Versi Online: http://journal.ipb.ac.id/index.php/jtip
https://doi.org/10.6066/jtip.2022.33.1.87
Hasil Penelitian

J. Teknol. dan Industri Pangan
Vol. 33(1): 87-99 Th. 2022 ISSN: 1979-7788
Terakreditasi Ristekdikti: 164/E/KPT/2021

Differentiation of Beef, Buffalo, Pork, and Wild Boar Meats Using Colorimetric and Digital Image Analysis Coupled with Multivariate Data Analysis

Fayca Rudhatin Swartidyana[1)], Nancy Dewi Yuliana[1,3)]*, I Ketut Mudite Adnyane[2)], Joko Hermanianto[1,3)], and Irwandi Jaswir[4)]

[1)] Department of Food Science and Technology, Faculty of Agriculture Engineering and Technology, IPB University, Bogor, Indonesia
[2)] Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
[3)] Halal Science Center, IPB University, Bogor, Indonesia
[4)] International Institute for Halal Research and Training, International Islamic University Malaysia, Selangor, Malaysia

Received April 10th 2022 / Accepted June 25th 2022

ABSTRACT

Beef price is relatively expensive, which makes this commodity vulnerable to be counterfeited. The development of rapid, cheap and robust analytical methods for meats authentication has therefore become increasingly important. In this study, colorimetric and digital image analysis methods were used to characterize and classify four types of meat (beef, buffalo, pork, and wild boar) and two muscle types from each sample (*Semitendinosus* and *Vastus lateralis*). Multivariate data analysis (PCA and OPLS-DA) was used to observe classification pattern among species using different color parameters data obtained from meat chromameter and digital image measurement. The results showed that PCA and OPLS-DA successfully classified meat from different species and different muscle type based on color, both in chromameter and in image analysis. It was shown that pork had the highest lightness level, and was the most different among the four types of meat tested. Beef was predominated by yellowish color, while buffalo meat had the highest reddish color level. *Semitendinosus* and *Vastus lateralis* muscles had different color intensity where *Vastus lateralis* exhibited darker color intensity. This study showed that meat color analysis using chromameter and imaging techniques can be used as cheap and quick tools to discriminate meats form different species and different muscles type.

Keywords: adulteration, color, halal, multivariate data analysis, muscle

INTRODUCTION

Meat is considered as an important source of high biological value protein, fat, and essential micronutrients (De Smet and Vossen, 2016). The high demand for meat is not followed with an adequate meat supply, making meat price expensive and thus, vulnerable to adulteration. The most common type of adulteration is a substitution of high valued meats with the cheaper ones, such as beef substituted by buffalo meat. This also includes substitution of halal meat by the nonhalal ones, such as beef substituted by pork or wild boar meat. The latter case was of particular concern in Indonesia as one of the world's largest Muslim communities, where Muslims are well aware of the halal status of their food. Since it is difficult to visually distinguish different types of red meats such as beef, buffalo, pork and wild boar, the development of a robust, fast and inexpensive methods for authenticating meat at the sales level is highly required.

Several meat authentication techniques have been developed and comprehensively summarized in a recently published review (El Sheikha *et al.,* 2017). Most of the analytical techniques summarized there were too tedious for daily routine analysis. Thus, the use of rapid, low cost, and non-destructive analysis to determine meat quality including in the distinction of halal and non-halal animals has recently gained more interest, including those of digital-image analysis techniques. As examples, multispectral image analysis combined with multivariate data analysis was shown to be able to detect pork adulteration in minced beef with detection limit of 10% (Ropodi *et al.,* 2015). Image analysis was also successfully used to classifying ham based on meat type (pork and turkey), and processing types (boiled, smoked, and roasted) (Sinanoglou *et al.,* 2018).

Several studies have shown that consumer preferences in different countries on whether or not to buy meat are highly affected by the color and physical appearance of the meat (Wang *et al.,* 2020). There are very limited studies on linking meat color obtained from instrument measurement with different animal types especially in the halal and non-halal context. Customers in many parts of Indonesia traditionally believe that beef has a more yellowish color compared to buffalo meat. It is also common knowledge that beef has a color that is more similar to that of wild boar meat, but somewhat

*Corresponding Author:
E-mail: nancy_dewi@apps.ipb.ac.id

different from pork, which is lighter than beef. There is little scientific proof to justify these perceptions circulating in the society. Besides, there is a high bias in visually or qualitatively assessing color and texture as the human eye has no specific benchmarks and different individuals have different abilities to perceive particular color.

Given the growing interest in the quality and authenticity of meat by using colorimetry and digital image analysis, their combination may be a powerful tool to differentiate between muscle and meat types. However, the number of studies in this topic is very limited. In the current study, a combination of colorimetry and digital image analysis was used for the first time to differentiate buffalo, pig, and wild boar meat that is commonly used in Indonesia as beef adulterant. The technique was also used to distinguish the type of muscle (*Semitendinosus* and *Vastus lateralis*) in each sample. Principal Component Analysis (PCA) and Othogonal Projection to the Least Square-Discriminant Analysis (OPLS-DA) were used to identify typical color characteristics strongly associate with each type of meat and muscle, which were selected based on the VIP and coefficient value of the respective multivariate data models. Additionally, ultimate pH of each sample was also determined.

MATERIALS AND METHODS

Materials

Four types of meat (beef, pork, buffalo and wild boar) were purchased from different suppliers in Indonesia. Beef from Brahman cross cattle (n= 2) were purchased from Bubulak Abattoir, Bogor, West Java, with the age range of 24-30 months and the slaughter weight was 400-450 kg. Pork (n= 2, the age range of 5-6 months, 95-100 kg of the slaughter weight) was purchased from traditional market in Bogor, West Java. The buffalo meat samples used (n= 2) were the swamp buffalo which were from Trondol Abattoir, Serang, Banten, with the age range of 30-36 months and the slaughter weight was 350-400 kg. Wild boar meat (n= 2) were purchased from the hunter in South Sumatera, with the weight after hunted was 70-80 kg. Two types of muscle (*Semitendinosus* and *Vastus lateralis*) were prepared from each samples immediately after slaughtering and breaking the carcass, then packed in polyethylene plastic, sealed, and stored at cool box equipped with ice gel during the trip. Samples were stored in chiller until analyzed (8-10 h). Except for wild boar meats, samples were frozen after removing the visceral organs. Shipped frozen, muscle separated, and thawed in refrigerator prior to analysis. This condition was similar to wild boar meat, which is frequently sold illegally in the market. The meats were cut crosswise into 10 slices and measured each side.

Ultimate pH measurement

The ultimate pH (pH_u) measurement of meat was carried out using a pH meter (model HI 99163, Hanna, Woonsocket, RI, USA).

Color measurement

A tristimulus chromameter (model CR-400, Minolta, Tokyo, Japan) with illuminant D_{65} was used to measure the meat color. The data was expressed in as L* (lightness), a* (greenness/redness), b* (blueness/yellowness), C* (chroma), h (hue), and XYZ scale. XYZ scale represent the weighs of our retinal response to wavelengths in a range. The X value represents the Red response, the Y value represents the Green/Yellow response, and the Z value represents the Blue response. Three random readings per sample were taken and averaged with bloom period 10-20 min.

Image acquisition

Meat cuts were digitally photographed using Nikon D4600 digital camera. Meat slices were placed at light box (image capture box with a length of 35 cm and a width of 35 cm equipped with white LED light) to isolate external light sources and to get the maximum image quality with the same lighting conditions from one object to another. Images of meat slices were acquired at lens aperture f = 4.5, ISO 200, 2992 x 2000 pixel resolution, lighting around 1700 lux, and taken at a distance of about 8-10 cm. The images were saved in jpg format and resized at 128x128 pixels. This image resizing step is important to speed up computation and to remove unwanted parts of the image, so as to produce an image that suits the needs. Next, the input parameter values for the color feature, those are HSV, L*, a*, b*, and C*, and h was calculated, while feature values from the texture was calculated using Gray Level Co-occurrence Matrix (GLCM) method refered to Haralick *et al.* (1973). HSV is a user-oriented color model focused on the artist's idea of tint, shade and tone. H (hue) differentiates between thee color perceived, such as red, yellow, green and blue; S (saturation) refers to how much light is concentrated at each particular wavelength of the hue; and V (value) reflects the overall brightness (Park and Lu, 2015).

Statistical Analysis

All of the data from color and image analysis were converted into numeric form in an excel file. The data was subjected to Principal Component Analysis (PCA) and Orthogonal Projection to the Least-Square Discriminant Analysis (OPLS-DA) (SIMCA ver. 16, Sartorius Stedim Biotech, Malmö, Sweden) using Pareto scaling method.

RESULTS AND DISCUSSION

pH_u Value

Except for wild boar meat, all samples had passed the rigor mortis phase and had a normal pH value, both in *Semitendinosus* and *Vastus lateralis* muscles (Table 1). A pH higher than the normal range was found in wild boar meat, both in *Semitendinosus* and *Vastus lateralis* (pH 6.30 and 6.16, respectively). Previous study reported a close relationship between meat color and pH, *i.e.* the higher pH value corresponds to the darker meat (Kasprzyk *et al.,* 2010). Wild boar meat is characterized by a higher initial pH at 45 min post-mortem (pH_{45}), darker color, and lower conductivity compared to the domesticated Pietrain breed (Sales and Kotrba, 2013). This result also conforms Müller *et al.* (2002), who reported that pH_{45} in *Semimembranosus* of wild boar was higher (6.14) than Pietrain pig (5.45). Similarly, Kasprzyk *et al.* (2010) showed that *Longissimus dorsi* of wild boar had higher pH_{45} (pH 6.54) than Pulawska pigs and Crossbred pigs (pH 6.33). Higher concentration of hydrogen ions was also found in *Semimebranosus* of wild boar. The high pH of wild boar compared to other species is related to the stress experienced by wild boar when hunted by hunters. In a study of an effect of hunting to red deer meat quality, hunting-related stress caused depletion of carbohydrate sources to strengthen muscles. This leads to a raise in pH, disruption of muscle tissue, and an increase of beta-endorphins and cortisol secretion which are usually associated with extreme physiological and psychological stress (Bateson and Bradshaw, 1997).

Table 1. pH_u value of meat depending on animal and muscle types

Types of Meat	Types of Muscle	pH_u Value
Beef	*Semitendinosus*	5.43±0.08
	Vastus lateralis	5.50±0.07
Buffalo	*Semitendinosus*	5.58±0.08
	Vastus lateralis	5.76±0.10
Pork	*Semitendinosus*	5.57±0.06
	Vastus lateralis	5.65±0.08
Wild Boar	*Semitendinosus*	6.30±0.17
	Vastus lateralis	6.16±0.20

Multivariate data analysis of beef, buffalo, pork and wild boar chromameter data

PCA score plot derived from chromameter data was able to distinguish beef, pork, and wild boar, although buffalo and beef were partially overlapped (Figure 1A). PCA loading plot revealed discriminating colour parameters for each cluster. Beef was marked with a high value b*, while buffalo with high value of C* and a*. Wild boar was differentiated from others by its high a* value, while pork was dominated by high X, Y, Z, and L (Figure 1B). This is in accordance with the perception that develops in the community that beef has a yellowish color compared to buffalo meat. Based on subjective observations, lower pH caused beef to appear more orange (Page *et al.,* 2001). However, it is not reported at what pH the beef looks more orange. OPLS-DA with 4 classes was then conducted with the same set of data. The model had satisfying performance with R^2X 0.987, R^2Y 0.539, Q^2 0.51 and *p* CV ANOVA $1{,}47741 \times 10^{-30}$. OPLS-DA showed similar classification pattern as revealed by PCA (Figure 2A). To identify colorimeter parameters which are important for the classification of each class, VIP (variable important for the projection) plot and coefficient plots were used. VIP value indicates the importance of the variables in explaining data X and to correlate to the Y value (Eriksson *et al.,* 2006). VIP value is expressed only in positive value. In this OPLS-DA model, b*, a*, h and C seemed to give the highest contributions for sample clustering since they had VIP value higher than 1 (Figure 2B). Coefficient plot gives information whether the contribution is positive or negative (Figure 2C and 2D). The b* value showed as a strong positive discriminating color parameter in beef (Figure 2C). Class 2 (buffalo) exhibited high a* (redness) (Figure 2D). Pork was discriminated by its high h value (Figure 2E), while wild boar was strongly differed from others with a high L value (Figure 2F).

OPLS-DA was also used to observe whether there is a classification pattern between *Vastus lateralis* and *Semitendinosus* muscles among all samples. OPLS-DA score plot showed no distinct classification (data not shown). OPLS-DA was only able to discriminate *Vastus lateralis* and *Semitendinosus* muscles in beef (Figure 3) and buffalo (Figure 4) as can be seen in the respective score plot (Figure 3A and Figure 4B). *Vastus lateralis* of beef muscles was marked with a high a* value, followed by C* value (Figure 3B), whereas beef *Semitendinosus* muscles exhibitied high h value, followed by b* and L (Figure 3C). *Vastus lateralis* of buffalo had remarkebly high b* value (Figure 4B), while its *Semitendinosus* muscle was oppositely had lower b* value dominated with a high a* (Figure 4C).

The results are in agreement with previous study, where beef *Semitendinosus* exhibited the higher L*, b* and h value, while *Vastus lateralis* had higher a* and C* value, but *Vastus lateralis* had less stable color intensity (King *et al.,* 2011). Perhaps that is why in beef and wild boar, *Semitendinosus* had higher values of a* and C* than those of *Vastus lateralis.* Interestingly, in wild boar meat, *Semitendinosus* had a darker color than *Vastus lateralis*. This may associate with a higher pH of *Semitendinosus* than *Vastus lateralis*.

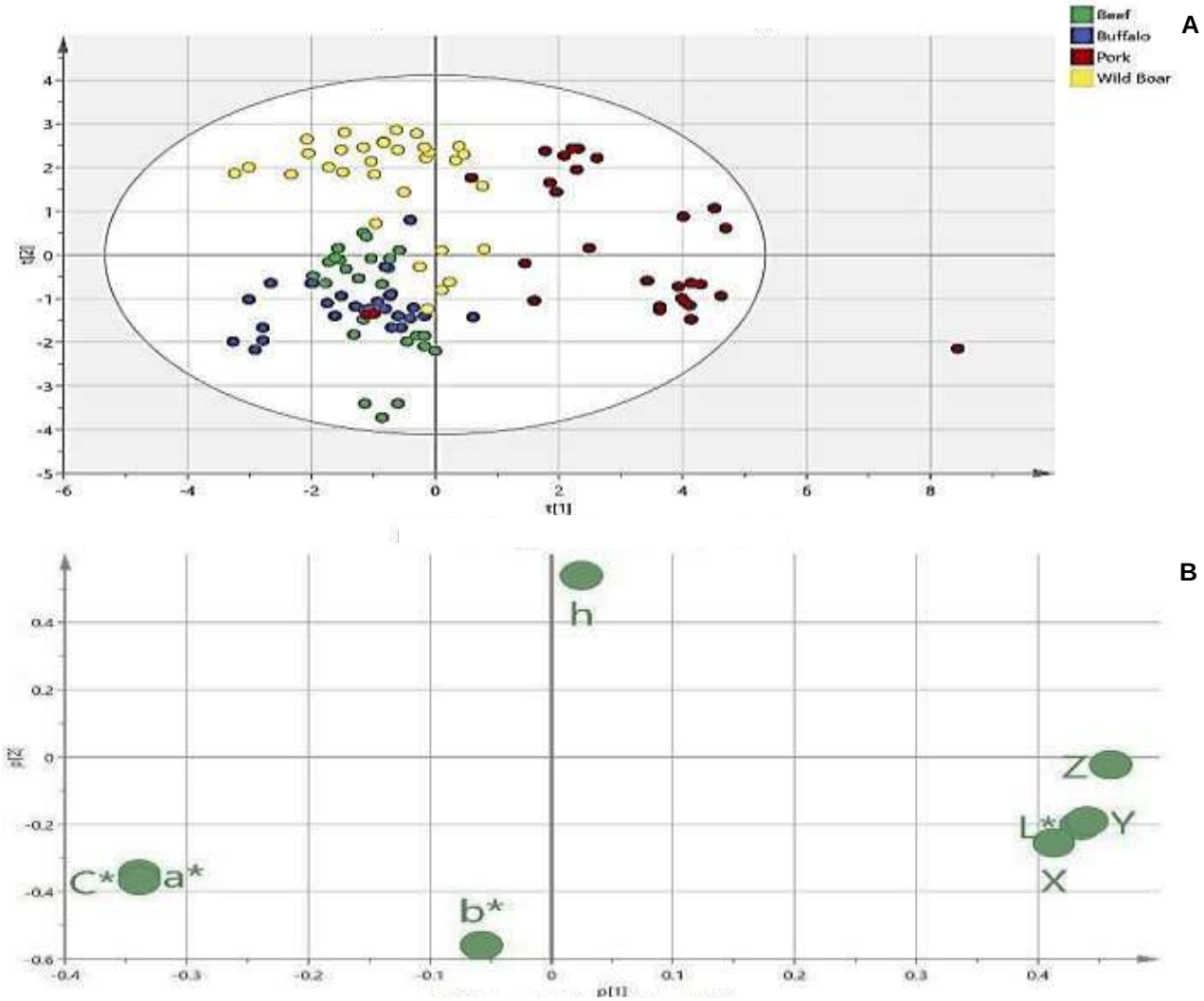

Figure 1. Score plot (A) and loading plot (B) chromameter data of different types of meat

Muscle effects have a greater contribution to color variation and stability, whereas animal effects are consistent across muscles (King *et al.,* 2011). Individual muscles have specific anatomical locations and physiological functions, resulting in metabolic differences; consequently, each muscle exhibits a unique biochemical color (Hunt and Hedrick, 1977). Muscles that are heavily used for movement such as the shoulders and legs require more O_2, which is carried mainly by red blood cells, resulting in a darker color of muscle tissue. Therefore, the myoglobin concentration in muscles that are widely used is generally higher than in muscles that are rarely used for movement. This also explains why pork shoulder meat is darker in color than pork loin. The terms 'dark' and 'light' flesh are usually used to describe this color difference (Feiner, 2006).

Many researches on meat color were conducted using colorimeter. However, colorimeters have limitations. Colorimeter is unable to measure the color of the whole surface in a single measurement if it is non-homogeneous. The colorimeter measures the light reflectance of a given portion of the matrix, giving a color evaluation without any information about its local variability (Antonelli *et al.,* 2004). Meanwhile meat does not have a homogeneous surface because of its structure, its connective content and its intramuscular fat. The enlargement of the measured area would possibly include fat and connective tissue, thus yielding unreliable measures (Girolami *et al.,* 2013). The interaction of the light emitted with the surface to be analyzed is another problem. Noted that color depends on the physical and chemical characteristics of the product. The object will be transmitting, refracting, reflecting, diffusing and absorbing the light beam (the one the colorimeter emits).

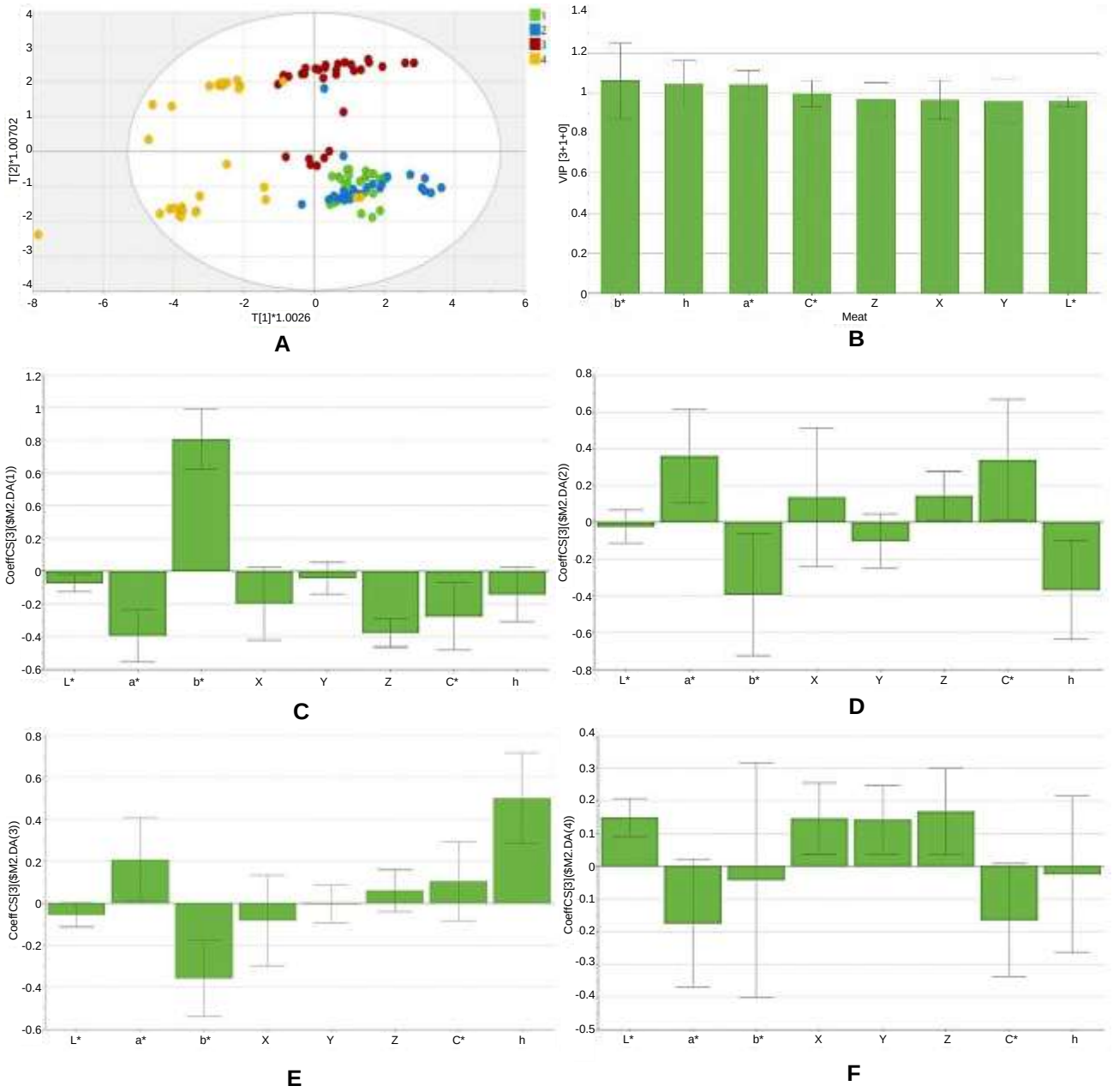

Figure 2. A= OPLS-DA score plot of beef (class 1), buffalo (class 2), pork (class 3) and wild boar (class 4) chromameter data. B= VIP plot of OPLS-DA mentioning colorimetry parameters important for the classification. C= Coefficient plot mentioning colorimetry parameters which positively or negatively associated with class 1 (beef). D= Coefficient plot mentioning colorimetry parameters which positively or negatively associated with class 2 (buffalo). E=. Coefficient plot mentioning colorimetry parameters which positively or negatively associated with class 3 (wild boar). F= Coefficient plot mentioning colorimetry parameters which positively or negatively associated with class 4 (pork)

Meat, which is an optically non-homogeneous medium (its refraction index is not uniform), has air, liquids, and granules of different materials scattered inside. Therefore it causes multiple reflections and refrac-tions where optical discontinuities are present, resulting in a diffusion of light (scattering). For this reason, the technology of the digital camera is being increasingly adopted, because the whole image of the product can be analyzed, not just the color of a reduced area such as the area spotted by the colorimeter only. Image analysis method allows estimating the overall color of the sample and its heterogeneity.

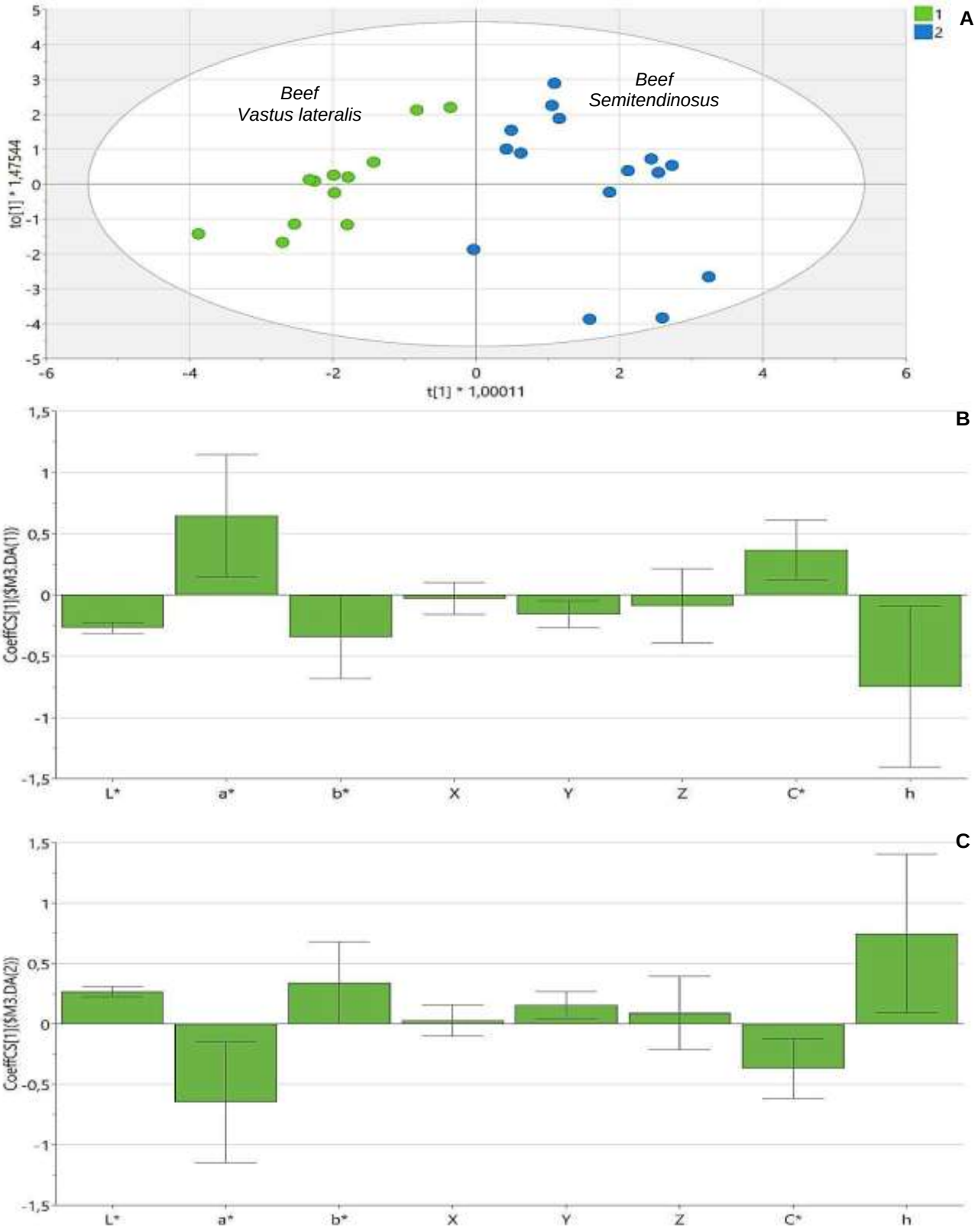

Figure 3. A= OPLS-DA score plot data chromameter beef *Vastus lateralis* (class 1) and *Semitendinosus* muscles (class 2) with satisfying validation value R^2Y 0.789, Q^2 0.728, and p CV ANOVA $8.4x10^{-5}$. B= Coefficient plot mentioning color parameteres discriminant for class 1(beef *Vastus lateralis*). C= Coefficient plot mentioning color parameters discriminant for class 2 (beef *Semitendinosus*)

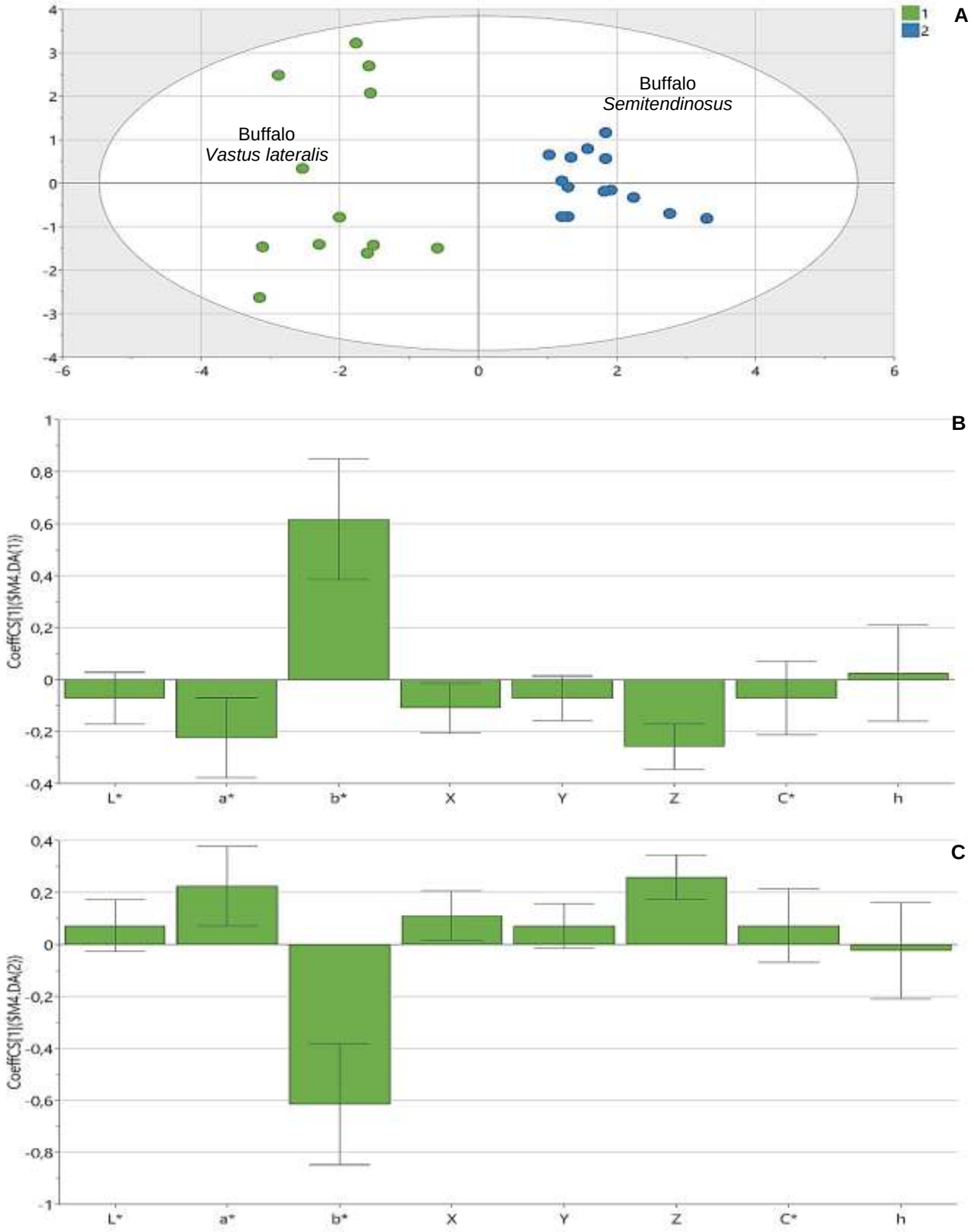

Figure 4. A= OPLS-DA score plot data chromameter buffalo *Vastus lateralis* (class 1) and *Semitendinosus* muscles (class 2) with satisfying validation value R^2Y 0.887, Q^2 0.816, and p CV ANOVA $4.4x10^{-6}$. B= Coefficient plot mentioning color parameteres discriminant for class 1 (buffalo *Vastus lateralis*). C= Coefficient plot mentioning color parameters discriminant for class 2 (buffalo *Semitendinosus*)

The image was captured, processed and analyzed, then the color was assessed with a non-destructive and objective method (Zheng and Sun, 2006). It had been proved that the colorimeter did not generate coordinates corresponding to the true color of meat. Instead, the computer vision system method had given valid measurements that reproduced a color very similar to the real one (Girolami *et al.,* 2013).

Multivariate data analysis of beef, buffalo, pork and wild boar image analysis data

We subjected digital image color data into PCA (Figure 5A) to obtain R^2X and Q^2 value of 0.983 and 0.913, respectively. However, we obtained more overlapping pattern as compared to PCA of colorimeter data. OPLS-DA with four classes according to the species type also gave similar result, although with R^2Y and Q^2 0.4. Since beef-pork, and pork-wild boar seemed to be in different cluster in the PCA score plot, we conducted separate PCA for beef and pork (Figure 5B), and for wild boar and pork (Figure 5C). Both models had satisfying performance which indicated by R^2Y and Q^2 value not less than 0.9. For better understanding on which image parameters significantly influence the grouping, we further conducted OPLS-DA for pork (class 1) and beef (class 2), and for pork and wild boar (Figure 6). The image parameters responsible for the classification in each OPLS-DA model were selected based on the respective S-plots (Figure 6B). S-plot is very useful to find extreme discriminant factors between two groups of observation. Pork was discriminated from beef for its high value of G, R, B, b*, and L. Other parameters, those are C, V, S, and h, were located close to the centre of the S-plot, meaning they were less important for beef and pork discrimination. In contrast, beef was marked with its high a* value (Figure 6B). Interestingly, when pork was compared to wild boar, it was predominated by high G, R, B, L and b* value, while wild boar was marked with high a* value (Figure 6C and Figure 6D).

Next, we tried to observe whether it is possible to create OPLS-DA model with 8 classes to differentiate meat based on both species type and muscle type. The resulted OPLS-DA model was very poor with R^2Y and Q^2 value less than 0.4 (data not shown). When separate model was created, it was only able to discriminate between between buffalo *Semitendinosus* and buffalo *Vastus lateralis* (R^2Y and $Q^2 = 0.856$). Based on the S-plot, the R, G, B, and L was predominant in buffalo *Semitendinosus*, while a* was dominant in *Vastus lateralis* (Figure 7).

pH_u value

Previous study reported a close relationship between meat color and pH, *i.e.* the higher pH value corresponds to the darker meat (Kasprzyk *et al.,* 2010). Similar to our result (Table 1), wild boar meat is characterized by a higher initial pH at 45 min post-mortem (pH_{45}), darker color, and lower conductivity compared to the domesticated Pietrain breed (Sales and Kotrba, 2013). The result of our study also conforms Müller *et al*. (2002), who reported that pH_{45} in *Semimembranosus* of wild boar was higher (6.14) than Pietrain pig (5.45). The high pH of wild boar compared to other species is related to the stress experienced by wild boar when hunted by hunters. In a study of an effect of hunting to red deer meat quality, hunting-related stress caused depletion of carbohydrate sources to strengthen muscles. This leads to a raise in pH, disruption of muscle tissue, and an increase of beta-endorphins and cortisol secretion which are usually associated with extreme physiological and psychological stress (Bateson and Bradshaw, 1997).

Multivariate data analysis of beef, buffalo, pork and wild boar chromameter data

The results of PCA analysis in our study showed that beef, buffalo, pork, and wild boar can be differentiated based on their colour profile (Figure 1). Clear classification pattern observed in unsupervised PCA scores plot is an important indicator of the reliability of supervised multivariate data analysis (Worley and Powers, 2016), allowing us to further conducted supervised multivariate data analysis (OPLS-DA) to fine tune classification pattern obtained from PCA. The result of OPLS-DA conformed the PCA results, which showed that beef has a yellowish color compared to the buffalo meat. This is in accordance with the consumer perception that develops in some parts of Indonesia. As mentioned earlier, beef and buffalo were partially overlapped. In fact, they had different discriminating factors. Colour parameters measured in this study could not explain which color descriptors they have in common.

It was reported earlier that objective color measurement results was found to be linear with myoglobin concentration measurement. High myoglobin concentration corresponds to a high a* value (Newcom *et al.,* 2004). Thus, high a* value of buffalo meat found in this study could be attributed, at least in part, to higher myoglobin content although we did not measure the myoglobin content in this study. However, another report states that the color of buffalo meat was redder than beef because it contained more myoglobin, which was 2.50% in buffalo meat and 1.50% in beef (Argañosa *et al*., 1973). Animals kept indoors in confined conditions, where they cannot move freely, generally exhibit a lighter color of meat than animals that move freely (Feiner, 2006).

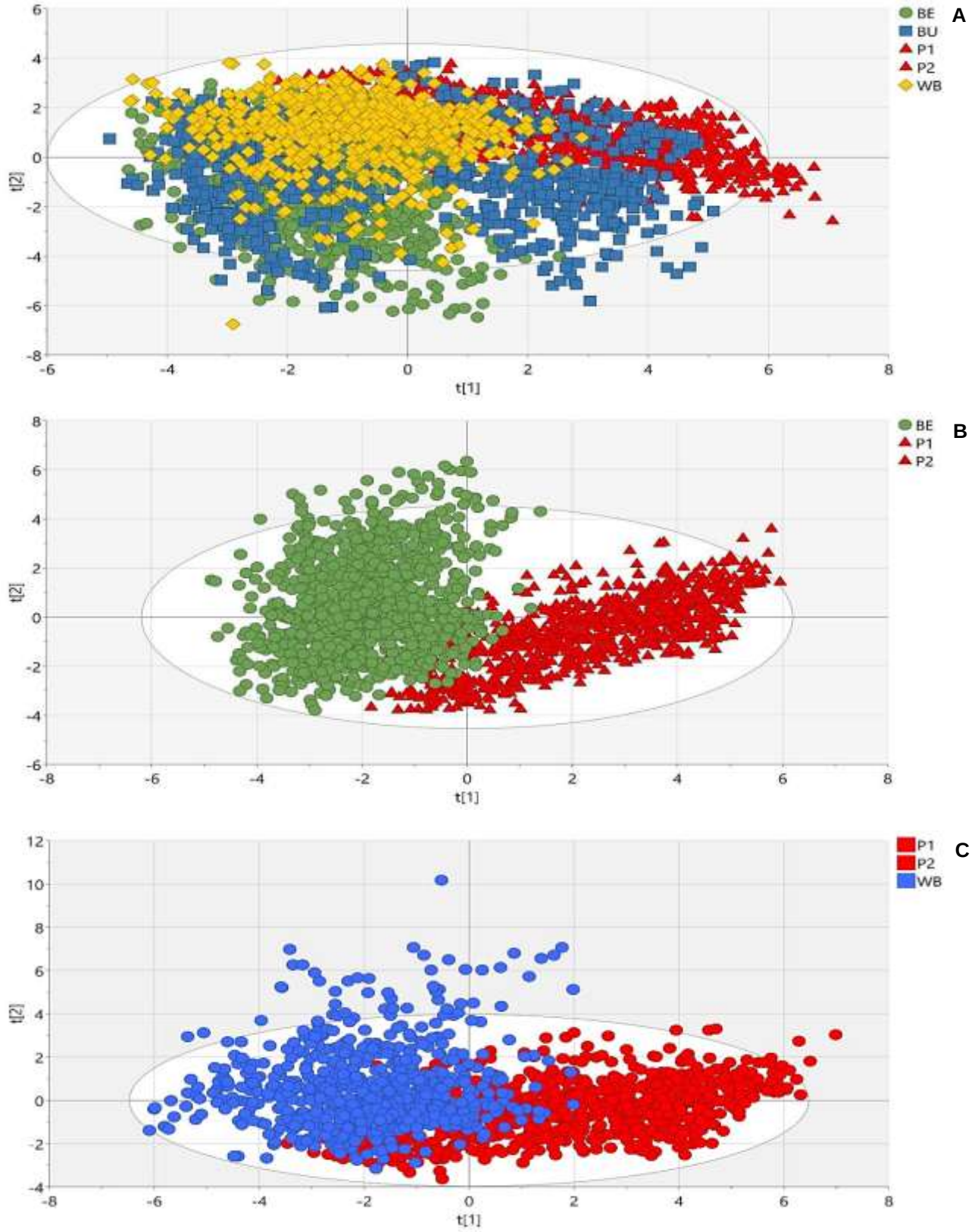

Figure 5. A= PCA score plot of beef, buffalo, pork, and wild boar image analysis data. B= PCA score plot of beef and pork image analysis data. C= PCA score plot of pork and wild boar image analysis data (beef= BE, buffalo= BU, pork= P1, P2, wild boar= WB)

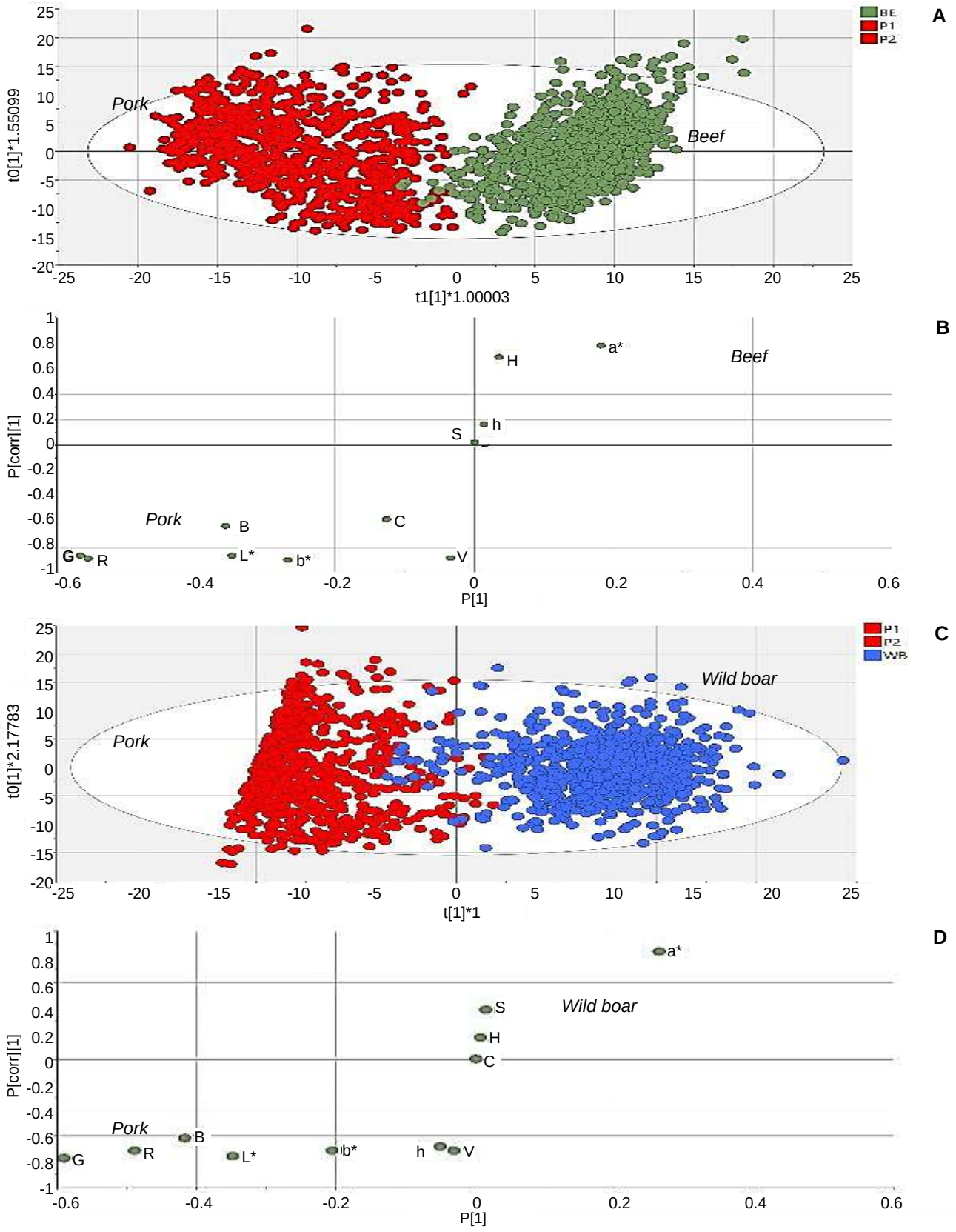

Figure 6. A= Score plot of OPLS-DA model for pork (class 1) and beef (class 2) (R^2Y 0.835 Q2 0.835). B= S-plot of OPLS-DA model for pork (class 1) and beef (class 2). C=. Score plot of OPLS-DA model for pork (class 1) and wild boar (class 2) (R^2Y 0.865 Q^2 0.862). D= S-plot of OPLS-DA model for pork (class 1) and wild boar (class 2)

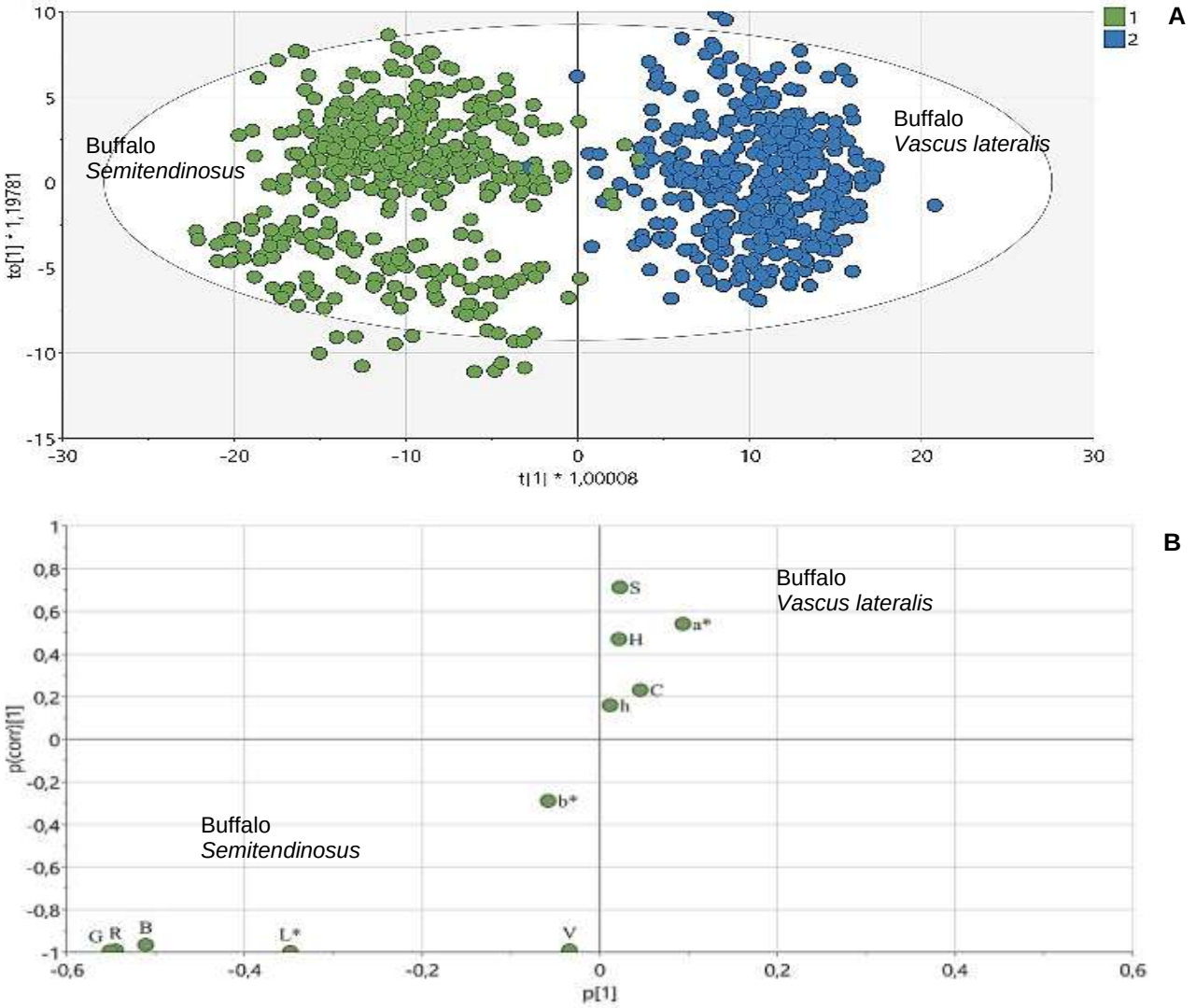

Figure 7. A= OPLS-DA score plot of the image data with 2 classes (class 1 buffalo *Semitendinosus* and class 2 buffalo *Vastus lateralis*). B= OPLS-DA S-plot of the image data with 2 classes (class 1 buffalo *Semitendinosus* and class 2 buffalo *Vastus lateralis*)

Color variation in meat can be also influenced by the pH as previously described (Seideman *et al.,* 1984; Page *et al.,* 2001). Meat with lower pH will have a paler color since the muscle were more exposed and scatter light. Low pH also makes the myoglobin fraction becomes easier to be oxidized into metmyoglobin which has a low color intensity. Oppositely, the higher pH value corresponds to the darker meat appearance (Kasprzyk *et al.,* 2010). The color of muscle tissue is determined by the reflection of light off free water and the oxygenation of myoglobin (Ledward *et al*., 1992). Proteins can form stronger bonds with water at a higher muscle pH, which results in less free water. Less space exists between muscle fibers as a result of the proteins' increased ability to bind water. Because there is less free water to reflect light, meat with a higher pH will therefore be darker in color (Ledward *et al*., 1992). In addition, oxygen-using enzyme activity is increased in muscles with higher pH levels, which results in decreased oxygenation of the surface myoglobin and a darker hue (Price and Schweigert, 1987; Ledward *et al*., 1992).

In this study, OPLS-DA showed that *Vastus lateralis* and *Semitendinosus* muscles in beef (Figure 3) and buffalo (Figure 4) had different color profile. Beside animal species, muscle type was reported to have a greater contribution to color variation than the breed (King *et al.,* 2011). Individual muscles have specific anatomical locations and physiological functions, leading to metabolic differences among them and thus, a unique biochemical color (Hunt and Hedrick, 1977). Muscles that are extensively used for movement such as shoulders and legs, needs more O_2, which is primarily carried by red blood cells, resulting in a darker color of

muscle tissue. As a result, the concentration of myoglobin in muscles that are frequently used is usually higher than in muscles that are rarely used. For example, pork shoulder meat has a darker color than pork loin (Feiner, 2006). In a more recent study, *Vastus lateralis* of beef contained higher myoglobin concentration than *Semitendinosus* (Wibowo *et al.,* 2019), which explains why it had higher value of a* and C* value than *Semitendinosus* found in our study (Figure 1).

Multivariate data analysis of beef, buffalo, pork and wild boar image analysis data

The results of image data OPLS-DA gave explanation why wild boar is more frequently used as adulterant of beef. Both are discriminated by high redness (a*) as can be seen in Figure 6B and 6C. This information could not be obtained from colorimetry data. Colorimetry technique is the most common technique to measure meat color. However, this technique has limitations. Colorimetry is unable to measure the color of the whole surface in a single measurement if it is non-homogeneous. It measures the light reflectance of a given portion of the matrix, giving a color evaluation without any information about its local variability (Antonelli *et al.,* 2004). Meanwhile meat does not have a homogeneous surface because of its structure, its connective content and its intramuscular fat. The enlargement of the measured area would possibly include fat and connective tissue, thus yielding unreliable measures (Girolami *et al.,* 2013). The interaction of the light emitted with the surface to be analyzed is another problem. Noted that color depends on the physical and chemical characteristics of the product. The object will be transmitting, refracting, reflecting, diffusing and absorbing the light beam (the one the colorimeter emits). Meat, which is an optically non-homogeneous medium (its refraction index is not uniform), has air, liquids, and granules of different materials scattered inside. Therefore, it causes multiple reflections and refractions where optical discontinuities are present, resulting in a diffusion of light (scattering). For this reason, the technology of the digital camera is being increasingly adopted, because the whole image of the product can be analyzed, not just the color of a reduced area such as the area spotted by the colorimeter only. Image analysis method allows estimating the overall color of the sample and its heterogeneity. The image was captured, processed and analyzed, then the color was assessed with a non-destructive and objective method (Zheng and Sun, 2006). It had been proved that the colorimeter did not generate coordinates corresponding to the true color of meat. Instead, the computer vision system method had given valid measurements that reproduced a color very similar to the real one (Girolami *et al.,* 2013). However, in this study, image analysis also possessed limitation. Unlike chromameter data, image data could not differentiate beef *Semitendinosus* and beef *Vastus lateralis.* The slight differences in classification power based on color patterns between colorimetric and image analysis may be caused by differences in the light source used. The chromameter used Illuminant D_{65}, while the image analysis used white LED lights.

CONCLUSIONS

Meat color analysis using chromameter and imaging techniques were able to discriminate meats form different species and different muscles type. Multivariate data analysis such as PCA and OPLS-DA modelling of chromameter and image data were able to identify discriminating colour parameters for each meat and muscle type. However the limitation of this study we only used samples from two animals for each type of meats. Further experiment using larger number of samples are recommended as a verification to the results obtained from our studies.

ACKNOWLEDGEMENT

This research was partially funded by Indonesian Ministry of Research and Technology/ National Research and Innovation Agency through PENELITIAN DASAR UNGGULAN PERGURUAN TINGGI (PDUPT) 2020 with Contract No. 2529/IT3.L1/PN/2020

REFERENCES

Antonelli A, Cocchi M, Fava P, Foca G, Franchini GC, Manzini D, Ulrici A. 2004. Automated evaluation of food colour by means of multivariate image analysis coupled to a wavelet-based classification algorithm. Anal Chim Acta 515: 3-13. https://doi.org/10.1016/j.aca.2004.01.005

Argañosa FC, Sanchez PC, Ibarra PI, Gerpacio AL, Castilio L, Argañosa VG. 1973. Evaluation of carabeef as a potential substitute for beef. Philippine J Nutr 26: 128-143.

Bateson P, Bradshaw EL. 1997. Physiological effects of hunting red deer (*Cervus elaphus*). Proc R Soc B Biol Sci 264: 1707-1714. https://doi.org/10.1098/rspb.1997.0237

De Smet S, Vossen E. 2016. Meat: The balance between nutrition and health. A review. Meat Sci 120: 145-156. https://doi.org/10.1016/j.meatsci.2016.04.008

El Sheikha AF, Mokhtar NFK, Amie C, Lamasudin DU, Isa NM, Mustafa S. 2017. Authentication technologies using DNA-based approaches for meats and halal meats determination. Food Biotechnol 31: 281-315. https://doi.org/10.1080/08905436.2017.1369886

Eriksson L, Johansson E, Kettenah-Wold N, Trygg J, Wikström C, Wold S. 2013. Multi- and Megavariate Data Analysis. Umetrics Academy Publisher.

Feiner G. 2006. Meat Products Handbook: Practical Science and Technology. Camridge: Woodhead publishing Ltd.

Girolami A, Napolitano F, Faraone D, Braghieri A. 2013. Measurement of meat color using a computer vision system. Meat Sci 93: 111-118. https://doi.org/10.1016/j.meatsci.2012.08.010

Haralick RM, Shanmugam K, Dinstein I. 1973. Textural Features for Image Classification. SEG Tech Progr Expand Abstr SMC-3: 610-621. https://doi.org/10.1109/TSMC.1973.4309314

Hunt MC, Hedrick HB. 1977. Profile of fiber types and related properties of five bovine muscles. J. Food Sci 42: 513-517. https://doi.org/10.1111/j.1365-2621.19 77.tb01535.x

Kasprzyk A, Stasiak A, Babicz M. 2010. Meat quality and ultrastructure of muscle tissue from fatteners of Wild Boar, Pulawska and its crossbreed Pulawska × (Hamshire × Wild Boar). Arch Anim Breed 53: 184-193. https://doi.org/10.5194/aab-53-184-20100.

King DA, Shackelford SD, Wheeler TL. 2011. Relative contributions of animal and muscle effects to variation in beef lean color stability. J. Anim Sci 89: 1434-1451. https://doi.org/10.2527/jas.2010-3595

Ledward DA, Johnston DE, Knight MK. 1992. The Chemistry of Muscle-Based Foods. 128–139. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge, U.K.

Müller E, Rutten M, Moser G, Reiner G, Bartenschlager H, Geldermann H. 2002. Fibre structure and metabolites in M. longissimus dorsi of Wild Boar, Pietrain and Meishan pigs as well as their crossbred generations. J Anim Breed Genet 119: 125-137. https://doi.org/10.1046/j.1439-0388.2002.00328.x

Newcom DW, Stalder KJ, Baas TJ, Goodwin RN, Parrish FC, Wiegand BR. 2004. Breed differences and genetic parameters of myoglobin concentration in porcine longissimus muscle. J Anim Sci 82: 2264-2268. https://doi.org/10.2527/2004.8282264x

Page JK, Wulf DM, Schwotzer TR. 2001. A survey of beef muscle color and pH. J Anim Sci 79: 678-687. https://doi.org/10.2527/2001.793678x

Park B, Lu R. 2015. Hyperspectral imaging technology in food and agriculture. In: Park B, Lu R, editor. 1-390. Hyperspectral Imaging Technology in Food and Agriculture. 1st Ed Springer-Verlag New York.

Price JF, Schwiegert BS. 1987. The Science of Meat and Meat Products. 3rd ed. Food and Nutrition Press, Westport, CT.

Ropodi AI, Pavlidis DE, Mohareb F, Panagou EZ, Nychas GJE. 2015. Multispectral image analysis approach to detect adulteration of beef and pork in raw meats. Food Res Int 67: 12-18. https://doi.org/10.1016/j.foodres.2014.10.032

Sales J, Kotrba R. 2013. Meat from wild boar (*Sus scrofa* L.): A review. Meat Sci 94: 187-201. https://doi.org/10.1016/j.meatsci.2013.01.012

Seideman SC, Cross HR, Smith GC, Durland PR. 1984. Factors Associated with fresh meat color: a Review. J Food Qual 6: 211-237. https://doi.org/10.1111/j.1745-4557.1984.tb00826.x

Sinanoglou VJ, Cavouras D, Xenogiannopoulos D, Proestos C, Zoumpoulakis P. 2018. Quality assessment of pork and Turkey hams using FT-IR spectroscopy, colorimetric, and image analysis. Foods 7: 152. https://doi.org/10.3390/foods7090152

Wang F, Holman BWB, Zhang Y, Luo X, Mao Y, Hopkins DL. 2020. Investigation of colour requirements of frozen beef rolls by Chinese consumers for hot pot. Meat Sci 162: 108038. https://doi.org/10.1016/j.meatsci.2019.108038

Wibowo A, Panpipat W, Kim SR, Chaijan M. 2019. Characteristics of Thai native beef slaughtered by traditional Halal method. Walailak J Sci Technol 16: 443-453. https://doi.org/10.48048/wjst.2019.4689

Worley B, Powers R. 2016. PCA as a Practical indicator of OPLS-DA model reliability. Curr. Metabolomics 4: 97-103. https://doi.org/10.2174/2213235X04666160613122429

Zheng C, Sun D. 2006. Recent applications of image texture for evaluation of food qualities-a review. Trends Food Sci Technol 17: 113-128. https://doi.org/10.1016/j.tifs.2005.11.006