits than halal slaughtered animals. These results have linked increased glucose levels with more physical activity in rabbits in the gas stun chamber and anaerobic oxidative metabolism than possible stress. Estimating blood glucose levels at different time points before and after slaughter can provide more meaningful information about animals' stress.

Water holding capacity (WHC)

Water holding capacity (WHC) is the ability of meat to hold water during external treatment such as cutting, heating, milling, and processing. The magnitude of WHC affects the colour, tenderness, elasticity, juiciness, and meat texture (Suadarna, 2009). The binding capacity of meat water is strongly influenced by pH, species, age, muscle function, feed, transportation, humidity, temperature, storage, sex, health, treatment before slaughter, and intramuscular fat content (Soeparno, 2009).

The factors that can affect the water-binding capacity of meat protein are pH, stress, locale, the formation of actomyosin (rigor mortis), temperature and humidity, carrion withering, muscle type, and muscle location, species, age, muscle function, feed, and intramuscular fat (Soeparno, 2009). Muscles with a high intramuscular fat content tend to show a high WHC. The relationship between intramuscular fat and WHC is complicated. Intramuscular fat will loosen meat's microstructure, thereby providing more opportunities for meat protein to bind water (Joo, 2013).

Resistance value

Apart from tenderness and meat colour, the conductance value can also provide a basis for the difference between normal and tiren chicken meat. This can be observed from the resistance value, as in previous research by Dwiatmaja and Rakhmadi (2012) which stated that the resistance value in tiren chickens was lower than that found in normal chickens whose values (for drained chickens) ranged from 60 - 78.4 kΩ on the breast, 81.8 - 115.3 kΩ on the thighs, and 60.9 - 97,4 kΩ on the wings; resistance in normal chickens is 569 - 858 kΩ on the breasts, 767 - 3610 kΩ on the thighs, and 736 - 958 kΩ on the wings. Conductance in tiren chicken was higher than in normal chicken.

According to Ghozali (2016), the resistance value of normal chicken is greater than that of tiren chicken, and is even greater when the meat is put in the refrigerator. The longer the storage, the greater the increase in resistance value.

The resistance value of normal and drained chicken meat during storage increased because storage will cause electrons in the cells of chicken meat to degrade, thus increasing the resistance value. The average value of normal chicken meat resistance is greater than the average value of tiren chicken (Farid, 2017).

Another illustration shows that lean chicken has a resistance value ranging from 11.08 - 69.49 kΩ, while normal chicken ranges from 67.20 - 123.39 kΩ. Tiren chicken meat shows a lower resistance value than normal chicken because tiren chicken meat has a low resistance value due to the level of degeneration, high necrose, the vigorous activity of proteolytic enzymes that damage protein filaments, and the release of intracellular water and several types of ions which play a role in muscle fibres. These factors cause muscle tissue to soften, thus resulting in lower resistance (Dwiatmaja and Rakhmadi, 2020).

Escherichia coli load

Several factors can influence the presence of *E. coli*, including the method and means of transportation used; meat that is placed on a table with an insufficient base resulting in a high total number of bacteria in the meat and fostering bacterial growth; lack of clean water; the location of selling chicken

meat where it is combined with other markets; and when the hygiene of the sellers is poor (Paerununan, 2018).

Among the critical points of microbial contamination in the case of ground meat is the handling of thawed meat, as well as cutting and mixing leftover ground beef, contacting processing equipment, and serving meat without packaging (Paerununan, 2018). When thawing and serving ground meat without packaging, the development of aerobic bacteria increases because the meat is in contact with the air (Paerununan, 2018). Common aerobic bacteria that grow in meat include *Shigella*, *Salmonella*, *Pseudomonas*, and *Bacillus*. A decrease in the number of bacteria in each meat sample has been observed as the result of extended storage and at temperatures in the treatment of frozen meat.

Regarding the microbiological characteristics of halal and non-halal poultry meat, nearly half of the poultry tested showed low contamination, and only a few samples had high microbial contamination. The total contamination of halal poultry (23×10^4 CFU/g) was less than that found in non-halal poultry, measuring 13.1×10^4 CFU/g. Also, the amount of yeast and mould contamination for non-halal poultry (61.1×10^4 CFU/g) exceeded that of halal poultry (9.1×10^3 CFU/g) (Moustafa, 2019). However, halal poultry was not tested with coliform bacteria, while non-halal meat contained 3.0×10^3 CFU/g coliforms. Moreover, *E. coli* was found in relatively large numbers in non-halal meat (4.0×10^4 CFU/g) in contrast to halal meat without *E. coli*. *Salmonella* spp. was not detected in halal meat while it was found in large quantities in non-halal meat (4.3×10^4 CFU/g) (Little, 2014). *Salmonella* spp. and *E. coli* were detected in 7 and 0.6% of 183 raw meat products tested respectively (Moustafa, 2019).

Total coliform load

The cause of the high load of coliform bacteria was due to the water used by traders to wash their hands, cleaning meat cutting tools together, and using stagnant sources of water. The washing water became a medium for coliform contamination; if the water has been contaminated with coliform, the meat will also be contaminated (Paerununan, 2018). The number of coliform bacteria in the research samples (sold in traditional markets and modern markets were in the following ranges, respectively: $0.11 \times 10^7 > 24 \times 10^7$ MPN/g and $0.35 \times 10^8 > 24 \times 10^8$ MPN/g.

The initial bacterial contamination of chicken meat is caused by microorganisms that enter the blood vessels when the cutting knife is not sterile. Contamination on the surface of chicken meat can occur during slaughter, processing, storage, distribution, or meat transportation (Paerununan, 2018). According to Jay (2005), bacterial contamination in chicken meat occurs during cutting, packing, distribution, and processing of animal products. Contamination can also occur due to poor sanitation in breeders, slaughterhouses, and chicken meat processing facilities. Water from poor sanitation increases the number of microbial contaminants in chicken and beef (Paerununan, 2018).

Conclusion

Based on the results of the discussion, it is possible to conclude that the detection and testing of carrion meat (tiren) can be accomplished with 14 potential analytical methods for distinguishing carrion meat (tiren) from fresh meat (normal). The first of these methods is the malachite green and hydrogen peroxide test, which results in a green reaction with carrion meat (tiren), and a clear blue reaction with meat that is halal. In the protein content detection method, carrion meat (tiren) will have higher protein content than fresh meat. In contrast, the peroxiredoxin-6 content test of tiren meat showed a higher level of peroxiredoxin-6 as compared to that found in fresh meat. In the biochemical test of blood, the contents of Fe, glucose, and Hb in meat from non-stunned animals are lower than those found in carrion meat (from stunned animals), For PUFA content, halal meat will have higher content as compared to carrion meat (stunning). Furthermore, halal meat has a lower blood pH (*i.e.*, more acidic) than carrion meat, and the meat's ability to hold an electrical charge (capacitance value) is lower for halal meat than carrion meat. Conversely, the level of resistance for halal slaughtered meat is higher than that of carrion meat. For parameters of variation in blood loss, fat content, and water holding capacity (WHC), halal meat has higher values than carrion meat. For the level of meat redness, halal (non-stunning) slaughtered meat has a higher reddish value (bright red) than carrion meat (dark red). Fresh meat has a lower cooking shrinkage value than carrion meat, and an absence of *E. coli* and coliform bacteria. The above conclusions show that some technical parameters,

such as malachite green and hydrogen peroxide tests can produce outputs as absolute values. In addition, some technical parameters have comparative values. These technical parameters must be characterised based on the purpose of the test in a given standard; namely, some are used for the detection of carrion meat, while others are used for market surveillance or sampling purposes. The results of the analyses also show that several technical parameters have limitations. Some can only identify the status of meat on the 0, 1st, 2nd, and 3rd day of slaughter; hence, it is necessary to highlight on this limitation when applied to a standard proposal. The factors that determine the level of validity of the test results for carrion meat (tiren) include the combination of storage of slaughtered animals, as well as the stunning *vs*. non-stunning status of the animals.

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