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Can Bioelectrical Impedance Analysis (BIA) Be Used to Predict Pig's Meat Quality In Vivo?

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Abstract: The aim of the current study was to evaluate the possibility of application of bioelectrical impedance analysis (BIA) in order to estimate pork quality. The BIA measurements were tested on 18 living animals for the prediction of the meat quality. The absolute resultant electrical resistance (Rz) and reactance (Xc) of the body was measured with a set of disposable surface electrodes at the frequency of 50 kHz and the current intensity of 400 µA. The characteristics of meat quality, pH measured 1 h and 24 h after slaughter, meat color parameters represented in CIE L*a*b* system, glycolytic potential, intramuscular fat, and natural drip loss, were assessed on the samples of the *Longissimus dorsi* (LD) muscle. The slaughter value of pigs was characterized on the basis of hot carcass weight (HCW) and percent of meat in carcass. The results showed a significant Pearson correlation between bioelectrical impedance parameter Rz and pH_1 (r = 0.48*, $p < 0.05$). A significant Spearman correlation was showed between color b* value and the Rz/Xc/HCW ratio (r = −0.62*, $p < 0.05$) and Xc (r = −0.51*, $p < 0.05$), as well as between the Rz/Xc ratio with pH_1 (r = 0.48*, $p < 0.05$). The multivariate statistical method (principal component analysis and cluster analysis) showed that bioimpedance measurements combined with meat quality traits make it possible to distinguish groups with different quality parameters. However, the relationships between them are complex and still require analysis.

Keywords: bioelectrical impedance; resistance; reactance; pork meat quality

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1. Introduction

The safety and quality of meat assessment play an important role in the food industry, since consumers are being more demanding and cautious in choosing food articles. Pork and beef quality and safety evaluation is of critical significance for these meat products in different countries worldwide. There are different techniques used in determining meat quality, which include invasive and non-invasive techniques. The popularity of non-invasive techniques has increased due to advances in technology. Instruments used to estimate the composition for the grading and classification of carcasses in general dissected composition as a reference. It is normally obtained by manual dissection carried out by a qualified group. As a consequence, obtaining meaningful and accurate information involves an invasive method, which often is costly and time-consuming. Hence, these expensive methods that are used in research or breeding programs include a lot of animals, and they are impossible or impractical to apply routinely in commercial operations. The challenge of the meat industry is to guarantee consumers the quality of products while maintaining the profitability of production. Meat control and its quality improvement, particularly sensory parameters, seem to be important aspects for farm animal breeding [1–3]. The solution of this problem is a non-invasive, precise, not expensive, and fast method of predicting the technological and sensory quality of meat.Therefore, non-invasive techniques used to monitor the safety and quality of beef and pork are currently a trend in research [4–6]. Hence, non-destructive new technologies used in monitoring the safety and quality of beef and pork, such as techniques of imaging, imaging of magnetic resonance, imaging of X-ray, imaging of ultrasound, and spectroscopic and electronic nose are still in demand [7–13]. The literature review demonstrated that the techniques were mostly developed for laboratory use [13]. There is a lack of evidence regarding their feasibility on an industrial level.

Bioelectrical impedance analysis (BIA) is used as a reliable, safe, and efficient way of studying human body structure in order to estimate fat-free mass as well as muscle and body fat ratio [14]. This method is used for measuring body composition in experiments on laboratory animals [15,16]. Therefore, the exploration and research of methodologies and technologies are crucial for the efficacious quality assessment of zoo technical products. Therefore, bioelectrical impedance (BIA) is characterized as being a technology of fast and simple analysis, is non- invasive, and is relatively cost-effective and objective. BIA has a predictive potential for the assessment of composition of chemical tissue of farm animal carcasses [3]. However, the possibility of predicting or measuring meat quality traits in live animals gives the capability to aid breeding decisions through the availability of phenotypes for those traits on chosen candidates [3].

It can also be an efficient tool used to evaluate the composition of the bodies of farm animals and their carcasses [17,18]. Such assessments are a good source of knowledge about the amount of extracellular water and its relation to the total water content in an organism [19,20]. The quantity of extracellular water in pig carcasses can be very important in estimating the natural drip loss, which has essential meaning for meat processing [21]. Bioelectrical impedance analysis could also be applied to estimate the empirical quality of pork. The analysis of livestock body composition can facilitate the selection of animals that are the best, adjusted for the intended purpose in the meat industry [11,13,22]. Currently, several biochemical methods are used to assess meat quality, but they have some drawbacks [23]. For instance, instrumental mechanical methods are destructive or invasive [3]. Optical methods such as infrared spectroscopy need more complex data analysis [11,24]. Methods based on nuclear magnetic resonances and ultrasounds are accurate but expensive [25,26]. Hence, the object of the research was to assess the possibility of application of bioelectrical impedance analysis as a non-invasive method used for the evaluation of pork quality.

2. Materials and Methods

2.1. Material

The research was conducted on a sample of 18 livestock subjects, Pen Ar Lan fattening pigs developed from crossbreeding between P76 boars and Naima sows. The characteristics of hot carcass weight and meatiness of animals taken in the consideration in BIA (n = 18) are summarized in Table 1. All of the pigs submitted to the analysis were provided with the same environmental conditions, fed with a complete feeding mixture, and given unlimited access to water. Additionally, the duration of the fattening period was identical. The animals were slaughtered in accordance with a technology applied in the slaughter house (2 h pre-slaughter rest period, pre-slaughter electrical stunning, bleeding in horizontal position). The animals were slaughtered in accordance with the European Union Council Regulations (EC) No 1099/2009 for the protection of animals at the time of slaughter. The material was transported from the slaughterhouse to the Department of Gastronomy Technology and Food Hygiene, Warsaw University of Life Sciences WULS–SGGW in polystyrene containers, ensuring refrigerated transport.

2.2. Methods

2.2.1. The Bioelectrical Impedance (BIA)

The bioelectrical impedance measurements were taken using the Akern BIA-101/s.c. body composition analyzer (Akern, Italy), which uses measurement technology based on vector bioimpedance measurement (BIA). The absolute resultant electrical resistance (Rz) at 50 kHz (Ω) and reactance (Xc) at 50 kHz (Ω) of the body were measured with a set of disposable surface electrodes (Akern, Italy, Lisboa). The electrodes were attached to pigs' limbs so that the electricity flowed through the whole half-carcass, producing the exact results. Procedures conducted during the study were performed carried out in accordance with the principles of the European Union (recommendation 2007/526/CE) and Polish Law on Animal Protection. The measurements were taken at the frequency of 50 kHz and current intensity 400μA. The living animals were lifted in cages (before slaughter), and the electrodes were placed on their limbs.

Table 1. Group characteristics of animals (BIA was carried out on livestock n = 18).

Variables	Statistical Measures	
	Mean	Standard Deviation
Hot carcass weight (kg)	84.61	8.89
Meat in carcass (%)	60.24	1.29
Rz/Xc/HCW	9.14	3.22
Rz (Ω)	166.00	31.92
Xc (Ω)	23.22	6.40
Rz/Xc	7.63	2.46
pH_1	6.49	0.17
pH_2	5.62	0.06
PG (mmol/L)	130.87	16.99
Color L*	55.12	1.58
Color a*	16.17	0.74
Color b*	9.35	0.74
Natural drip loos (%)	3.66	2.05
Intramuscular fat (%)	1.71	1.26

Explanation: Rz = resistance (Ω), Xc = reactance (Ω), HCW = hot carcass weight (kg).

2.2.2. Meat in Carcass

After slaughter, the content of meat in carcass was determined on the basis of measurement of back fat and *Longissimus* muscle thickness in the carcasses with a CGM apparatus using (Sydel, France). The characteristics of meat quality were tested on the LD muscle from the section behind the bottom rib.

2.2.3. pH

The pH in the muscle tissue was determined 1 h (pH_1), 3 h (pH_3), and then 24 h (pH_{24}) after slaughter using the WTW 330 pH-meter (Weilheim, Germany) equipped with SenTix® SP Number 103645 electrodes. The pH value was measured in triplicate.

2.2.4. Color of meat

The color of meat parameters were determined within 48 h after slaughter using the Minolta CR310 (Konica Minolta, Osaka, Japan) chroma meter and were represented in the CIE L*a*b (L*-lightness, a*-redness, b*-yellowness) system at three locations on the cross section of each piece of meat (in triplicate). The apparatus was adjusted before every measurement against a white and black tile.

2.2.5. Drip Loss

Natural drip loss was defined according to the Prange methodology 48 h after slaughter. Blood was collected into tubes containing EDTA immediately after slaughter, during the opening of the carotid artery. The collected blood was centrifuged, MPW 350 (Med. Instruments, Poland) 10 min at 3000 rpm (1470 g). The resulting sera were frozen at −82 °C and gradually were used for analysis.

2.2.6. Glycolytic Potential

The glycolytic potential (GP) of muscles was calculated according to the formula recommended by Monin and Sellier [27]. The sum of lactic acid and glucose was calculated and submitted as mmol lactic acid per liter of fresh muscle exudation [28]. Muscle glucose (mmol/L) and lactic acid (mmol/L) in drip loss were assessed by the strip test using an Accutrend Plus apparatus (Roche Diagnostic GMBH, Mannheim, Germany). The measurements were performed with appropriate reactive strips. Distilled water was used for sample dilution to achieve the appropriate analyte concentration. The test results were available at: 60 s (lactic acid); 12 s (glucose); after placing l drop on a reactive strip. Each test was carried out in duplicate.

2.2.7. The Content of Intramuscular Fat

The assessment of the content of fat was performed using the Soxhlet methodology according to the PN-ISO 1444:2000 Standard for the determination of intramuscular fat content for meat and meat products [29]. The dried sample was extracted with n-hexane or light petroleum. The residual solvent was removed by evaporation. The dried extracted fat was weighed.

2.2.8. Statistical Method

The results were statistically processed using the statistical package STATISTICA 13.3 software version (TIBCO Software Inc. 2017, Palo Alto, CA, USA, Statistica data analysis software system, http://statistica.io, accessed on 19 September 2022). Mean and standard deviation were calculated. The Shapiro–Wilk test was applied for checking Pearson's linear and Spearman range correlation coefficients between the variables of BIA measurements, and meat quality traits were calculated. Principal component analysis (PCA) and cluster analysis (CA, using the K-means method) were applied as multivariate methods to deepen the evaluation of the relationship between measured traits. Based on the results of the analysis, three groups of samples varying in BIA and meat quality traits were identified. The results were obtained using one-way analysis of variance (ANOVA). The significance of differences between means were computed based on the least significant differences test (LSD). Significances were tested at the level of $p < 0.05$.

3. Results and Discussion

The mean values obtained in the study are summarized in Table 1. The results showed that the studied animals were meat fatteners with good quality meat. The meat was characterized by good ultimate pH value and meat color parameters, with low natural drip loss and good intramuscular fat level. The analysis of the obtained pH values, color brightness (L*), and natural drip loss showed that, between the samples, there were no cases of defective meat such as PSE, DFD, or the so-called "acid meat". However, it should be noted that the variability in drip loss and intramuscular fat content was high (Table 1).

The results that characterized meat quality are in accordance with the work of Hamilton et al. [30] and Nani Costa et al. [31]. Resistance and reactance values reported in the study were higher compared to those observed in pigs of Yorkshire Duroc Hampshire barrows of 109.4 kg [32], Yorkshire Duroc pigs of 103 kg [33], and 109.2 kg Iberian pigs of Torbiscal line [34].

Tests of correlations between different BIA variables were carried out on the tested group of reference values. The results of the correlation analysis of BIA variables and measurement values with statistically significant correlations in the examination group are presented in Table 2. The results of the research showed a significant Pearson correlation between resistance (R_z) and pH_1 value r = 0.48 ($p < 0.05$) and a Spearman correlation between R_z/Xc ratio and pH_1 r = 48 ($p < 0.50$). Additionally, a significant Spearman correlation was found between color b* value and reactance (Xc) r = −0.51 ($p < 0.05$), as well as with ratio R_z/Xc/HCW r = −0.62 ($p < 0.05$). The pH_1 values are the traits that reflects the intensity of *post mortem* glycolysis, and in the case of rapid changes occurring in meat after slaughter, a low pH is typical for PSE defect [21,35]. The intensity of post-slaughter glycolysis also affects the condition of muscle proteins, their water holding capacity, and therefore their natural drip loss and color parameters. As has been shown in many studies, fast glycolysis is associated with an increase in muscle temperature and leads to the degradation of muscle proteins and to a lightening of their color and a reduction in water retention capacity [30,31]. The BIA method allows to read parameters such as water content (total, intra, and extracellular), adipose tissue, or muscle tissue. Resistance and reactance during the measurement depend on the water content and its distribution in the body, as well as the amount of fat and other tissues. The relationship between the in vivo resistance and reactance and the post-slaughter pH_1 value seems to be logical and indicates that it would be possible to detect the propensity for rapid post-slaughter glycolysis already in vivo. There are no studies investigating the correlation between the results of BIA measurements and these meat quality determinants in live pigs, but Suliga et al. [35] applied bioimpedance as a marker for ham defects after slaughter. Suliga et al. [35] also showed a low correlation between bioimpedance parameter and pH and color parameters. They ranged from r = 0.32 to r = −0.45. Fang et al. [36] found the differences and relationships between bio-impedance values, water loss rate, and pH value of bovine muscles near the freezing point. The results revealed that the correlation between bio-impedance and pH and water loss rate was significant ($p \geq 0.05$). Similar results were reported by Yang et al. [37], who studied 44 pieces of porcine LD muscle. They used a mobile bioelectrical impedance system of spectroscopy with a four-terminal electrode to measure MC. Xie et al. [38] developed a methodology for the rapid detection of chilled pork freshness based on bioimpedance technology. For the assessment of 20 chilled pork samples, the bioimpedance characteristic was established by measuring impedance, phase angle, and the total volatile basic nitrogen content.

Table 2. The correlation between BIA variables and measurement meat quality traits (n = 18).

Variables	Pearson Correlations				Spearman Correlations			
	Rz/Xc/HCW	Rz/Xc	Rz (Ω)	Xc (Ω)	Rz/Xc/HCW	Rz/Xc	Rz (Ω)	Xc (Ω)
pH_1	−0.06	−0.03	0.48 *	0.29	−0.03	0.48 *	0.29	−0.03
Color b*	−0.40	−0.39	−0.32	0.35	−0.62 *	0.36	0.44	−0.51 *

Explanations: * Coefficient of correlation statistically significant at $p < 0.05$, Rz = resistance (ohm), Xc = reactance (ohm), HCW = hot carcass weight (kg).

Cox et al. [39] indicated that making the relation between the variables of BIA and the body composition of animals is possible. The principle is the laboratory evaluation of the moisture level, protein, mineral, and lipids content The position of the electrodes is crucial, and it is recognized that the more accurate BIA results are obtained with the electrodes placed on the back of the animals compared to the ventral readings. According to Bertotti [40], right after the slaughter, there are no significant changes in the BIA, even considering temperature variation. However, when the carcass starts to cool, biochemical changes in the cell membranes occur due to the rigor mortis effect, as the carcass loses its ionic gradients with the rise of the temperature and the time-lapse of aging.

In other studies, Daza et al. [34] demonstrated a positive correlation between the resistance, swine live weight, and amount of content of fat. Moreover, Alfonso et al. [41] found that the parameters assessed by BIA can be used for meat characteristics in the combination of Rs and Xc to predict intramuscular fat, demonstrating 79.3% of adjustment, while, for the physicochemical characteristics, the best adjustments were in the length of the sarcomere with 64.4% and sheer force of 60.5.

The obtained correlations can lead to the conclusion that bioelectrical impedance can be useful for meat quality prediction, but it needs additional research because the relationship is not simple for interpretations. The other correlations between BIA and the analyzed features were not statistically significant. Results of research conducted by Daza et al. [34] on live Iberian pigs showed that bioimpedance measurements were correlated with live weight and length values and body composition, such as fat quantity and fat proportion, bone amount and bone percentage, and skin quantity and skin proportion. As indicated by Bohuslavek et al. [18], after the improvement of the proportionality of adjusted distance electrodes to carcass length, the achieved results can be more favorable. Thorough research showed that BIA technology can be an accurate predictor of beef carcass composition. This work showed that it was possible to derive a regression formula (r = 0.8) for an estimate of carcass conformation on the basis of BIA analysis [18]. Zollinger et al. [42] developed equations to predict % salable carcass yield (SY%) and percentage trimmable fat (FT%). The obtained equations accounted for 81% and 84% of the variation in SY% and FT%, respectively.

In our study, we applied the statistical method of analysis (PCA) to better understand the relationship between the measured traits in a multidimensional space. The results showed that two main components explained about 50% of the total variability in the set of variables (Figure 1). The first component that explained 33,18% of a total variability is strongly negatively associated with color b* value, PG, and Xc and, on the other hand, is positive associated with IMF, Rz/X/MTC, and Rz/Xc values (Table 3 and Figure 1). The second component explained about 17% of total variability and was strongly associated with pH_1, pH_{24}, and color L* and b* values (Figure 1 and Table 3). Similar effects were obtained by Suliga et al. [35] in the study of application of bioimpedance measurements as a marker for ham defects. These authors showed in a PCA analysis also that both principal components that explained about 51% of total variability and similar traits (pH, drip loss, color values) were related with the bioimpedance parameter but were measured in meat after slaughter.

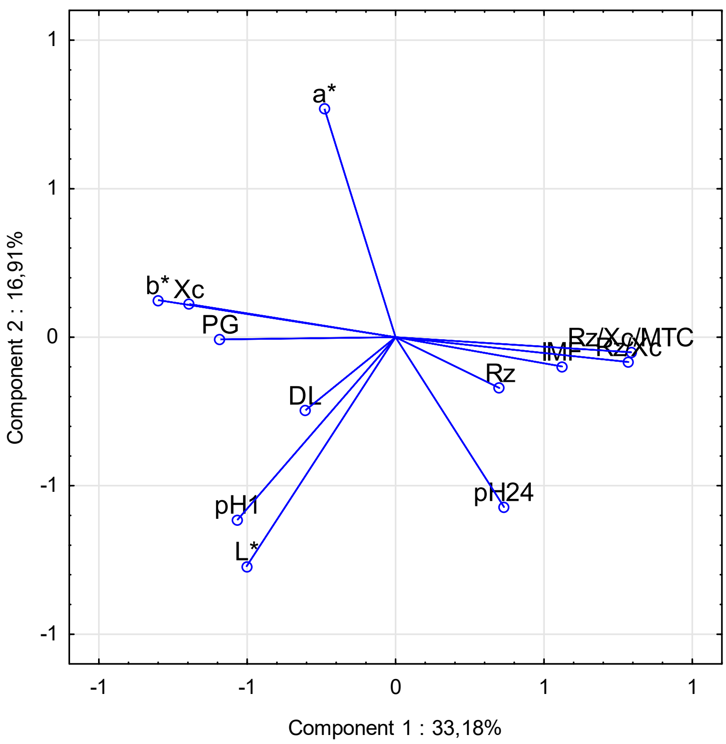

Figure 1. Principal components analysis results—location of individual traits based on their relationship with each other and principal components. Rz = resistance (ohm); Xc = reactance (ohm); HCW = hot carcass weight (kg); DL-drip loss; PG, glycolytic potential; IMF, intramuscular fat.

The second component was strongly and negatively related to pH_1, pH_{24}, and color L* value, as well as positively with color a* value (Table 3, Figure 1). Then, the K-means cluster analysis was used to check whether the bioimpedance measurements in combination with the meat quality traits allowed for the separation of subgroups with different meat quality in the studied group of pigs. The meat quality traits with bioimpedance measurements were used as focusing criteria. The results showed that three groups of pigs with various meat quality parameters and bioimpedance measurements were obtained as the effect of cluster analysis (Table 4 and Figure 2).

Table 3. Factor coordinates of variables with respect to principal components based on correlation.

Variables	Principal Component	
	Component 1	Component 2
Rz/Xc/HCW	0.79	−0.05
Rz (Ω)	0.35	−0.17
Xc (Ω)	−0.70	0.11
Rz/Xc	0.78	−0.08
pH_1	−0.53	−0.61
pH_{24}	0.37	−0.57
PG (mmol/L)	−0.59	−0.01
Color L*	−0.50	−0.77
Color a*	−0.24	0.77
Color b*	−0.80	0.13
Natural drip loos (%)	−0.31	−0.25
Intramuscular fat (%)	0.56	−0.10

Rz = resistance (ohm), Xc = reactance (ohm), HCW = hot carcass weight (kg).

Table 4. Mean values for clusters obtained from the cluster analysis using the K-means method.

Variables	Clusters		
	Cluster 1	Cluster 2	Cluster 3
R_z/Xc/HCW	11.05	8.66	7.80
R_z (Ω)	197.00 [a]	170.33 [b]	117.50 [c]
Xc (Ω)	22.00	25.33	20.00
R_z/Xc	9.08	7.45	6.21
pH_1	6.48	6.52	6.44
pH_{24}	5.66 [a]	5.58 [b]	5.65 [a]
PG (mmol/L)	115.13 [a]	140.98 [b]	127.78 [ab]
Color L*	55.15	55.25	54.81
Color a*	15.64	16.48	16.14
Color b*	8.69 [a]	9.71 [b]	9.39 [ab]
Natural drip loos (%)	2.59	4.02	4.19
Intramuscular fat (%)	2.48	1.41	1.45

Explanation: a,b—means with different letters differs significantly at $p < 0.05$; Rz = resistance (ohm), Xc = reactance (ohm), HCW = hot carcass weight (kg).

The analysis of variance showed that the obtained cluster group differed significantly for the Rz bioimpedance trait, pH_{24}, PG, and b* color values (Table 4). The results showed that groups from cluster 1 and 2 differed significantly between traits Rz, pH_{24}, PG, and color b* values (Table 4 and Figure 2), while the group from cluster 3 did not differ significantly from the other two groups. The meat of fatteners from cluster 1 appeared with higher R_2 resistance with a better value of ultimate pH, lower glycolytic potential, and lower color b* value (Table 4). The meat of fatteners from cluster 2 was characterized by lower ultimate pH, higher GP, and color b* value, while the meat from cluster 3 was intermediate between both previous in meat quality traits (pH_{24}, GP, and color b* value) and completely different from both in terms of resistance (R_z). Regarding the meat quality traits, the obtained relationship between the characteristics with regard to pH, GP, and color were consistent with the results of the studies by Hamilton et al. [30], Nani-Costa et al. [31], and Copenhafer et al. [43]. Cluster analysis showed that bioimpedance measurements combined with meat quality traits make it possible to distinguish groups with different quality parameters. However, the relationships between them are complex and still require analysis. Suliga et al. [35], in their work on the use of bioimpedance to diagnose the quality of hams with good-quality and poor-quality, obtained difficult-to-interpret results. They also used PCA analysis with standard technological meat quality variables and bioimpedance measurements (pHu, lightness (L*), Py, and drip loss) for the classification. Moro et al. [44] pointed out that bioimpedance analysis is a promising technology compared to traditional methods. It gives an effective way to evaluate the composition of protein, fat, and moisture in commercial cuts and provides precise information and meets the demands for the consumers' market.

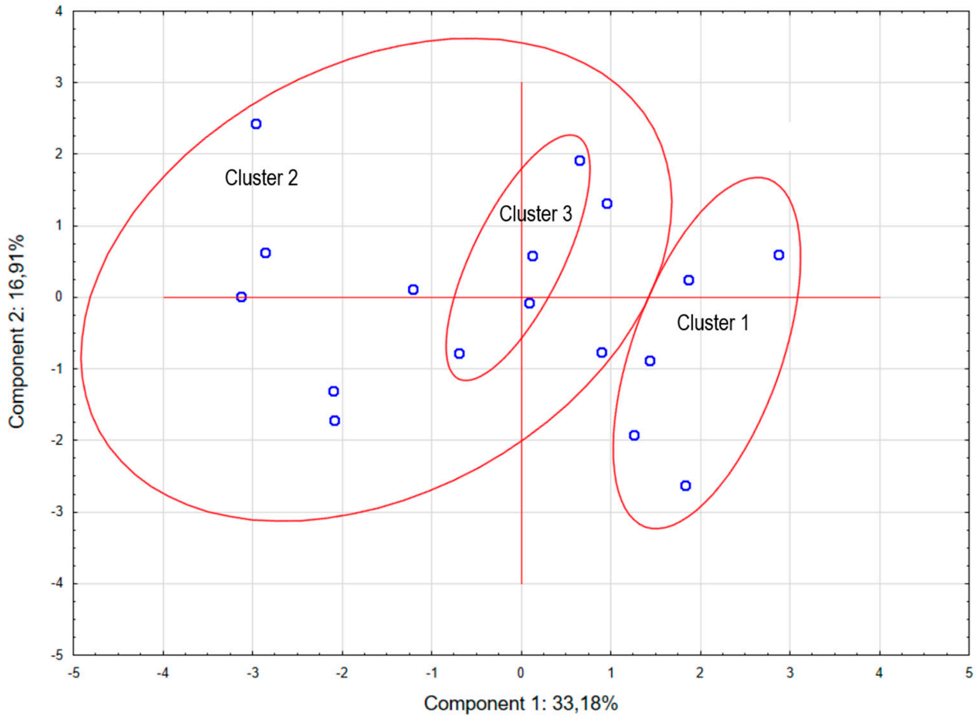

Figure 2. The effects of cluster analysis using the K-means method.

4. Conclusions

The results showed a significant Pearson correlation between the bioelectrical impedance parameter R_z and pH1 (r = 0.48, $p < 0.05$). A significant Spearman correlation was shown between color b* value and the Rz/Xc/HCW ratio (r = −0.62, $p < 0.05$) and Xc (r = −0.51, $p < 0.05$), as well as between the Rz/Xc ratio with pH_1 (r = 0.48, $p < 0.05$).

PCA and cluster analysis showed that bioimpedance measurements combined with the following meat quality traits make it possible to distinguish three groups with different BIA and quality parameters: resistance (R_z), ultimate pH (pH_{24}), glycolytic potential (GP), and color b* value. However, the relationships between them are complex and still require analysis.

The conducted research indicates potential possibilities to use bioelectrical impedance measurements for the empirical estimation of particular features of pork meat quality. However, the enhancement of a number of the methodological aspects seems to be necessary.

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