Identification and investigation of mechanically separated meat (MSM) with an innovative ultrasonic method

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ABSTRACT

An innovative analytical ultrasonic method for identification and investigation of Mechanically Separated Meat (MSM) samples is presented. To this end, the ultrasonic wave velocity ($f = 5\ MHz$) in the investigated meat samples was measured. The measured ultrasonic velocity ranged from 1553.4 to 1589.9 m/s. The investigations were performed for: 1) minced hand deboned chicken fillets, 2) low pressure MSM from chicken carcasses, 3) low pressure MSM from chicken collarbones, 4) high pressure MSM from chicken carcasses and 5) high pressure MSM from chicken collarbones. Statistically significant ($p < 0.001$) differences in the ultrasonic velocity were observed for each of investigated kinds of meat. High significant correlations were found between the ultrasonic velocity and the content of protein, fat, sodium and density of the investigated meat. The applicability of the developed ultrasonic method for identifying various kinds of meat and to determine the content of protein, fat, sodium and density was demonstrated.

1. Introduction

Growing demands of the food industry as well as consumer expectations present increasing challenges for the quality control methods of food products. In fact, the methods for assessing the quality and properties of foodstuffs should be fast, simple, reliable and non-destructive. These methods should also be possible to automate and control with computers, a primary requirement in on-line industrial production lines.

The acronym MSM used throughout the paper stands for the Mechanically Separated Meat that is a raw material obtained by mechanical separation of soft tissue residues from the bones, remaining after cutting and punching of poultry, pig and beef carcasses.

At present, the MSM is widely used as a component in a variety of meat products. The use of MSM as an ingredient in meat products has a significant influence on their quality as well as consumer satisfaction and safety. It is therefore of paramount importance to develop an effective and reliable industrial method to identify MSM and their properties (de Oliveira et al., 2015; Wubshet et al., 2019; Tomaiuolo et al., 2019).

Addressing these demands, the current paper presents a new innovative, ultrasonic method for identification and investigation of MSM samples. The method, developed to this end by the authors in the

Laboratory of Acoustoelectronics at the Institute of Fundamental Technological Research of the Polish Academy of Sciences, Warsaw, Poland, enables for rapid and automated measurements.

The authors put forward the hypothesis that the measurements of the longitudinal ultrasonic wave velocity can be successfully applied to discriminate various types of meat as well as to evaluate the physicochemical parameters of meat samples.

It should be noticed that up to date, a large number of physical and biochemical methods have been proposed and used in identification of MSM products, such as:

(1) chemical composition analysis (Crosland et al., 1995),
(2) gel electrophoresis (Savage et al., 1995),
(3) microscopy (Pickering et al., 1995a),
(4) immunological techniques (Pickering et al., 1995b),
(5) capillary gel electrophoresis (Day & Brown, 2001),
(6) proteomic approach (Surowiec et al., 2011a),
(7) total reflection X-ray fluorescence (Dalipi et al., 2018),
(8) X-ray micro computed tomography (Pospiech et al., 2019),
(9) multivariate analysis of protein profiles (Skarpeid et al., 2001),
(10) histological analysis (Tremlová et al., 2006),
(11) evaluation of radiostrontium levels (Iammarino et al., 2019),

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(12) metabolomic approach (Surowiec et al., 2011b),
(13) electron spin resonance spectroscopy (Tomaiuolo et al., 2019),
(14) inductively coupled plasma-mass spectrometry (Sarakatsianos et al., 2018).

However, all these methods display many deficiencies. Their main disadvantage is lack of suitability for on-line measurements. They are all in fact laboratory methods. Despite their capability to deliver fairly precise results, they are plagued by a number of additional disadvantages (Surowiec et al., 2011b), such as:

a) inability to operate in real time (on-line),
b) complicated setup and operation,
c) lengthy, time consuming measurements,
d) high cost,
e) bulky equipment,
 f) requirement for a highly qualified personnel,
g) tedious off-line sample preparation and
h) difficult and complex data analysis (Damez & Clerjon, 2008).

Two more promising methods that may be used to investigate properties of MSM products are as follows: 1) Near Infra-Red hyperspectral imaging method (Kamruzzaman et al., 2012a, Kamruzzaman et al., 2012b) and 2) Near Infra-Red Reflectance spectroscopy method (Alomar et al., 2003; Prieto et al., 2006).

It should be emphasized that these methods are designed generally to determine the chemical composition of meat, not to discriminate various kinds of meat.

Although, these two methods can be in principle used online, they have substantial limitations (Kumar & Karne, 2017) and share most of the disadvantages (a–h) listed above. Their main disadvantages are:

1) very complex calibration procedures and time-consuming sample preparation
2) very complicated algorithms of pre-processing and post-processing analysis,
3) highly complex and difficult interpretation of the results obtained and
4) sophisticated and very costly equipment.

Preferably, the identification process of various meat products in industrial conditions should be fast, relatively inexpensive, non-destructive and performed with compact, portable and fully- automated equipment. The ultrasonic methods, such as those based on ultrasonic measurements of velocity fulfill all these requirements.

In this paper the authors propose the application of a new innovative ultrasonic method for investigation and identification of various types of MSM. The method is based on the ultrasonic velocity c measurement. The proposed ultrasonic method is free of all of the above mentioned deficiencies (a–h). As a matter of fact, the ultrasonic methods have already been successfully employed in food investigations in a variety of food products, for a general review see, (Taufiq et al., 2016; Kiełczyński, 2017).

Surprisingly, a fairly large number of mechanical and physicochemical properties of meat and meat products was already evaluated by ultrasound in a numerous papers, e.g.,

1) composition of cod fillets (Ghaedian et al., 1997),
2) mechanical properties of meat (Nowak et al., 2015).
3) structure and composition of pork meat (Koch et al., 2011a).
4) dry salting monitoring (Prados et al, 2016).
5) textural properties of a meat-based product (Llull et al., 2002).
6) detection of bone fragment in mechanically deboned chicken breasts (Correia et al., 2008)
7) non-destructive determination of fat content in green hams (Prados et al., 2015).

However, to the best of our knowledge, the ultrasonic methods have not been yet employed to investigate the properties of MSM products. In the proposed ultrasonic method, the authors measure the velocity c of longitudinal ultrasonic waves propagating in various kinds of investigated MSM meat samples.

In order to determine the correlation between the ultrasonic velocity and the chemical composition of the meat samples, the content of calcium, phosphorus, sodium, water, protein, fat and density of the investigated meat samples were determined. The measured ultrasonic velocity c was subsequently compared with the actual physicochemical parameters of the investigated MSM samples. The measured ultrasonic velocity c displayed a high level of correlation with a number of the actual physicochemical parameters (i.e., content of protein, fat, sodium and density) of the investigated MSM samples. We anticipate that this discovery can constitute a basis for a future on-line industrial method for 1) identification of individual types of MSM meat products and 2) assessment of the basic chemical composition of the investigated meat samples.

The proposed by the authors ultrasonic method has the following favored features:

 1) possibility for on-line implementation
 2) possibility of full automation and computerization,
 3) fast measurement process,
 4) no special sample preparation and preprocessing are necessary,
 5) high reliability,
 6) simplicity and low cost,
 7) not destructiveness,
 8) no moving mechanical parts,
 9) use of industrial grade instrumentation,
10) environmental friendliness.

The goal of this paper was to identify different types of MSM meat samples from measurements of the ultrasonic velocity c and to find possible correlations between the ultrasonic velocity c and the following physicochemical parameters of MSM meat samples: the content of protein, fat, calcium, phosphorus, sodium, water, and the density of the investigated meat samples.

2. Materials and methods

2.1. Meat samples

The sample materials utilized in this work were provided by a Polish poultry processing plant located in Lublin, Poland.

The raw material under investigation was the chicken (*Gallus gallus domesticus*) breast meat obtained from industrial hand deboning (HD) poultry meat and four types of mechanically separated meat (MSM) obtained from mechanical deboning of non-frozen chicken carcasses and chicken collarbones using two different types of separator devices, namely:

1) Sepamatic Sepa 1200 belt separator device (Overath, Germany) classified as low pressure device produces MSM meat (drum perforation diameter is 3.0 mm). Due to the requirements of the device, the processed bones were initially fragmented into smaller portions. The end product had a consistency similar to minced meat.
2) Lima RM 600 s separator (Quimper, France), classified formally as high pressure device produces MSM meat. The actual operating pressure is 1.5 MPa. The size of the outlet slots is 0.5×20.0 mm^2. By contrast to device no 1, the processed bones were initially not fragmented into smaller portions. The obtained MSM meat material had a paste-like consistency.

Before examination, HD poultry meat and MSM samples were minced with a grinder (Edesa PL-22-TU-T, Czosnów, Poland), using a

strainer with 3 mm holes and subsequently mixed with a Keripar mixer (Troy, Ohio, USA) for homogenization.

In this work, we investigated the following five types of meat samples (obtained with different methods), i.e.,

1) minced HD chicken fillets,
2) low pressure MSM samples from chicken carcasses,
3) low pressure MSM samples from chicken collarbones,
4) high pressure MSM samples from chicken carcasses and
5) high pressure MSM samples from chicken collarbones.

Prior to ultrasonic measurements the investigated five types of meat samples were stored at a constant temperature of 4 °C.

2.2. Chemical analysis of the investigated meat samples

The following basic physicochemical parameters of the investigated meat samples were measured:

a) Density

The density of meat samples (g/cm^3) was measured with a modified pycnometric method. Measuring flask had a volume of 100 cm^3. The density measurements were performed for meat samples with a mass of approximately 10 g. In the first stage, we determine the mass of the sample. The volume of the meat sample was determined from the determination of the water level increase after inserting the investigated meat sample into the measuring cylinder with water. Knowledge of the mass and the volume of meat samples allows to determine their density.

b) Calcium content

The calcium content (mg/kg) was evaluated using flame atomic absorption spectrometry (FAAS) and Z-2000 apparatus (Hitachi, Japan). The meat sample was dry mineralized in a muffle furnace at 420–450 °C. After the mineralization (on the heating plate and in the muffle furnace), dissolving and adding the matrix modifier - Lanthanum buffer, the sample was analyzed on a spectrophotometer using a Ca lamp with a hollow cathode. The calcium concentration was determined on the basis of the evaluated standard curves.

c) Phosphorus content

The phosphorus content was evaluated according to the [PN-A-82060:1999] standard. The determination of the total phosphorus content [%], expressed as P_2O_5, included the following steps: 1) mineralization of the sample, 2) precipitation of phosphorus in the form of choline phosphoromolybdate and 3) weight determination of total phosphorus.

d) Sodium content

The sodium content (mg/kg) was determined using flame atomic absorption spectrometry (FAAS) with the Hitachi Z-2000 apparatus, Japan. The meat sample was dry mineralized in a muffle furnace at 420–450 °C. After the mineralization (on the heating plate and in the muffle furnace), dissolving and adding the matrix modifier - Cesium buffer, the sample was analyzed by spectrophotometer using an Na lamp with a hollow cathode. The sodium concentration was determined employing the evaluated standard curves.

e) Water content

The water content [%] was determined using a standard drying method. The measured samples were drying at 103 °C for 30 min, (Oven Series 9000, Thermolyne, USA).

f) Protein content

The protein content [%] was measured with a Kjeldahl method, (Foss Tecator, Sweden) according to the [PN-75-A-04018/Az3] standard.

The method relies on determination of the total nitrogen content by the Kjeldahl method, using subsequently the conversion factor of nitrogen content into protein content (for meat = 6.25).

g) Fat content

The fat content [%] was measured using a weight method according to the [PN-ISO 1444-2000] standard. Extraction of fat was performed employing a Soxhlet technique, (Tecator Co., Sweden).

2.3. Ultrasonic measurements

The phase velocity c the ultrasonic waves propagating in the investigated meat samples, was measured with a custom designed computerized measurement system, constructed by the authors in the Section of Acoustoelectronics at Institute of Fundamental Technological Research of the Polish Academy of Sciences in Warsaw, Poland (see Fig. 1).

The ultrasonic waves, propagating through the examined meat samples, were generated and detected with custom designed piezoelectric transducers, with $LiNbO_3$, Y-36 ° cut piezoelectric elements, provided by (Roditi, USA). The resonant frequency of the transducers was $f = 5\ MHz$.

The ultrasonic transmitting transducers was driven by the state-of-the-art TB1000 pulser-receiver board (Matec, USA). The electric signal from the receiving ultrasonic transducer was amplified in the pulser-receiver board and sent further to the fast PDA1000 digitizer board (Signatec, USA) operating at a sampling rate of 62.5 Mega samples per second. After acquisition the digitized ultrasonic signal was digitally filtered and subsequently averaged 1024 times to further improve the signal to noise ratio (S/N), of the signal. The digital ultrasonic signals, after processing, were displayed in real time on the monitor, enabling therefore for visual control and eventual adjustments by the operator.

The measurement process and the instrumentation were controlled by a custom designed software written in the Microsoft Visual C ++, using object-oriented-technology. The digitizer board and ultrasonic board were enclosed within an industrial PC computer (Berta, Transduction Computers, Mississauga, Ontario, Canada), providing stable, temperature controlled and noise free environment.

Fig. 1 sketches only a general diagram of connections of the ultrasonic measurement system, with the ultrasonic pulser-receiver and the digitizer shown as separate stand-alone devices. In the actual measurement system the TB1000 ultrasonic board and PDA1000 digitizer board were totally enclosed within an industrial PC computer (Berta), providing signal processing, signal visualization and data saving capabilities.

The velocity c of longitudinal ultrasonic waves, propagating in the investigated meat samples was determined from measurements of the Time-of -Flight (TOF) τ_d for two selected ultrasonic impulses travelling between the transmitting and receiving transducers (a through-transmission method), see (Kiełczyński et al., 2014a, Kiełczyński et al., 2014b). The Time-of-Flight (TOF) was determined digitally using a cross-correlation method (Kiełczyński et al., 2015, Kiełczyński et al., 2019). The maximum of the cross-correlation function, between the two selected impulses, corresponds to the Time-of-Flight (TOF) between the ultrasonic impulses in the investigated meat samples.

Relative changes in the Time-of-Flight can be determined with a very high precision (of the order of hundreds picoseconds). The uncertainty in determination of the ultrasonic path and the impact of other systematic errors bound the uncertainty in determination of ultrasonic velocity to ± 0.1%.

The velocity c of propagation of the ultrasonic wave in the investigated meat samples was determined from the elementary relation $c =$
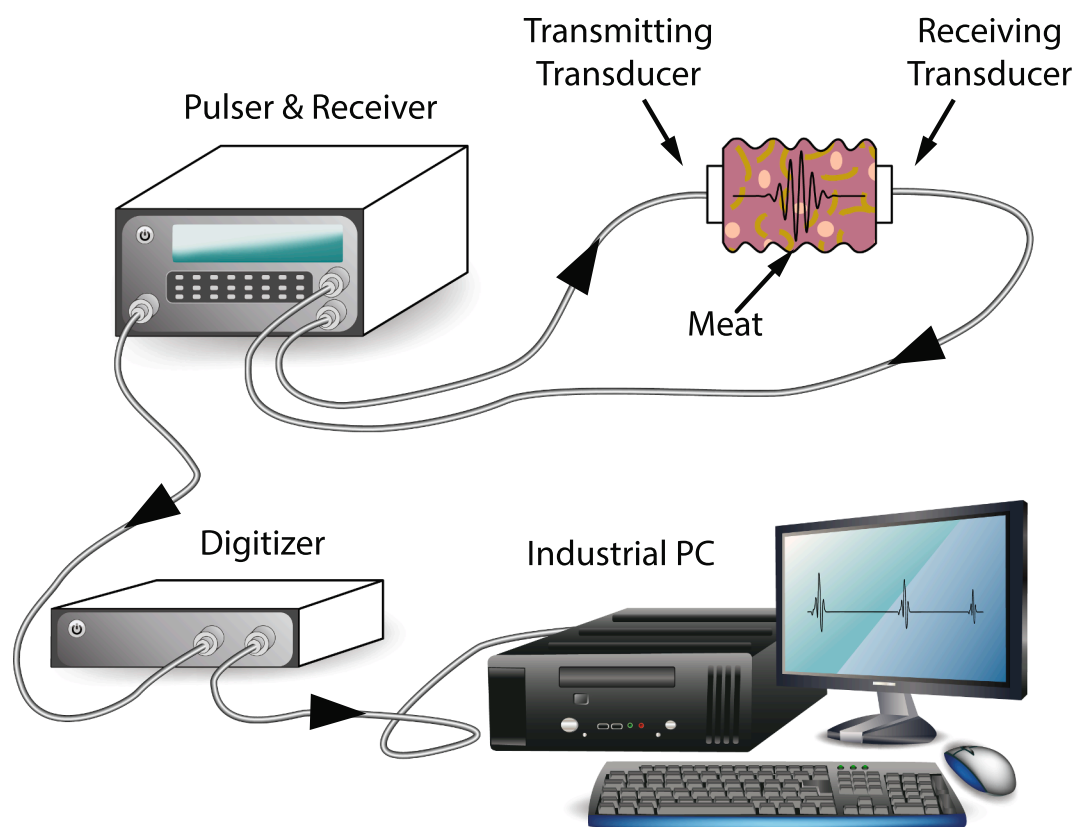
Fig. 1. Schematic diagram of the computerized measurement system.

TOF/L; where L is the distance between the two transducers in the examined meat material.

The distance L between two (sending and receiving) piezoelectric transducers was evaluated based on calibration measurements performed in distilled water at controlled temperature and pressure.

Knowing, with a high accuracy the speed of sound in water at a given temperature (from NIST Steam Tables) and measuring the Time-of-Flight, the distance L between the transducers was calculated from the elementary formula $L = c \times TOF$.

By constructing the custom designed measurement system, the authors aimed to achieve the following two main goals: 1) high accuracy in measurements of the Time-of-Flight (velocity) of ultrasonic signals and 2) computerization of the measurement process in order to mitigate and finally eliminate the operator's errors that might influence the whole measurement process. Such a high accuracy of measurements ($\pm0.1\%$) is virtually not achievable with the commercially available measuring equipment, accessible on the market.

The ultrasonic velocity was measured in five (5) different types of the investigated meat, i.e., in 1) minced HD chicken fillets, 2) low pressure MSM samples from chicken carcasses, 3) low pressure MSM samples from chicken collarbones, 4) high pressure MSM samples from chicken carcasses and 5) high pressure MSM samples from chicken collarbones. In each of these 5 types of meat, the ultrasonic velocity was measured 5 times for 5 randomly selected different samples of a given type of meat. After completion of every ultrasonic measurement of velocity, the investigated meat sample was removed from the measurement chamber. Subsequently, before the next consecutive measurement, the chamber was carefully rinsed with water and dried-up.

2.4. Statistical analysis

In this work, all statistical analyses were performed with STATIS-TICA 12.5 (TIBCO Software, Palo Alto, USA) software package.

All measurements (for ultrasonic velocity and each of 7 physico-chemical parameters of meat) were performed using five (5) randomly extracted meat samples, for each of the five (5) types of meat. Consequently, $5 \times 5 = 25$ independent ultrasonic velocity measurements were conducted (see Table 2). Similarly, $5 \times 5 \times 7 = 175$ independent measurements were carried out to evaluate the seven (7) physico-chemical parameters of meat (see Table 1). The ultrasonic measurements were performed in a stable temperature controlled environment. The ultrasonic measurements of the time-of-flight and hence the ultrasonic velocity were of very high repeatability and precision, due to the employment of sophisticated signal processing procedures and proprietary controlling software. As a result, we observed high repeatability and reproducibility of the ultrasonic measurements presented in this study.

In order to determine the significant differences between mean values of the ultrasonic velocity in various kinds of meat, we performed the following statistical analyzes: 1) Shapiro-Wilk test to check the normality of the distribution, 2) Levene's test to investigate homogeneity of variances and 3) one-way ANOVA analysis along with post-hoc Tukey tests.

To assess the correlation between the measured ultrasonic velocity and the chemical composition of meat samples we measured the content of 1) protein, 2) fat, 3) calcium, 4) phosphorus, 5) sodium, 6) water, and 7) the density of the investigated meat samples using standard methods of the analytical chemistry. Subsequently, the obtained results of measurements (ultrasonic and chemical) were analyzed statistically in order to determine the corresponding correlation coefficients and linear regression equations.

Table 1

Measured physicochemical properties of the investigated meat samples. Mean values and standard deviations are given in square and round brackets respectively.

Type of meat	Calcium content [mg/kg]	Total phosphorus content expressed as P_2O_5 [%]	Sodium content [mg/kg]	Water content [%]	Protein content[%]	Fat content [%]	Density[g/cm^3]
Minced HD chicken fillet	48	0.56	378	75.4	23.1	0.9	1.0561
	52	0.53	326	75.3	22.9	1.0	1.0609
	45	0.50	411	74.1	22.3	1.5	1.0584
	48	0.58	356	74.5	21.8	1.8	1.0572
	55	0.60	385	74.6	22.9	1.2	1.0597
	[49.6] [A] ± (3.91)	[0.554] [A,B] ± (0.04)	[371.2] [A] ± (32.00)	[74.8] [A] ± (0.55)	[22.6] [A] ± (0.54)	[1.28] [A] ± (0.37)	[1.0585] [A] ± (0.0019)
Low-pressure MSM (collarbone)	1280	0.59	517	73.5	20.5	5.5	1.0135
	960	0.55	610	73.2	20.1	6.0	1.0344
	1080	0.54	509	73.8	19.5	5.8	1.0295
	1150	0.56	556	73.1	19.1	7.1	1.0140
	990	0.58	510	72.0	18.9	8.0	1.0310
	[1092] [B] ± (129.11)	[0.564] [B] ± (0.02)	[540.4] [B] ± (43.43)	[73.1] [B] ± (0.68)	[19.62] [B] ± (0.67)	[6.48] [B] ± (1.04)	[1.0245] [B] ± (0.0099)
High-pressure MSM (collarbone)	152	0.44	449	74.7	18.6	6.8	1.0128
	170	0.45	443	74.3	19.8	5.6	1.0095
	144	0.40	490	73.5	19.2	7.2	1.0148
	160	0.48	475	73.9	18.4	7.1	1.0097
	158	0.50	523	75.1	18.1	6.1	1.0144
	[156.8] [A] ± (9.65)	[0.454] [C] ± (0.04)	[476] [B] ± (32.50)	[74.1] [B,C] ± (0.63)	[18.82] [B] ± (0.68)	[6.56] [B] ± (0.69)	[1.012] [C] ± (0.0025)
Low-pressure MSM (carcass)	1840	0.59	761	75.1	16.8	7.1	0.9750
	1760	0.42	733	74.6	17.0	6.3	0.9747
	1910	0.65	752	75.4	17.1	5.9	0.9743
	1880	0.45	823	74.3	16.8	7.5	0.9749
	1947	0.53	801	73.1	16.7	8.5	0.9742
	[1867.4] [C] ± (71.76)	[0.528] [A,B,C] ± (0.09)	[774] [C] ± (37.00)	[74.5] [B,C] ± (0.89)	[16.88] [C] ± (0.16)	[7.06] [B] ± (1.02)	[0.9746] [D] ± (0.00036)
High-pressure MSM (carcass)	720	0.45	635	68.8	15.0	14.8	0.9504
	560	0.42	679	69.4	14.2	14.0	0.9600
	620	0.45	723	70.1	15.5	12.0	0.9591
	692	0.50	698	69.0	15.2	13.2	0.9501
	650	0.38	756	71.0	14.8	12.2	0.9572
	[648.4] [D] ± (62.55)	[0.44] [C] ± (0.04)	[698.2] [D] ± (45.60)	[70.0] [C] ± (0.9)	[14.94] [D] ± (0.49)	[13.24] [C] ± (1.19)	[0.9554] [E] ± (0.0048)

Mean values in the same column marked with different superscript letters are statistically different ($p < 0.05$).

3. Experimental results

3.1. Measurement of physicochemical parameters of the investigated meat samples

Employing the chemical analytical methods described in subsection 2.2, a series of physicochemical parameters of the investigated meat samples were measured.

The obtained results of chemical measurements were processed statistically using one-way analysis of variance (ANOVA) procedures and post-hoc Tukey tests.

Table 1 contains 1) the results of chemical analysis performed in order to determine the basic physicochemical parameters of the investigated meat samples i.e., the content of calcium, phosphorus, sodium, water, protein and fat as well as the density and 2) the results of statistical analysis.

The mean value and standard deviation were evaluated for each physicochemical parameter (e.g., protein content) based on five consecutive measurements (performed on five randomly selected samples of each type of meat). As it is stated in Table 1, the protein content varies from 15% in high-pressure MSM (carcass) samples to 23% (in chicken fillets). On the other hand, the fat content varies from 1% (in chicken fillets) to 13% in high-pressure MSM (carcass) samples, what is in agreement with expectations.

3.2. Measurements of ultrasonic velocity c of longitudinal ultrasonic waves

The measurements of ultrasonic velocity c in the investigated meat samples were carried out in the experimental setup shown schematically in Fig. 1. The measurements were performed at an ambient temperature 24°C. To evaluate significant differences between ultrasonic velocity measurements in various kinds of meat, one-way analysis of ANOVA along with post-hoc Tukey tests were performed. The results of ultrasonic measurements (velocity c of longitudinal ultrasonic waves) performed in the investigated meat samples and results of statistical analysis are presented in Table 2.

The results of ultrasonic measurements for velocity c of longitudinal ultrasonic waves, included in Table 2, are shown graphically in Fig. 2.

The results of statistical analysis (one-way ANOVA + post-hoc Tukey tests) show that the ultrasonic velocity mean values obtained for different types of meat differ significantly from each other ($p < 0.001$).

From a technical point of view the ultrasonic velocity can be measured with an uncertainty ± 1.5 m/s. As can be seen from Fig. 2, the differences in ultrasonic velocity between various types of meat samples range from 6.4 m/s to 11.6 m/s. Therefore, the ultrasonic velocity measurements can be effectively used to discriminate various types of meat samples.

4. Relationship between ultrasonic velocity and chemical composition of the investigated meat samples

4.1. Statistical correlation between ultrasonic velocity and chemical composition

The obtained experimental results inserted in Table 1 and Table 2 were processed statistically using correlation procedures provided by Statistica software package. Consequently, the correlations between the ultrasonic velocity c and the physicochemical parameters of the investigated meat samples were determined.

Table 2

Results of ultrasonic measurements (velocity c of longitudinal ultrasonic waves) performed in the investigated meat samples. Wave frequency $f = 5\ MHz$.

Type of meat	Measured ultrasonic velocity $c\ [m/s]$	Mean value of $c\ [m/s]$*	Standard deviation of $c\ [m/s]$
High-pressure (HP) MSM (carcass)	1552.51 1550.34 1554.35 1555.02 1554.71	1553.39^{A}	1.96
Low-pressure (LP) MSM (carcass)	1560.34 1558.68 1559.26 1562.17 1561.24	1560.34^{B}	1.42
High-pressure (HP) MSM (collarbone)	1571.90 1572.16 1573.42 1571.34 1570.66	1571.90^{C}	1.03
Low-pressure (LP) MSM (collarbone)	1579.29 1576.51 1577.21 1577.55 1581.03	1578.32^{D}	1.83
Minced HD chicken fillet	1589.63 1590.42 1590.25 1589.23 1590.03	1589.91^{E}	0.48

*Mean values of the ultrasonic velocity c given in the third column, with different superscript letters, are statistically different ($p < 0.001$).
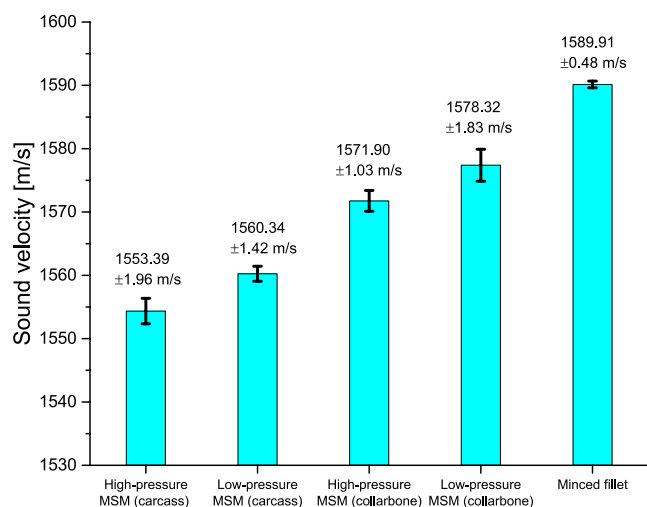

Fig. 2. Measured velocity c (expressed as mean value ± standard deviation) of longitudinal ultrasonic waves propagating in various types of investigated meat samples. Wave frequency $f = 5\ MHz$.

The following statistical parameters: Pearson's correlation co-efficients r, p-values and linear regression equations were evaluated, see Table 3 below.

Based on the performed statistical analysis of the experimental data, included in Table 1 and Table 2 , it was plotted in Fig. 3 a, b, c, d and e, linear regression curves of the measured ultrasonic velocity c as a function of protein content, fat content, sodium content, calcium content, and density.

The results presented in Table 3 and in Fig. 3 a, b, c and e show that the ultrasonic velocity exhibits a linear statistically significant ($p < 0.028$) dependence on the basic physicochemical parameters (i.e., protein content, fat content, sodium content and density) of the meat

Table 3

Statistical correlation between the results of the measured ultrasonic velocity c and measured physicochemical parameters of the investigated meat samples.

Meat property	Linear regression equation	p-value	Pearson correlation coefficient r
Protein content	y = 4.71 × + 1453.26	0.00075	0.99269
Fat content	y = -3.04 × + 1591.63	0.0270	−0.91836
Water content	y = 3.88 × + 1286.88	0.27838	0.60628
Calcium content	y = -0.01 × + 1578.36	0.36302	−0.52256
Phosphorus content	y = 159.93 × + 1489.21	0.2512	0.63458
Sodium content	y = -0.08 × + 1616.91	0.02774	−0.9182
Density	y = 349.20 × + 1219.17	0.00023	0.99667

y = ultrasonic velocity, x = protein content, fat content, water content, calcium content, phosphorus content, sodium content or density.

samples. This suggests the possibility of applying the ultrasonic velocity measurements as a reliable analytical tool to assess the chemical composition of meat (i.e., the content of protein, fat, sodium, and the density of investigated meat samples).

5. Discussion

5.1. Ultrasonic velocity measurements

The velocity c of longitudinal ultrasonic waves, measured in the investigated meat samples, was ranging from $c = 1553.4$ m/s (MSM samples obtained with high-pressure separation of meat from bones of chicken carcasses) to $c = 1589.9$ m/s (meat samples obtained from HD chicken fillets). This indicates that meat samples obtained from chicken fillets are mechanically more stiff than MSM samples obtained by high-pressure separation of meat from bones of chicken carcasses, which suffered destruction of muscle fiber structure during the process (Sifre et al., 2009). In fact, the reduction of stiffness (elasticity) leads directly to the reduction of ultrasonic velocity c according to the standard formula $c = \sqrt{c_{11}/\rho}$, where c_{11} stands for the modulus of elasticity and ρ for the density of the measured sample.

The difference $\Delta c = 36.5$ m/s between the maximum and minimum measured velocities is quite large (2.3%). This is an advantage of the proposed ultrasonic method since from the ultrasonic measurements we are able to deduce reliably the type of the meat sample under investigation (see Table 2 and Fig. 3).

The ultrasonic velocity c, in the measured meat samples, arranged in the ascending order is as follows:

- $c = 1553.4$ m/s (in high pressure MSM from chicken carcasses)
- $c = 1560.3$ m/s (in low pressure MSM from chicken carcasses
- $c = 1571.9$ m/s (in high pressure MSM from chicken collarbones)
- $c = 1578.3$ m/s (in low pressure MSM from chicken collarbones)
- $c = 1589.9$ m/s (in minced HD chicken fillets).

The above arrangement shows that the difference in the ultrasonic velocity c between two consecutive values of c is quite pronounced and ranges from 6.4 m/s to 11.6 m/s. This suggests that the proposed ultrasonic method that uses the measurement of the ultrasonic velocity c can be effectively applied to discriminate various types of meat samples.
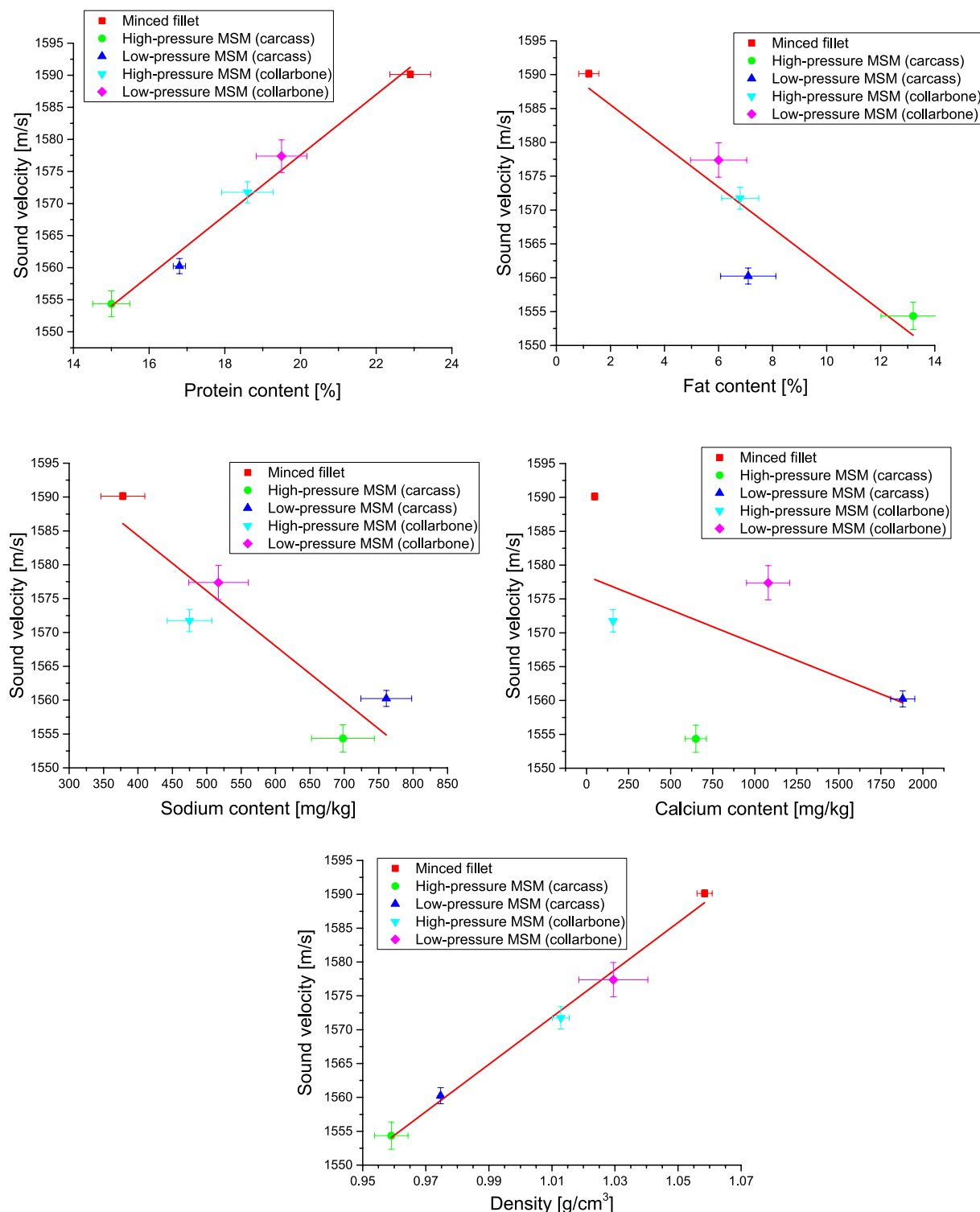
Fig. 3. Relationship between the measured ultrasonic velocity c and the content of a) protein, b) fat, c) sodium (Na), d) calcium (Ca) and e) density of the investigated meat samples.

5.2. Physicochemical properties versus ultrasonic velocity

Another advantage of the proposed ultrasonic method is high degree of correlation between the measured ultrasonic velocity c and the content of basic chemical components in the measured meat samples (see Fig. 3 a-e). For example, the coefficient of correlation r between the ultrasonic velocity c and the protein content in the investigated meat samples was significant ($p = 0.00075$) and is equal to $r = 0.99269$, (see Fig. 3 a). In fact, a high protein content increases the elasticity c_{11} of meat samples. At the same time the density ρ increases but to a lesser extent. Consequently, we observe an overall increase in the ultrasonic velocity $c = \sqrt{c_{11}/\rho}$.

A high significant ($p = 0.0270$) correlation coefficient was also found between the ultrasonic velocity c and the fat content ($r = -0.91836$), see Fig. 3 b. This can be explained by the fact that higher fat content reduces both the density and elasticity of the investigated meat

samples. However, the decrease in the elasticity c_{11} is greater than that in the density ρ. Consequently, we observe the reduction in the ultrasonic velocity c in the measured meat samples.

It is interesting to note that high significant correlations were also discerned between the ultrasonic velocity c and sodium content ($r = -0.9182$ and $p = 0.02774$), (see Fig. 3 c), and between the ultrasonic velocity c and density ρ ($r = 0.99667$ and $p = 0.00023$), (see Fig. 3 e), in the measured meat samples.

It was difficult to determine the significant linear correlation between the ultrasonic wave velocity and the phosphorus and water content, see Table 3, due to the low variability of the phosphorus and water content in the investigated samples.

5.2.1. Calcium content

The experimental data (see Table 1 and Fig. 3 d) show a large spread of the calcium (Ca) content in the investigated meat samples. The lowest calcium (Ca) content was found in the samples of minced HD chicken fillets, as expected. Surprisingly, the highest calcium (Ca) content was found in samples obtained by low-pressure methods, i.e., a) low pressure MSM samples from chicken carcasses and b) low pressure MSM samples from chicken collarbones.

This was not the case in samples obtained by high pressure methods, i.e., c) high pressure MSM samples from chicken carcasses and d) high pressure MSM samples from chicken collarbones.

The above finding can be explained by the fact that the low-pressure methods used with the appropriate exploitation parameter setup may result in meat products with a relatively high bone content, larger than that resulting from the use of high-pressure methods. Moreover, the calcium content in the MSM and HD meat samples varies depending on the animal species, part of the carcass and a method (technical conditions) used for meat recovery.

The results of the statistical analysis show that the ultrasonic velocity and calcium content are weakly correlated ($r = -0.52256$) with a very low significance ($p = 0.363$). Therefore, we can conclude that there is no significant linear relationship between the ultrasonic velocity and calcium content. Consequently, the calcium content cannot be used as a reliable indicator for the meat identification in the industrial environment.

The measurements of the ultrasonic velocity performed in this paper show high statistically significant ($p < 0.001$) differences between mean values of the ultrasonic wave velocity in various kinds of investigated meat samples. Therefore, the ultrasonic measurements of velocity can be recommended as a rapid and effective analytical technique for identifying various classes of meat, e.g., in order to differentiate hand-deboned meat (HD) from mechanically separated meat (MSM).

The results obtained in this study are new and original. The literature reports show that up to date, the ultrasonic examination of meat was mainly focused on:

1) estimation the composition of meat (Benedito et al., 2001; Lakshmanan et al., 2012)
2) determining the structure of meat (Koch et al., 2011b)
3) evaluation of marbling in meat samples (Ludwiczak et al., 2017).

By contrast, our study aims at achieving ultrasonic discrimination of MSM meat from HB meat employing ultrasonic velocity measurements.

According to the best of our knowledge, the ultrasonic method, based on ultrasonic velocity measurements, have not yet been used to discriminate various types of meat e.g., to differentiate chicken fillets from mechanically separated chicken meat.

6. Conclusions

The main implications arising from the results of research performed in this work can be summarized as follows:

1. The developed analytical ultrasonic method for identification and investigation of meat samples is rapid and non-destructive and has a potential for an on-line implementation in industrial conditions for real time measurements.
2. The measurements of the ultrasonic velocity c constitute a promising analytical tool to discriminate various types of MSM samples, obtained by different methods, in relation to hand deboned meat.
3. The measurements of the ultrasonic velocity can be used as well to evaluate quantitatively the basic physicochemical parameters of meat samples, i.e., the content of protein, fat and sodium, as well as the density of meat samples.
4. High statistically significant correlation was observed between the ultrasonic wave velocity and a) protein content ($r = 0.99269$ and $p = 0.00075$), b) fat content ($r = -0.91836$ and $p = 0.0270$), c) sodium content ($r = -0.9182$ and $p = 0.02774$) as well as the density ($r = 0.99667$ and $p = 0.00023$), of the meat samples measured (including MSM).
5. The correlation between the ultrasonic velocity c and the content of phosphorus and water in the selected meat samples is moderate and statistically not significant. $r = 0.63458$ and $p = 0.2512$ for phosphorus content, and $r = 0.60628$ and $p = 0.27838$ for water content, see Table 3.
6. Since different technological processes lead to different levels of calcium (Ca) content (see the Discussion Section), it is difficult to linearly correlate the Ca content with the ultrasonic velocity c (see Table 3) and consequently with various MSM types. Therefore, the calcium (Ca) content cannot be used as a reliable criterion in identification and investigation of different types of MSM meat.
7. High degree of cross-correlations were also found between protein content, fat content, sodium content and the density. Hence, the evaluation of e.g., protein content can assess the fat content, sodium content and the density of the investigated meat samples.

Taking into account the conclusions No. 2, 3 and 4, presented above, the research hypothesis stated in the Introduction section has been confirmed.

The analytical ultrasonic method, developed by the authors in this paper, can be an attractive alternative to the conventional methods, due to its inherent advantages such as: rapidity, simplicity, low cost, possibility of computerization and on-line measurements, simple measurement procedure, as well as its ability to identify and quantify different physicochemical parameters in various types of meat (e.g., MSM).

CRediT authorship contribution statement

K. Wieja: Conceptualization. **P. Kiełczyński:** Supervision, Investigation, Formal analysis, Writing - original draft. **P. Szymański:** Methodology. **M. Szalewski:** Investigation, Writing - review & editing. **A. Balcerzak:** Data curation. **S. Ptasznik:** Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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