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Detection of Formalin Content in Chicken Meat Using Portable Near Infrared Spectrometer

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ABSTRACT

Food safety is essential for consumers. One of the compound that are prohibited from being used to preserve chicken meat is formalin. It demands a fast classification and sorting process for chicken meat, whether it is processed further or not. The main objective of this research is to develop a model that can predict the formalin content of chicken meat at room temperature using a portable NIR spectrometer. The NIRS method utilizes electromagnetic waves in infrared radiation with wavelengths ranging from 740-1070 nm. The method used to process the data is partial least square discriminative analysis (PLS-DA) to determine the presence of formalin in chicken meat. The results showed that the best pre-treatment was using the 1st derivative which had calibration results with an accuracy value of 92.86%, sensitivity 94.05%, specificity 91.67%, and FPR 8.33%. While the validation results obtained an accuracy value of 92.86%, sensitivity 92.86%, specificity 92.86%, and FPR 7.14%.

1. INTRODUCTION

One of the main protein products in Indonesia is chicken meat. Chicken meat is the cheapest animal protein compare to other kind of meat. This is due to the fact that chicken meat can be produced very quickly and is more affordable than other animal protein sources. In addition, chicken meat is also easy to obtain in the market by the public. Even though production has decreased compared to 2018 and 2019 which previously reached 3.49 million tons due to the covid-19 pandemic (Ilham & Haryanto, 2020). According to the statistical data, chicken meat production in 2020 remain high, namely 3.22 million tons (Ramadhany & Ermansyah, 2022).

According to the Ministry of Agriculture's census, the value of the livestock sub-sector's contribution to the gross national product in 2019 was 7.96%. Chicken is one of the livestock products that is produced and consumed the most in Indonesia (Andriniawati & Saskara, 2016). Chicken meat as a source of animal protein contains amino acids needed by the body and helps improve public health. Chicken meat is one of the foods that rarely

cause allergies, and its mild aroma makes it a popular choice among consumers of all ages (Soesanto, 2013).

The quality of chicken meat is affected by the treatment when the chicken is still alive, during slaughter and after slaughter. Subagyo *et al.* (2021) states that good quality chicken carcass is one that does not have conformity abnormalities such as no broken bones due to slaughter. The condition of the bones being intact or not torn in the chest, free from discoloration caused by bruising or freeze burn (discoloration of the meat due to contact with very cold surfaces, below -18^{o}C) and free from shoot hairs (BSN, 2009; Fikri *et al*., 2018).

Chicken meat is prone to contamination in the middle of the journey before it reaches consumers. The supply chain of chicken meat to the hands of consumers starts from breeders who are then directed to chicken slaughterhouses before the chicken meat is handed over to collectors. From the chicken meat collectors themselves, they will proceed to the chicken meat traders or restaurants and supermarkets before reaching the final consumers. The long process of fresh chicken meat reaching consumers is the reason for some irresponsible people to mix chicken meat with preservatives so that the chicken meat looks fresh longer. One of the compound used to preserve chicken meat is formalin. Formalin is a trade name for formaldehyde and is a group of strong disinfectant compounds (Rahmahani *et al*., 2018). Formalin is used in chicken meat because it can kill various putrefactive bacteria that will inhibit the process of putrefaction in chicken meat. The use of hazardous additives such as formalin will also interfere with health. Yulianti (2021) added that the dangers of formalin to health include if it is inhaled, swallowed or gets on the skin it can cause respiratory tract irritation, allergic reactions, and burns, while in the long term it can trigger the development of cancer cells.

The method that can be used to determine the presence or absence of formalin content in chicken meat is to perform non-destructive screening using a portable near infrared spectrometer (NIRS). Andasuryani *et al.* (2013) and (Suhandy, 2021) also explained that this method is cheaper, environmentally friendly by not requiring sample preparation when used because it can be directly tested on samples. The NIRS method itself utilizes electromagnetic waves in the form of infrared radiation with wavelengths ranging from 740-1070 nm.

NIRS is a technique to predict the chemical composition of a substance based on its absorbance spectrum. The advantage of adopting NIRS technology in chemical testing is that it reduces the amount of time required for agricultural testing and eliminates the need for chemicals. According to Schwanninger *et al*. (2011), testing using NIRS has two main advantages, namely having high speed for spectral acquisition which collects descriptive data, and has high speed for spectrum acquisition which collects descriptive. NIRS has been widely used to determine the quality of food ingredients, including to test the quality of chicken meat. Silva *et al.* (2020) conducted a trial to find out the mixing that was carried out on chicken, pork and ground beef with the end result being able to distinguish the meat content mixed by NIRS, De Marchi *et al.* (2012) used NIRS to evaluate the fatty acid content in chicken breast using waves of 350-1830 nm. The use of spectral values with wavelengths between 1130 and 1400-1450 nm can be used to observe O-H bonds (indicating the presence of water molecules) and C-H bonds (indicating the presence of fat molecules) (De Marchi et al., 2011; Murray & Williams, 1990). In addition to performing classifications more accurately and quickly without causing sample damage, near infrared spectroscopy

(NIRS) technology can replace conventional methods for determining the content in recently butchered chicken meat. Using a portable NIR spectrometer, this study seeks to identify the formalin content in chicken meat.

2. MATERIALS AND METHODS

2.1. Materials and Tools

The materials used in this research were fresh chicken meat obtained from chicken slaughterhouses in the Bogor area, formalin (2 ppm), and aquades. The use of formalin with a concentration of 2 ppm refers to the smallest concentration that can be tested using a conventional formalin test kit using an antiline reagent (Yulianti, 2021). The tools used in this research were a portable SCiO visible near infrared spectrometer to collect spectrum data with a wavelength of 740-1070 nm, analytical balance, petri dish, volumetric flask, pipette, beaker glass, and knife.

2.2. Location and Time of Research

The research was carried out in August-December 2022, at the Laboratory of Meat and Draught Animal Nutrition, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University.

2.3. Research Methods

This research used the Partial Least Square Discriminant Analysis (PLS-DA) data processing method. Prior to data processing, the data will undergo a pre-treatment process which aims to reduce or eliminate noise (disturbances) in the obtained spectral data. Pre-treatment of spectral data is used to solve problems related to solid-state radiation scattering as measured by the reflectance of the other spectral baselines that affect the data (Pasquini, 2003). The pre-treatments used were normalization, 1^{st} derivative, 2^{nd} derivative, smoothing Savitzky-Golay, SNV, and detrending. Processing of this data, according to Pizarro *et al.* (2004), can reduce spectral fluctuations caused by scattering and variance that are not related to the response being modeled. In some circumstances, processing of the spectral data allows a more accurate comparison of the calibration model to the original spectrum. Munawar (2014) emphasized that the pre-treatment step is very important before modeling.

2.4. Research Procedure

The research was divided into 3 stages, namely the sample preparation process, reflectance measurement using portable near infrared and data analysis.

2.4.1. Sample Preparation

The main ingredient was freshly slaughtered chicken which is cleaned and divided into weights of ±20 g each. Samples were divided into two observation groups, namely samples of fresh meat and samples with formalin immersion process. The two sample groups were then stored at room temperature and observed and measured for changes during the first 6 hours after storage (0, 1, 2, 3, 4, 5, 6 hours). Samples were stored at room temperature (±25^{o}C). Tests are carried out every hour to determine differences in the absorption of NIR waves, which indicate changes in the chemical content present in the samples during the storage period. Based on SNI 2897:2008, fresh chicken meat is meat that is obtained 4 hours after slaughtering.

2.4.2. Reflectance Measurement with Portable NIR Spectrometer

Spectral data were obtained from the reflectance results of chicken meat samples with the spectrometer calibrated online with the SCiO Lab software using a smartphone connected to Bluetooth. Samples were scanned on the surface three times at three different points to obtain spectral data. Samples were scanned every 1 hour during 5 hours of storage. The Unscrambler X 10.4 program was used to process the data after the spectrum data had been acquired.

2.4.3. Data Analysis

The calibration model was built using the PLS-DA method. Of the 84 samples consisting of fresh chicken meat without formalin and chicken meat using formalin, it was divided into 2/3 parts for calibration and 1/3 parts for validation. After the data was divided into calibration and validation data, chicken meat data was classified into 2 groups, namely group 1 (fresh chicken) and group 2 (formalinized chicken meat). Grouping is done according to predetermined thresholds. The results of the PLS-DA analysis must be rounded so that the accuracy of the data classification can be known (Masyitah *et al*., 2023). The success rate of the PLS-DA method will be determined by the calibration and validation model's use of confusion matrix analysis. According to Olson (2008), the accuracy, sensitivity, specificity, and false positive rate of the constructed model are used to assess its performance.

3. RESULTS AND DISCUSSION

3.1. Spectral Scan Results

The presence of peaks and valleys on the results of a spectral scan can indicate the presence of certain chemical compounds in the material. The results of the spectral scan on chicken meat can be seen in Figure 1. The figure shows the scan results of the spectra of fresh chicken meat and formalin chicken meat at a wavelength of 740-1070 nm. The resulting waveform tends to be the same as the difference in trough depth or crest height. Figure 2 explains the difference in height from the scan results of the two samples (formalin and non-formalin chickens). The peaks and valleys obtained on the spectral scan occur due to vibrations that occur between chemical bonds and NIRS that interact with each other. In Figure 2 it can be seen that formalin chicken has a higher peak than fresh chicken without formalin.

The reason formalin can retain materials is because its aldehyde groups will bind to the protein components in the sample. Formalin or also known as formaldehyde is able to modify proteins and nucleic acids. This can occur through the process of alkylation between the -NH2 and -OH groups of proteins and nucleic acids with the hydroxymethyl group of formaldehyde. This bond between formaldehyde and protein will then form a methylene bond and a cross-link that is difficult to break (Kiernan, 2000). Differences in peaks and valleys with spectral waveforms that tend to be the same can be caused because other substances such as carbohydrates, fats and nucleic acids trapped in.

These cross-links do not change chemically when mixed with formaldehyde (formalin), but the process of breaking down into simpler molecules will be slower (Mundriyastutik *et al.*, 2020). Chicken meat treated with formalin showed relatively more stable spectral results because the decomposition of the ingredients in the meat was restrained in the presence of formalin. Nurfi & Sopandi (2014) in their writing explained that formalin is a reactive compound, this allows formalin to bind to compounds present in food ingredients such as proteins, fats and carbohydrates.

Formalin has antimicrobial properties. If formalin is mixed in the meat, it will condense with the free amino acids in the protein and become another mixture, this will inactivate the protein so that the meat is not easily damaged (Mundriyastutik *et al*., 2020).

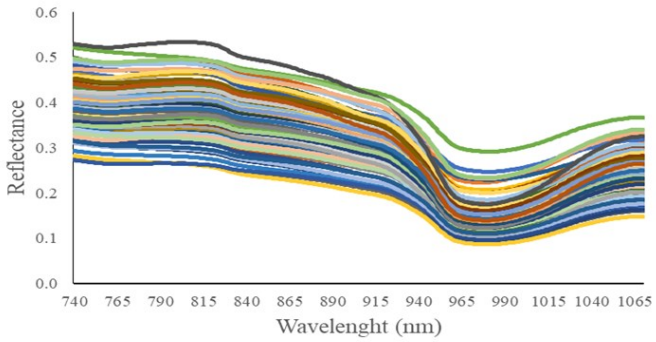

Figure 1. Original spectra on chicken meat

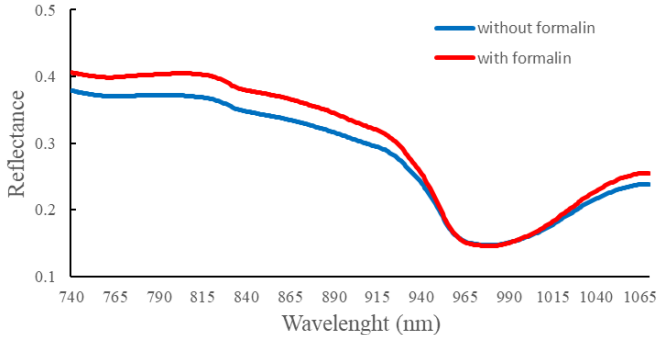

Figure 2. Average spectra on chicken meat

At waves of 950-1000 nm there is a similarity in the depth of the valley in Figure 2. At this wavelength it is thought that there are O-H bonds which refer to the water content (Akeme *et al*., 2021; Ardiamsyah, 2021; Doan *et al*., 2021; Williams & Norris, 1987). Formalin when mixed with water does not have any reaction so that on the graph it can be seen if the waves obtained tend to be the same and are not affected by their contents even though they have been treated with formalin.

In Figure 3 it can be seen more about the content of amine groups at waves 1040-1070 nm. The amine group can refer to the presence of protein content in the chicken meat studied. As is known, chicken meat contains protein above 19% (Lukman *et al*., 2009). This is reinforced by the results of research from Doan *et al*. (2021) who explained that there were N-H bonds at waves 1040-1070 nm and the results of Ardiamsyah (2021) which showed the presence of N-H bonds indicating the protein content in chicken eggs. Formalin is very influential in the process of breaking down proteins. Formalin mixed with meat will condense with amino acids present in protein (Mundriyastutik *et al*., 2020). Whereas in the 921-951 nm waves there are O-H groups (Masyitah *et al*., 2023) which indicate the presence of water content in the material. The water content in the sample was not much affected by the presence of the formalin mixture and continued to undergo the evaporation process. This can be caused by the absence of a special reaction that occurs when water and formaldehyde are mixed in a material.

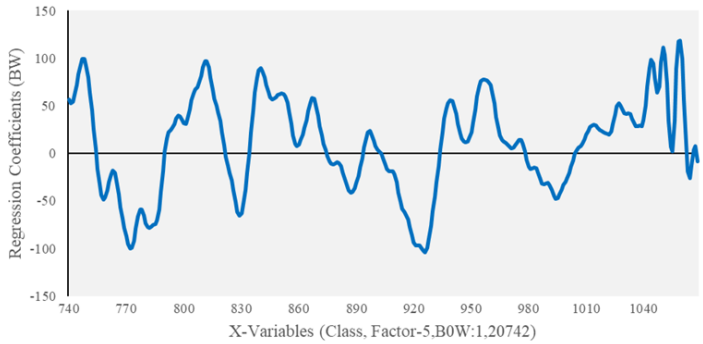

Figure 3. Graph of absorption spectrum in chicken meat

3.2. Spectra Scan Results Analysis

From the results of the spectral scan, further analysis is needed to determine the exact difference in value from the spectral scan results obtained from these two types of chicken meat. The spectral data is then pretreated to remove the existing noise. Classification of the predicted sample is done by rounding the predicted data to 0 after the decimal point. The limiting value used is 0.5 where this value is a reference for classifying chicken meat samples into group 1 (fresh) or 2 (formalinated). The results of the classification of the predicted samples can be entered into the confusion matrix table to then calculate each of the parameters that determine the success of the PLSDA model. The parameters in question are accuracy, sensitivity, specificity, and false positive rate (FPR). The results of calculating the PLS-DA parameters for calibration data can be seen in Table 1.

Table 1. The calculation results of the PLS-DA model parameters on the calibration data

Pre-treatment	Accuracy (%)	Sensitivity (%)	Specificity (%)	FPR (%)
No pre-treatment	85.12	79.76	90.48	9.52
Smoothing	85.12	79.76	90.48	9.52
Normalize	84.52	79.76	89.29	10.71
1st Derivative	92.86	94.05	91.67	8.33
2nd Derivative	92.26	90.48	94.05	5.95
SNV	84.52	77.38	91.67	8.33
Detrending	84.52	77.38	91.67	8.33

Based on the results of the various types of pre-treatment, the results can be seen in Table 1 that the 1st derivative pre-treatment gave optimal results with an accuracy value of 92.86%, a sensitivity of 94.057%, a specificity of 91.67%, and an FPR of 8.33%. The high accuracy value indicates that the model is able to predict differences in the formaldehyde content in chicken meat. The use of pre-treatment can help overlapping spectra so as to clarify the peaks and valleys of the resulting spectrum (Ozaki *et al*., 2007). The sensitivity value indicates if the model is able to predict the presence of formalin in chicken meat. While the FPR value indicates the possibility of error in predicting the data. The results of calculating the PLS-DA parameters for validation data can be seen in Table 2.

Table 2. The results of calculating the parameters of the PLS-DA model for validation data

Pre-treatment	Accuracy (%)	Sensitivity (%)	Specificity (%)	FPR (%)
No pre-treatment	88.10	85.71	90.48	9.52
Smoothing	88.10	85.71	90.48	9.52
Normalize	86.90	83.33	90.48	9.52
1st Derivative	92.86	92.86	92.86	7.14
2nd Derivative	88.10	85.71	90.48	9.52
SNV	85.71	78.57	92.86	7.14
Detrending	85.71	78.57	92.86	7.14

The validation results show an accuracy value of 92.86%. The sensitivity value obtained was 92.86% with a specificity value of 92.86%. The FPR value on the calibration data validation results was 7.14%. Prediction errors that occur can be caused by data on chicken meat in formalin which is read as chicken meat without formalin, and vice versa. Based on this, it can be said that the PLS-DA method can be used to distinguish chicken meat with formalin and chicken meat without formalin because the error value that occurs is still below 10%.

4. CONCLUSION

NIR spectrometer method with a wavelength of 740-1070 nm can be used to predict the presence of formalin in chicken meat. The use of the PLS-DA method can be an option for conducting an analysis of the data obtained. The calibration results were obtained quite well using several pre-treatments in the form of smoothing Savitzky-Golay, normalization, 1st derivative, 2nd derivative, SNV, and detrending. The best pre-treatment calibration results are found in the 1st derivative, with an accuracy of 92.86%, a sensitivity of 94.057%, a specificity of 91.67%, and an FPR of 8.33%. The validation results obtained an accuracy of 92.86%, a sensitivity of 92.86%, a specificity of 92.86%, and an FPR of 7.14%.

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