

Article

Investigation and Discrimination of Hand-Deboned and Mechanically Separated Pork Meat Using Ultrasonic Velocity Measurements

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Abstract

In this paper, we present an original ultrasonic technique to investigate and discriminate between different kinds of pork meat, i.e., hand-deboned (HD) meat and mechanically separated (MSM) meat. To this end, we measured the speed c of high-frequency $f = 5$ MHz ultrasonic waves propagating in the examined pork meat samples. The measured speed of longitudinal ultrasonic waves ranged from 1578 to 1610 m/s. Significant statistical differences were found between the speed of ultrasonic waves in the HD pork loin and pork neck, and those in various types of MSM. The newly proposed measurement (analytical) method, based on the determination of ultrasonic velocity, can be effectively applied to distinguish between manually separated (HD) and mechanically separated (MSM) pork meat. Importantly, the obtained experimental results were supported by a statistical analysis, showing highly significant correlations between the speed of ultrasonic waves and the content of water, protein, fat and sodium in the measured pork meat samples.

Keywords: physicochemical parameters of meat; mechanically separated meat (MSM); hand-deboned (HD) meat; speed of sound; protein content; fat content; water content; sodium content

1. Introduction

1.1. Background on the Current Status of Investigations into the Properties of Meat

In the 21st century, we have witnessed the emergence of the Fourth Industrial Revolution, characterized by the employment of smart sensors, interconnectivity (networking), computer-controlled instrumentation, advanced signal processing, robotics, AI (Artificial Intelligence), etc. [1,2]. It is important to realize how these new developments may improve current food processing and monitoring techniques used in real industrial practice.

As a matter of fact, many conventional food investigation techniques used in industrial practice are destructive, off-line, time-consuming and complicated, requiring the employment of highly qualified personnel. Thus, the development of non-invasive, accurate, and real-time monitoring systems for non-destructive, accurate, and on-line measurements of the physicochemical and mechanical properties of food (meat) still remains a very challenging task.



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On the other hand, for safety reasons and in accordance with the recommendations of the European Commission and the European Food Safety Authority (EFSA) [3], the search for new analytical methods examining meat quality remains a very important open endeavor.

In fact, non-destructive ultrasonic techniques with computer-controlled instrumentation offer very good opportunities to fulfill the requirements of the digital Fourth Industrial Revolution in the context of the food industry. Indeed, ultrasonic techniques exhibit many advantages over previously used ones, such as non-invasive examination, real-time measurements, high precision, low cost of instrumentation, the possibility of examining products contained in closed and opaque containers [4] and easy network connectivity, allowing for integration with a central control system of the plant.

1.2. Importance of the Investigation of Hand-Deboned (HD) and Mechanically Separated (MSM) Pork Meat

Mechanically separated (MSM) meat is commonly used in the meat industry as a raw material in the production of pates, canned meat, hot dogs, hamburgers and sausages. Therefore, the development of new methods for the characterization and discrimination of various kinds of meat, e.g., hand-deboned (HD) meat and MSM, is of crucial practical importance, since these new methods may improve the quality of final meat products and lower their overall cost.

The research hypothesis put forward in this paper claims that it is possible, using ultrasonic measurements of velocity c , to discriminate between mechanically separated (MSM) and hand-deboned (HD) meat samples, as well as to quantify the content of ingredients in the measured meat samples.

1.3. Existing Methods Used in the Investigation of Hand-Deboned (HD) and Mechanically Separated (MSM) Pork Meat

To date, many physicochemical methods have been used to test the quality of MSM, i.e.,

- 1) Inductively coupled mass spectroscopy [5];
- 2) Ion chromatography with conductivity detection [6];
- 3) Evaluation of radio-strontium levels [7];
- 4) X-ray micro-computed tomography [8];
- 5) Targeted liquid chromatography–mass spectroscopy (LC-MS/MS) [9];
- 6) Electron spin resonance [10].

Needless to say, none of the above-cited methods are close to on-line measurements. In fact, they are purely laboratory-based. In addition, despite their ability to provide relatively precise results, they are hampered by many drawbacks, such as the following:

1. Complicated setup and operation procedures;
2. Time-consuming measurements;
3. Bulky equipment and highly complicated hardware;
4. Tedious off-line sample preparation.

1.4. Existing Ultrasonic Techniques to Investigate the Properties of Meat

Ultrasonic techniques have already proven their usefulness in controlling the quality and properties of meat. For example, in references [11,12], ultrasonic waves were used to investigate the properties and structure (e.g., intramuscular fat content) of the meat acquired from German pigs. However, the properties of MSM meat obtained from raw pork material were not examined.

Similarly, in references [13–15], ultrasonic techniques were used to examine the physicochemical parameters of pork and beef. Ultrasonic methods were also applied to evaluate

the texture, mechanical and elastic properties of meat products [16,17], and also provide a score for pork meat marbling [18]. Ultrasonic techniques are also very useful in the control and determination of meat properties under high-pressure (HP) conditions [19–21].

1.5. Basic Characteristics of the Proposed New Ultrasonic Method for Investigation and Discrimination of Hand-Deboned (HD) and Mechanically Separated (MSM) Pork Meat

To the best of the authors' knowledge, ultrasonic techniques have not yet been employed in the examination of the quality of MSM pork meat and related products. To fill this gap, the authors developed a new ultrasonic method for investigation and discrimination of hand-deboned (HD) and mechanically separated (MSM) pork meat, a method which was designed for use in real industrial conditions.

The new developed method is based on measurements of the speed c of high-frequency $f = 5$ MHz ultrasonic waves propagating in the examined pork meat samples. Significant statistical differences were found between the speed of ultrasonic waves in HD pork loin and pork necks and various types of MSM meat. The obtained experimental results were supported by statistical analysis, showing significant correlations between the speed of ultrasonic waves and the content of water, protein, fat and sodium in the measured pork meat samples.

The propagation of ultrasonic waves through an investigated material depends on the physicochemical and mechanical properties of the medium. Therefore, changes in the material properties of the tested sample can be assessed by monitoring variations in the velocity of longitudinal ultrasonic waves.

The new ultrasonic method has the following advantages:

- 1) Possibility for real-time measurements;
- 2) Fully automated measurement process;
- 3) Fully computerized portable instrumentation;
- 4) High accuracy due to implementation of advanced signal processing procedures;
- 5) Fast measurement process;
- 6) Relatively low cost and simplicity.

However, it should be mentioned that an excessive content of air bubbles in the measured meat sample may negatively affect the accuracy of the measurement process with the new ultrasonic method.

1.6. Main Objectives of the Present Study

The main goals of the present study were as follows:

- 1) Develop a new ultrasonic method for real-time measurements of raw meat materials and products employing advanced instrumentation and signal processing procedures that have emerged in the 21st century during the digital Fourth Industrial Revolution.
- 2) Discriminate between HD and MSM meat samples from ultrasonic measurements of longitudinal velocity c .
- 3) Correlate the content of main ingredients in the pork meat samples such as protein, fat, sodium and ash with measurements of ultrasonic longitudinal velocity c .

2. Materials and Methods

2.1. Investigated Pork Meat Samples

The pork meat investigated in this paper was delivered by one of the leading Polish meat processing plants located in eastern Poland.

The raw meat under investigation was (1) pork (*Sus domesticus*) loin and pork neck meat obtained by hand deboning (HD) and (2) four kinds of mechanically separated meat (MSM) obtained by mechanical deboning of non-frozen pork loin bones and neck bones.

In order to obtain MSM meat processed by low-pressure and high-pressure techniques, we used two devices with different principles of operation and parameters:

- 1) Low-pressure Sepamatic Sepa 1200 belt separator device (Overath, Germany).

Meat separation from bones is achieved with this device by pressing the raw material between a flexible pressure belt and a perforated separator drum, which allows for tissue separation. Soft meat fractions are scraped from the surface and passed through the drum holes, creating a mechanically separated meat fraction, while the remaining bones are disposed of as waste material. The drum perforation diameter was 5.0 mm and the end product had a consistency similar to minced meat.

- 2) High-pressure LIMA RM 600 S separator (Quimper, France).

This device separates meat from bones by forcing the raw material (pre-ground bones) through a perforated cylindrical chamber using a rotating screw piston. The raw material is fed into the chamber, where it is displaced by a screw and gradually compressed. The generated pressure deforms soft tissues and forces them through chamber openings, while bones and tendons are removed as waste. The diameter of perforations in the separation chamber was 1.5 mm. The obtained final MSM meat had a paste-like consistency.

Before our investigation, HD pork meat and MSM samples were homogenized using two different devices, i.e., an Edesa PL-22-TU-T grinder (Czosnów, Poland) and a Keripar mixer (Troy, OH, USA).

In this paper, we have investigated the following six types of pork meat, obtained with different processing methods:

- A) Minced HD pork loin;
- B) Low-pressure MSM meat from pork loin bones;
- C) High-pressure MSM meat from pork loin bones;
- D) Minced HD pork neck;
- E) Low-pressure MSM meat from pork neck bones;
- F) High-pressure MSM meat from pork neck bones.

Figure 1a–c below show exemplary types of meat, such as HD and MSM meat, used in our investigation.

Prior to ultrasonic measurements, the six kinds of pork meat examined were stored in a refrigerator and kept at a temperature of 4 °C for 24 h. The meat samples were then equilibrated at 24 °C for about 4 h before measurements. All samples followed the same handling schedule.

2.2. Physicochemical Parameters of the Investigated Pork Meat Samples

We measured the following eight physicochemical parameters of the investigated pork meat using standard analytical chemistry methods [22]:

- a) Density

The density of meat samples (g/cm^3) was determined using a modified pycnometric method. The measuring flask had a volume of 100 cm^3 . The mass of meat samples was approximately 10 g [23].

- b) Calcium content

The calcium content (mg/kg) was measured following the standard, applying flame atomic absorption spectrometry (FAAS) and Z-2000 apparatus (Hitachi, Japan).

- c) Phosphorus content

The phosphorus content was assessed according to the standard. The evaluation 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 the total phosphorus content.



(a)



(b)



(c)

Figure 1. Different types of pork neck meat used in our investigation: (a) HD meat before mincing, (b) low-pressure MSM from pork loin bones, and (c) high-pressure MSM from pork loin bones.

d) Sodium content

The sodium content (mg/kg) was measured according to the standard using flame atomic absorption spectrometry (FAAS) with the Hitachi Z-2000 apparatus (Hitachi High-Technologies Corp., Tokyo, Japan).

e) Water content

The water content [%] was evaluated employing a drying method according to the [PN-ISO 1442:2000] standard [24]. The measured samples were dried at 103 °C for 30 min in a Thermoline Series 9000 oven (Spectral Lab Scientific Inc., Markham, ON, Canada).

f) Protein content

The protein content [%] was determined using the Kjeldahl method (Foss Tecator AB, Hoganas, Sweden) according to the standard.

g) Fat content

The fat content [%] was evaluated using the Soxhlet extraction technique following the [PN-ISO 1444:2000] standard [25].

h) Collagen content

The content of hydroxyproline [%] in the raw meat sample was determined according to PN-ISO 3496:2000 [26]. Finally, the collagen content was calculated following a formula according to Regulation (EU) No 1169/2011: collagen content [%] = hydroxyproline content [%] × 8.

2.3. Ultrasonic Measurement System

Figure 2 shows a diagram of the computerized ultrasonic system used in measurements of the speed of sound c of ultrasonic waves propagating in the investigated meat samples.

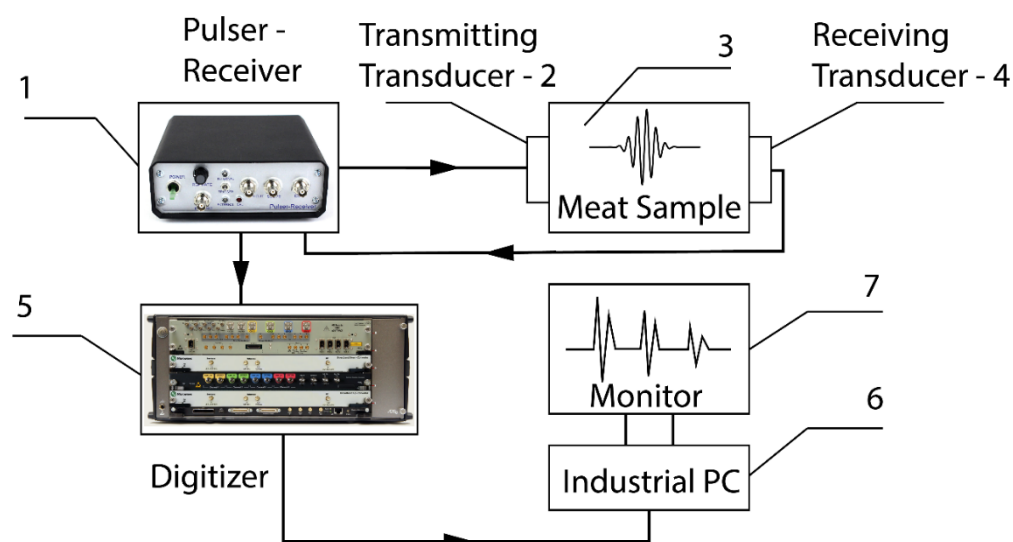


Figure 2. Schematic diagram of instrumentation of the new computerized ultrasonic system custom-designed and built by the authors at the Institute of Fundamental Technological Research of the Polish Academy of Sciences in Warsaw, Poland.

The new ultrasonic system, shown schematically in Figure 2, operates in the through-transmission (TT) mode. The transmitting piezoelectric transducer (2), driven by the pulser-receiver (1), generates a longitudinal ultrasonic wave that propagates through the investigated meat sample (3) and is finally detected by the receiving piezoelectric transducer (4). After amplification in the pulser-receiver (1), the analog electrical signal is converted into digital form in the analog-to-digital converter (digitizer) (5). The digitized signal is sent to the industrial computer (6) for further processing, storage and display.

The speed of sound c of ultrasonic waves propagating in the investigated meat samples is calculated from the following formula: $c = L/TOF$. Here, L is the difference between the distance traveled by the two selected ultrasonic impulses in the investigated meat sample

and the TOF (time of flight) is the corresponding time difference between the two selected ultrasonic impulses.

More information about the new ultrasonic system employed in measurements of the speed of sound c of ultrasonic waves propagating in the investigated meat samples can be found in Appendix A.

Figure 2 shows a sketch of a general connection diagram of the ultrasonic measurement system, where the ultrasonic transmitter/receiver and digitizer are shown as separate, stand-alone devices. In the actual measurement system, the TB1000 (Matec Instruments, Northborough, MA, USA) ultrasonic pulser-receiver board and the PDA1000 (Signatec Inc., New Port Beach, CA, USA) digitizer board were completely enclosed within an industrial PC computer, providing signal processing, signal visualization and data storage.

2.3.1. Transducer Unit

The schematic view of the transducer unit of the ultrasonic system is presented in Figure 3.

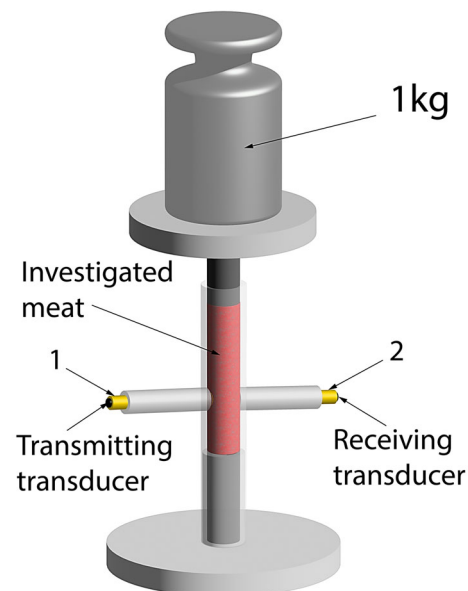


Figure 3. Schematic view of the transducer unit of the ultrasonic system. The measured meat sample is placed inside the vertical tube. A weight of 1 kg loads the investigated meat sample from the top in order to provide stabilization and homogenization of the sample. Symbols 1 and 2 correspond to two brass waveguides buffers with a length of $L_1 = 5$ cm.

The thickness of the investigated meat sample, in the direction defined by the line connecting the two ultrasonic transducers, was evaluated by auxiliary measurements of the TOF through distilled water filling the vertical tube in Figure 3, at a known temperature and pressure. Knowing the TOF through the water sample and the speed of sound c_w at a specific temperature and pressure (from tables), the thickness L of the investigated meat sample in the vertical tube was determined as $L = TOF \times c_w = 1.5201$ cm.

2.3.2. Determination of the Time of Flight (TOF) Between Two Selected Ultrasonic Impulses

Figure 4 presents an example of two consecutive ultrasonic impulses of longitudinal ultrasonic waves propagating in the transducer unit shown in Figure 3.

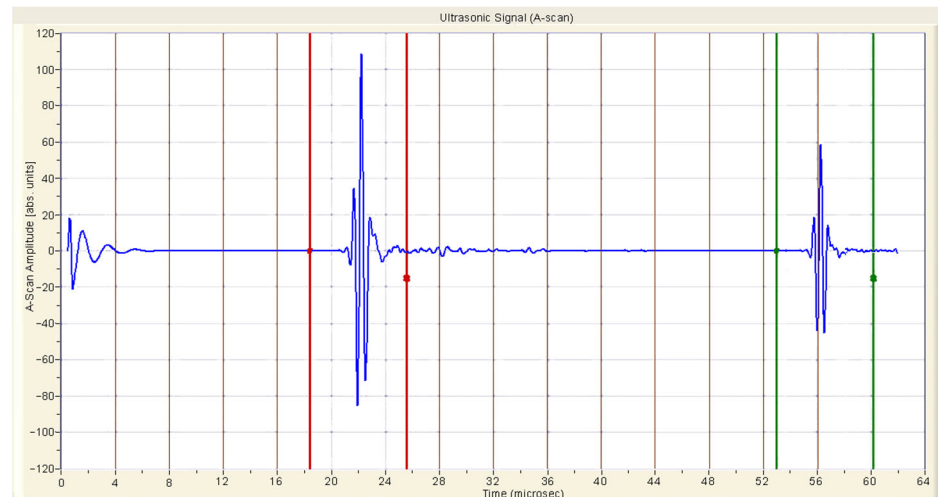


Figure 4. An example of two consecutive ultrasonic impulses of RF frequency ~ 5 MHz received by the piezoelectric transducer no. 2 at ~ 23 μs (between red cursors) and ~ 56 μs (between green cursors), respectively. The ultrasonic signal was digitized at a sampling rate of 62.5 MHz (every 16 ns). The total number of points in the ultrasonic signal is 4096.

To increase the signal-to-noise (S/N) ratio, the received ultrasonic signals were averaged 1024 times, prior to further processing.

The first received ultrasonic impulse (delimited by two red vertical cursors) is denoted hereafter as $f(t)$. Similarly, the second received ultrasonic impulse (delimited by two green vertical cursors) is denoted hereafter as $g(t)$.

The time difference (time of flight—TOF) between these two selected impulses shown in Figure 3 equals $TOF = 32.778$ μs and was determined via the cross-correlation method. In the theory of signal processing, the cross-correlation function $h(t)$, between two continuous functions of time t , $f(t)$ and $g(t)$, is defined as

$$h(t) = \int_{-\infty}^{+\infty} f(\tau)g(t + \tau)d\tau \quad (1)$$

It can be shown, using the Schwartz inequality of the functional analysis, that at time $t = \tau$, equaled to the time delay (TOF) between the two impulses, the cross-correlation function $h(t)$ in Equation (1) attains its maximum [27–29].

The velocity of sound c was measured at a temperature of 24 °C in 6 various types of pork meat, i.e., in (1) minced HD pork loin, (2) minced HD pork neck, (3) low-pressure MSM samples from pork loin bones, (4) low-pressure MSM samples from pork neck bones, (5) high-pressure MSM samples from pork loin bones and (6) high-pressure MSM samples from pork neck bones. In each of these 6 types of meat, the speed of sound c was measured 10 times for each of 10 randomly selected different samples of a given type of pork meat.

2.4. Statistical Analysis

In this study, we employed the STATISTICA 12.5 (TIBCO Software, Palo Alto, CA, USA) and OriginPro 2021 (OriginLab Corp., Northampton, MA, USA) software packages to perform statistical analyses of the obtained measurement results.

To specify significant differences between mean values of the speed of sound c in different types of meat, we first performed (1) the Shapiro–Wilk test to check normality of distribution and (2) Levene’s test to investigate homogeneity of variances. These studies were followed by (3) one-way ANOVA analysis with Scheffe and Tukey post hoc tests to identify which mean values of the speed of sound differ significantly.

To determine the correlations between the measured speed of sound c and the chemical composition of pork meat samples, the content of (1) protein, (2) fat, (3) calcium, (4) phosphorus, (5) sodium, (6) water, (7) collagen and (8) ash and (9) the density of the tested meat samples was measured, using standard methods of analytical chemistry.

To find the relationship between the speed of sound c and the content of (1) protein, (2) fat, (3) calcium, (4) phosphorus, (5) sodium, (6) water, (7) collagen and (8) ash, we used standard linear regression procedures. The results of calculations for the main ingredients of pork meat such as: the content of protein, fat, water, sodium and collagen are presented in Section 3.

Linear regression procedures were used also to determine the relationship between the speed of sound c and the content of (1) protein, (2) fat, (3) calcium, (4) phosphorus, (5) sodium, (6) water, (7) collagen and (8) ash. The results of calculations for the main ingredients of pork meat such as the content of protein, fat, water, sodium and collagen are presented in Section 3.

3. Experimental Results

3.1. Evaluation of Physicochemical Parameters of the Tested Pork Meat Samples

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

Table 1 (below) summarizes (1) the results of chemical measurements for basic physicochemical parameters of the tested meat samples, i.e., the content of calcium, phosphorus, sodium, water, protein, collagen, ash, fat and density, as well as (2) the results of the corresponding statistical analysis.

The measurements of physicochemical parameters (e.g., protein content) of each type of pork meat (e.g., HD meat and MSM meat) were performed five times with five randomly selected meat samples. A total number of $5 \times 9 \times 6 = 270$ measurements for randomly selected meat samples were performed. An individual random sample was measured only once.

The mean value and standard error were estimated for each physicochemical parameter (e.g., fat content) based on five consecutive measurements (carried out on five randomly selected samples of each kind of meat).

As shown in Table 1, the content of protein changes from 16% for MSM from pork neck bones and MSM from loin bone samples to 17% in pork neck and 21% in pork loin. On the other hand, the fat content varies from 4% in pork loin and 12% in pork neck to 16–18% for MSM from pork neck and loin bones samples, which confirms our expectations.

3.2. Measurements of the Speed of Sound c of Longitudinal Ultrasonic Waves

Measurements of the speed of sound c in the tested pork samples were performed using the measurement setup shown schematically in Figure 2. The measurements were conducted at a temperature of 24 °C. To determine the significant differences between sound velocity measurements in different types of meat, a one-way analysis of variance (ANOVA) and the Scheffe and Tukey post hoc tests were performed.

The results of ultrasonic measurements in the tested meat samples for the speed of sound c of longitudinal ultrasonic waves are presented in Figure 5. The capital letters below the horizontal axis indicate the following: A—pork tenderloin; B—low-pressure MSM from pork tenderloin bones; C—high-pressure MSM from pork tenderloin bones; D—pork neck; E—low-pressure MSM from pork neck bones; F—high-pressure MSM from pork neck bones. The mean values and standard errors of the measured speed of sound are given in square and round brackets.

Table 1. Measured chemical composition of the examined pork meat samples. Mean values and standard errors are given in square and round brackets, respectively. Mean values in the same column that do not share a superscript letter are significantly different ($p < 0.05$).

Type of Meat	Calcium [mg/kg]	Sodium [mg/kg]	Phosphorus P ₂ O ₅ [%]	Water [%]	Content of Protein [%]	Fat [%]	Collagen [%]	Ash [%]	Density [g/cm ³]
pork loin	8	296	0.47	73.7	21.2	3.8	1.01	1.0	1.116
	7	222	0.48	73.4	21.4	4.1	0.87	1.0	1.076
	8	225	0.47	74.4	21.6	3.0	0.97	1.0	1.055
	8	275	0.53	70.3	21.1	5.4	0.99	1.0	1.051
	6	210	0.40	72.8	21.0	4.5	1.05	1.0	1.061
mean	[7.4] ^A ± (0.4)	[245.6] ^A ± (16.8)	[0.47] ^{A,B} ± (0.02)	[72.9] ^A ± (0.7)	[21.3] ^A ± (0.1)	[4.2] ^A ± (0.4)	[0.98] ^{A,B} ± (0.03)	[1.0] ^A ± (0.1)	[1.072] ± (0.012)
low-pressure MSM from pork loin bones	378	774	0.57	64.5	15.9	17.0	0.60	1.2	1.058
	332	852	0.50	64.7	16.2	17.4	0.66	1.2	1.059
	390	857	0.49	64.9	16.3	16.6	0.59	1.2	1.059
	310	980	0.51	63.2	16.1	18.0	0.68	1.2	1.058
	430	901	0.43	65.0	16.5	16.5	0.58	1.2	1.058
mean	[368.0] ^B ± (21.3)	[872.8] ^B ± (33.7)	[0.50] ^{B,C} ± (0.02)	[64.5] ^{B,C} ± (0.3)	[16.2] ^B ± (0.1)	[17.1] ^B ± (0.3)	[0.62] ^C ± (0.02)	[1.2] ^B ± (0.1)	[1.058] ± (0.001)
high-pressure MSM from pork loin bones	379	824	0.45	65.4	15.9	16.6	0.69	1.2	1.106
	364	860	0.50	65.6	16.2	16.2	0.65	1.2	1.083
	292	775	0.48	65.7	15.9	15.9	0.59	1.1	1.007
	260	899	0.41	65.0	16.0	16.5	0.68	1.2	1.025
	399	880	0.47	66.0	16.4	16.3	0.60	1.2	0.987
mean	[338.8] ^B ± (26.7)	[847.6] ^B ± (22.0)	[0.46] ^{A,B} ± (0.02)	[65.5] ^B ± (0.2)	[16.1] ^B ± (0.1)	[16.3] ^B ± (0.1)	[0.64] ^C ± (0.02)	[1.2] ^B ± (0.1)	[1.041] ± (0.023)
pork neck	11	306	0.43	69.0	17.6	11.0	1.10	1.0	1.080
	8	276	0.43	68.4	18.1	12.1	1.06	0.9	1.096
	8	295	0.42	69.1	18.5	12.6	0.99	0.9	1.036
	10	340	0.41	69.1	17.5	13.5	1.00	0.9	1.036
	9	301	0.40	68.0	17.1	13.0	1.01	0.9	1.022
mean	[9.2] ^A ± (0.6)	[303.6] ^A ± (10.4)	[0.42] ^A ± (0.01)	[68.7] ^D ± (0.2)	[17.8] ^C ± (0.2)	[12.4] ^C ± (0.4)	[1.03] ^B ± (0.02)	[0.9] ^C ± (0.1)	[1.054] ± (0.014)

Table 1. Cont.

Type of Meat	Calcium [mg/kg]	Sodium [mg/kg]	Phosphorus P ₂ O ₅ [%]	Water [%]	Content of Protein [%]	Fat [%]	Collagen [%]	Ash [%]	Density [g/cm ³]
low-pressure MSM from pork neck bones	361	654	0.53	63.3	16.1	17.6	0.46	1.2	1.060
	384	576	0.59	63.2	16.1	18.4	0.43	1.2	1.020
	356	589	0.54	63.5	16.0	18.0	0.47	1.2	0.992
	330	550	0.61	62.8	15.8	19.0	0.48	1.2	0.998
	389	591	0.53	63.0	16.3	19.5	0.40	1.2	0.972
mean	[364.0] ^B ± (10.6)	[592.0] ^C ± (17.1)	[0.56] ^C ± (0.02)	[63.2] ^C ± (0.1)	[16.1] ^B ± (0.1)	[18.5] ^D ± (0.3)	[0.45] ^D ± (0.01)	[1.2] ^B ± (0.1)	[1.008] ± (0.015)
high-pressure MSM from pork neck bones	240	664	0.50	65.5	16.2	16.0	0.90	1.1	0.974
	248	605	0.48	65.4	16.4	16.8	0.84	1.1	0.957
	282	627	0.47	65.2	16.4	16.9	0.93	1.1	0.978
	255	661	0.44	66.4	16.1	15.8	0.90	1.1	0.977
	286	601	0.51	65.9	15.7	16.3	0.89	1.1	0.980
mean ± standard error	[262.2] ^C ± (9.2)	[631.6] ^C ± (13.4)	[0.48] ^{A,B} ± (0.01)	[65.7] ^B ± (0.2)	[16.2] ^B ± (0.1)	[16.4] ^B ± (0.2)	[0.89] ^A ± (0.01)	[1.1] ^D ± (0.1)	[0.973] ± (0.004)

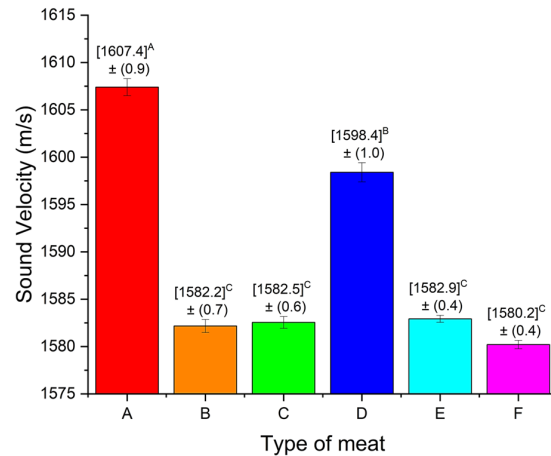


Figure 5. Results of ultrasonic measurements of speed of sound c of longitudinal ultrasound waves performed in the examined meat samples. RF frequency of ultrasonic waves $f = 5$ MHz. On top of each column are mean values \pm standard errors. Mean values marked with different superscript letters are statistically different ($p < 0.05$).

The results of the statistical analysis (one-way ANOVA + Scheffe and Tukey post hoc tests) show that the speed of sound c and its mean value obtained for different types of meat, i.e., for HD pork loin and pork neck and MSM meat, are significantly different ($p < 0.001$).

In practice, the speed of sound can be measured with an uncertainty of ± 1.5 m/s. As shown in Figure 5, the differences in the ultrasonic velocity between the HD and MSM meat samples range from 16 m/s (between meat types marked with the letters D and E in Figure 5) to 25 m/s (between meat types marked with the letters A and B in Figure 5). This is an important result, demonstrating that measurements of the speed of sound can be effectively used to distinguish between different types of meat samples, e.g., between HD meat and MSM meat.

A number of different, randomly selected meat samples were used in ultrasonic measurements of the speed of sound ($6 \times 10 = 60$). Each meat sample was investigated only once.

3.3. Density

The results of the density measurements of various types of meat are presented in Figure 6.

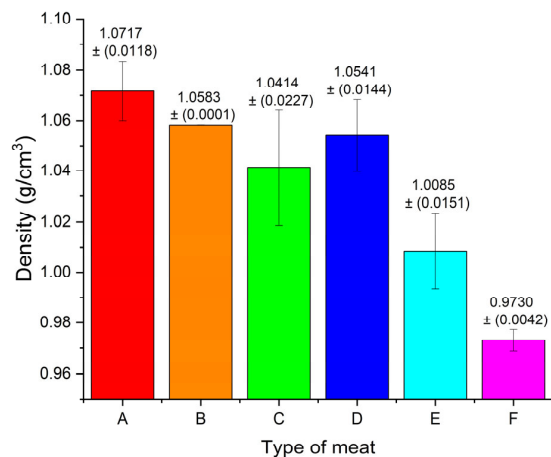


Figure 6. Density ρ of the tested meat samples measured for different types of pork meat. Mean values and the corresponding \pm standard errors.

The symbol A is assigned to minced HD pork loin, B to low-pressure MSM from pork loin bones, C to high-pressure MSM from pork loin bones, D to minced HD pork neck, E to low-pressure MSM from pork neck bones and F to high-pressure MSM from pork neck bones.

For hand-deboned meat and the meat obtained by mechanical methods, the measured average density values do not differ significantly; see Figure 6. Therefore, it is not recommended to discriminate various types of pork meat from their density measurements alone.

3.4. Linear Regression Curves

We also derived linear regression equations for the highest significant cross-correlations, i.e., for the correlations of the speed of sound with the content of (1) protein, (2) fat, (3) water, (4) sodium and (5) collagen.

Table 2 presents the resulting statistical parameters for the most important constituents, i.e., Pearson’s correlation coefficients *r*, *p*-values and linear regression equations.

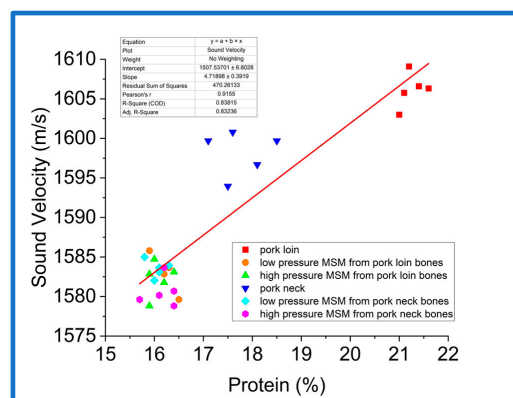
Table 2. Pearson’s coefficients *r*, *p*-values and linear regression equations for the speed of sound *c* as a function of protein, fat, water and sodium content.

Chemical Properties of Meat	Linear Regression Equation	<i>p</i> -Value	Pearson’s Correlation Coefficient <i>r</i>
Protein content	$y = 4.7200x + 1507.54$	1.37×10^{-12}	0.9155
Fat content	$y = -1.8496x + 1615.11$	1.48×10^{-12}	-0.9150
Water content	$y = 2.6528x + 1411.89$	3.85×10^{-11}	0.8916
Sodium content	$y = -0.0343x + 1608.95$	2.19×10^{-11}	-0.8528

y = speed of sound, *x* = content of protein, fat, water or sodium.

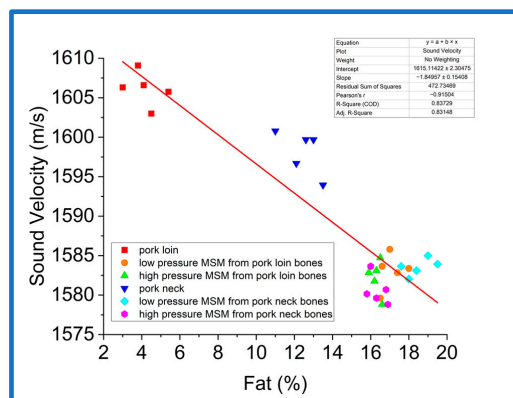
According to the statistical analysis of the experimental data presented in Table 1 and Figure 5, we have plotted in Figure 7a–e the linear regression curves for the measured speed of sound *c* as a function of the content of the following chemical ingredients: protein, fat, water, sodium and collagen.

The results of the statistical analysis shown in Table 2 and in Figure 7a–e demonstrate that measurements of the speed of sound *c* can be effectively used in the assessment of the chemical composition of pork meat.

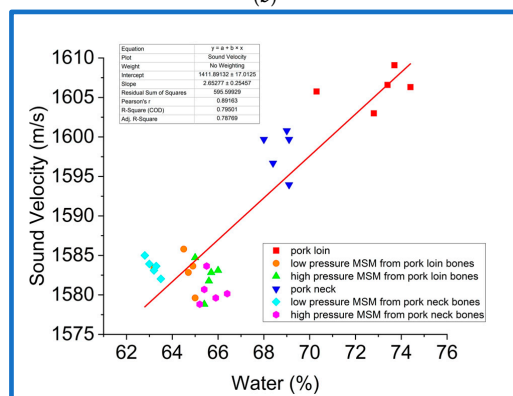


(a)

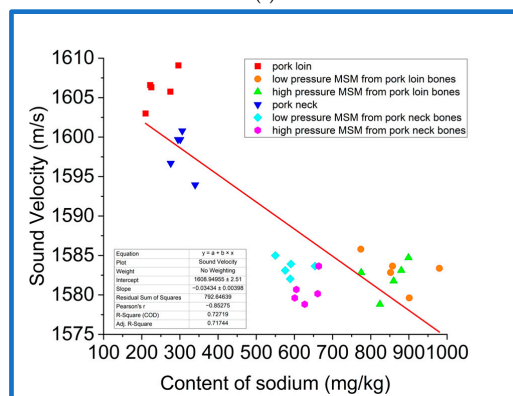
Figure 7. Cont.



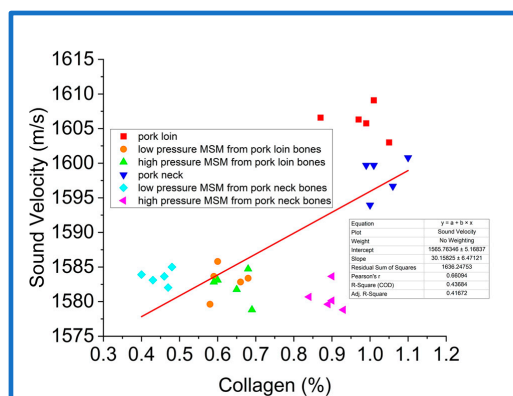
(b)



(c)



(d)



(e)

Figure 7. Linear regression curves relating measured speed of sound c with the chemical composition of the investigated pork meat, including the content of (a) protein, (b) fat, (c) water, (d) sodium and (e) collagen.

4. Discussion

4.1. Elastic Properties of Meat (Young's Modulus E)

The elastic properties of meat, such as Young's modulus E , depend on its composition and microstructure (e.g., the strength of the myofibrillar proteins, characteristics of the quasicrystalline collagen structure [30], etc.). Young's modulus is affected by changes in the mechanical properties of meat, such as a decrease in its elastic modulus, which can result from the disruption of the myofibrillar protein and fragmentation of collagen macromolecules.

In general, Young's modulus of meat is a measure of its stiffness and elasticity and also quantifies how meat deforms under tensile and compressive forces. Young's modulus E of MSM meat also depends on factors such as bone marrow content, connective tissue content, texture, direction and homogeneity. Each kind of meat (HD and MSM) has its own unique values of density ρ and E . These two material parameters allow for the determination of the specific value of the speed of sound c in all kinds of meat [31]. Young's modulus is considered to be one of the most important indicators of food texture [32], hardness, stiffness, toughness, firmness and marbling score [17].

The speed of sound is described by the formula $c = \sqrt{c_{11}/\rho}$, where c_{11} is the elastic coefficient proportional to Young's modulus E , and ρ represents the density of meat.

Figure 5 shows that the ultrasonic velocity c in the measured meat samples changes from 1580.2 m/s (for high-pressure MSM samples from pork neck bones) to 1607.4 m/s for meat samples from HD pork loin. Thus, differences in the composition of meat affect the mechanical parameters of meat such as its density ρ and Young's modulus E . Therefore (knowing Poisson's ratio ν), measurements of the speed of sound c can be a basis for the determination of the density ρ and Young's modulus E of meat. In fact, knowledge of Young's modulus E can be very important in the design and optimization of technological processes in the meat industry.

From Figure 5, we can also deduce that the HD meat samples (from pork loin and pork neck) are stiffer. In other words, they have a higher Young's modulus E than the high- and low-pressure MSM meat samples. Microscopically, this can be explained by the fact that fibers in MSM meat samples are destroyed during the applied technological process [17,33].

In summary, Young's modulus plays a crucial role in the evaluation of the following properties of meat:

1. The texture (chewiness, gumminess, etc.) and tenderness of meat.

A higher Young's modulus means that the meat is stiffer and causes greater resistance in the chewing process. Thus, Young's modulus is a direct indicator of meat tenderness

2. The anisotropy of meat, which is affected by its structural changes, such as muscle fiber orientation, etc.

3. The quality of structural proteins.

Young's modulus determines the condition of collagen and myofibrillar proteins. Changes in Young's modulus of meat during thermal treatment reflect the process of protein denaturation and fiber contraction.

4. Hardness and chewiness of meat.

A higher Young's modulus corresponds to stiffer meat and consequently to higher chewing force.

To conclude, Young's modulus has a decisive impact on most of the important mechanical and functional parameters of meat (e.g., texture, hardness, elasticity, tenderness). Therefore, Young's modulus is a crucial technological parameter playing a pivotal role in the design of technological processes in the modern meat industry [17].

4.2. Discrimination Between HD Meat and MSM Meat

HD meat and MSM meat exhibit many differences. For example, HD meat has a well-defined texture. Its muscular structure and fibers retain their integrity. By contrast, in MSM meat, the fiber structure is damaged and the original structure of the muscular tissue is modified. In addition, MSM meat contains some bone remnants, bone marrow, connective tissues and cartilage. The texture of low-pressure MSM meat resembles minced meat, while the texture of high-pressure MSM meat resembles dough. The velocity c of longitudinal ultrasonic waves in HD meat is higher than that of MSM meat.

Conventional methods for testing the properties of MSM meat, such as chemometrics, NIR spectroscopy and microscopy, use sophisticated and complicated equipment and are labor-intensive. Their use requires the employment of highly qualified personnel and lengthy sample preparation. By contrast, the ultrasound testing of the physicochemical properties of MSM meat is rapid, direct, straightforward and can be easily automated.

Figure 7a–e show the regression curves relating the velocity c of longitudinal ultrasonic waves with the content of the main ingredients in the investigated meat samples.

Since the coefficient of determination R^2 is relatively high (e.g., for protein content $R^2 = 0.83$), the regression curves accurately approximate the obtained measurement results.

These figures also indicate that the results obtained for the hand-deboned (HD) meat and mechanically separated meat (MSM) are clearly separated into two distinctive groups.

This fact enables the distinction between HD meat and MSM meat by measuring the velocity c of ultrasonic waves in the investigated meat samples.

The large difference in velocity (27.2 m/s) between the maximum and minimum measured speed of sound is 1.7% (see Figure 5), which allows for discrimination between various types of pork meat (i.e., HD meat from MSM meat; see Figure 7a–e). This feature of ultrasonic velocity measurements could be the basis for the new analytical research technique proposed by the authors.

4.3. Speed of Sound as a Function of Physicochemical Properties of Pork Meat

The statistical analysis performed in this paper displays a high degree of correlation between the measured speed of sound c and the content of the main constituents in the tested pork meat samples, such as protein, fat and water.

Figure 7a shows that a higher content of proteins leads to a higher speed of sound c . In fact, a higher content of protein increases the Young's modulus E of the measured meat samples. Simultaneously, the density ρ of the measured meat samples increases slightly. As a result, we observe an overall increase in the speed of sound c with an increasing content of proteins.

By contrast, Figure 7b displays that, with an increasing content of fat, the speed of sound c decreases. This fact can be explained as follows. The higher content of fat decreases both the density ρ and the Young's modulus E of the tested meat samples. Simultaneously, the drop in E is larger than that in ρ . As a result, we observe an overall decrease in the speed of sound c in the tested meat samples.

Figure 7e demonstrates that the speed of sound c rises with the increasing content of collagen. This phenomenon can be explained as follows. Since the content of collagen (connective tissue) and its degree of cross-linking in the intracellular matrix are key factors influencing meat stiffness, the higher the collagen content, the greater the stiffness of the meat. Consequently, the greater the speed of sound c ; see Figure 7e [17].

The content of collagen also affects the meat tenderness and toughness. These parameters are important quality characteristics that determine the technological quality of meat products. Meat tenderness has been shown to be strongly correlated with the number of collagen crosslinks [30].

4.4. Advancement in Existing Ultrasonic Techniques for Investigation of Meat Properties

As a matter of fact, the existing ultrasonic measurement systems used currently in the food industry do not reflect recent advances in signal processing, computerized equipment, software development environment, etc. Therefore, they exhibit a number of disadvantages, such as

- 1) A lack of portability;
- 2) Slow operation;
- 3) Limited accuracy.

The existing ultrasonic systems are composed of several separate (stand-alone) devices, such as an ultrasonic pulser-receiver, a digital oscilloscope and a controlling computer. Since the communication between these stand-alone devices is normally slow, this leads to a long measurement time and difficulties in system optimization.

The signal processing procedures used in the existing systems are of limited accuracy since they are prone to noise and use limited information about the ultrasonic signal.

For example, the ultrasonic measurement system described in reference [34] consists of three separate components: a generator, an oscilloscope and a PC computer. Determination of the time of flight is of limited accuracy since it is performed using the Energy Threshold Method, based on one point estimation. In addition, the measurement system is controlled by software written in Lab-View environment, which significantly lengthens data transfer, signal processing and the overall measurement process.

Similarly, the ultrasonic measurement system described in reference [17] consists of several separate devices (i.e., a generator, oscilloscope and PC computer), which takes up a lot of space and slows down the operation of the measurement system, making it difficult to use on-line.

By contrast, the new ultrasonic measurement system developed by the authors is entirely enclosed in one industrial computer, which includes the ultrasonic board, digitizer board and switching board. The industrial computer provides a stable operational environment, devoid of noise and interferences. The new ultrasonic measurement system developed by the authors also provides full portability.

The ultrasonic system developed by the authors for investigating meat samples emerges from recent achievements in hardware, software development and signal processing procedures, which may be attributed to the digital Fourth Industrial Revolution.

Simultaneously, the new ultrasonic measurement system developed by the authors implements modern signal processing procedures, such as cross-correlation, Fast Fourier Transform (FFT), Hilbert transform, and cubic-spline interpolation. As a result, we achieved a 1 ps (1×10^{-12} s) resolution in the time-of-flight (TOF) measurements. By contrast, current ultrasonic phase velocity (time of flight) measurement systems used in the food industry have a resolution of only 1 ns (1×10^{-9} s); see [31].

Moreover, our system is characterized by very fast operation and high accuracy due to noise reduction. Since the software of the new ultrasonic measurement system was developed in the Microsoft Visual Studio environment, the new device operates under the Microsoft Windows operating system, providing a user-friendly graphical user interface (GUI), easy network connectivity and huge data storage capability (one drive).

In fact, the new ultrasonic measurement system developed by the authors enables measurements of ultrasonic velocity in meat samples with an accuracy of ~ 0.25 m/s. By contrast, the accuracy of existing ultrasonic systems was ~ 1.5 m/s.

More information about the ultrasonic measurement system developed by the authors for investigation of meat properties is provided in Appendix A.

5. Conclusions

The results of the research and analyses performed in this study enable us to draw the following conclusions:

1. The research hypothesis, presented in Section 1.2 of this paper, has been confirmed by the results of the measurements and analysis using the newly developed ultrasonic method and the designed instrumentation.
2. From ultrasonic measurements of velocity c , it is possible to discriminate between hand-deboned (HD) and mechanically separated (MSM) meat samples as well as to quantify the content of main ingredients in the measured meat samples.
3. The developed new ultrasonic method and digital instrumentation allow for fully automated, real-time measurements in the actual industrial environment.
4. The development of the new method and instrumentation was possible due to the employment of tools enabled by the digital Fourth Industrial Revolution, such as fully computerized instrumentation, sophisticated signal processing procedures, network connectivity, graphical user interface (GUI), huge data storage and archiving capacity.
5. A statistically significant correlation was found between the speed of sound c and the main ingredients of pork meat, such as (a) protein content ($r = 0.9155$ and $p = 1.37 \times 10^{-12}$), (b) fat content ($r = -0.9150$ and $p = 1.485 \times 10^{-12}$), (c) water content ($r = 0.89$ and $p = 3.85 \times 10^{-11}$) and (d) sodium content ($r = -0.8528$, $p = 2.19 \times 10^{-11}$).
6. The measured average density values do not exhibit significant statistical differences. Therefore, density measurements cannot be used to discriminate various types of pork meat.

The newly developed ultrasonic method can also be used to assess the quality of meat products, which could have a positive impact on the health status of consumers. This is in accordance with contemporary consumers' expectations, because of their growing awareness and concern about health and nutrition.

Obviously, the investigation of the physicochemical parameters of meat requires further research. For example, in order to widen the spectrum of ultrasonic investigations of meat, it is recommended in future research to examine the relationships between the mechanical properties of meat (e.g., Young's modulus E , Poisson's ratio ν and shear modulus G) and the physicochemical properties of meat. We intend to address this problem in our future research.

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Appendix A

The characteristics of the new ultrasonic system developed by the authors for investigation of meat properties are presented here.

Appendix A.1. Instrumentation of the New Ultrasonic System

The new ultrasonic system developed by the authors is fully computerized, with all electronic devices such as digitizers, ultrasonic boards and switching boards entirely enclosed within a portable industrial computer, which provides a stable working environment, network connectivity and high volume storage capacity.

The ultrasonic transducers (transmitting and receiving) were connected to the high-power TB1000 ultrasonic pulser-receiver board working in the through-transmission (TT) mode. The amplitude of electric impulses exciting the generating ultrasonic transducer was ~ 300 V (peak-to-peak) with a maximum power of 0.5 kW per impulse.

The analog ultrasonic signal provided by the receiving ultrasonic transducer (see Figure 4) was immediately digitized with the fast 8-bit PDA1000 digitizer board, providing a maximum sampling rate 1 GHz and analogue bandwidth of DC-500 MHz. Since the RF frequency of the ultrasonic impulses was only ~ 5 MHz, the sampling rate of the digitizer was reduced to 62.5 MHz (one sample every 16 ns), a frequency well above the required Nyquist frequency of 10 MHz.

Since the maximum pulse repetition rate of the ultrasonic board is 5 kHz, one thousand (1024) ultrasonic signals can be generated, received, digitized (1024 discrete samples) and processed within a real-time duration less than 1 s.

Appendix A.2. Signal Processing Procedures Employed in the New Ultrasonic System

All signal processing procedures employed in the new developed ultrasonic system were implemented digitally:

1. Signal averaging;
2. Cross-correlation;
3. Fast Fourier Transform (FFT);
4. Hilbert transform;
5. Cubic-spline interpolation.

To reduce the noise in the received ultrasonic signals, 1024 digitized consecutive ultrasonic signals were averaged, providing $\sqrt{1024} = 32$ improvement in the signal-to-noise (SN) ratio of the final signal, ready for further processing.

The averaged ultrasonic signal was next used in the calculation of the cross-correlation function $h(t)$ (see Equation (1)) in order to determine the time of flight (TOF) between two selected ultrasonic impulses (see Figure 4). The determination of TOF using the cross-correlation function is a very robust and precise method to calculate the TOF between two selected ultrasonic impulses, since the cross-correlation function employs all digitized points in the considered ultrasonic impulses, not just one, such as the maximum value or zero crossing points. Moreover, to calculate the cross-correlation function $h(t)$, we employed the Fast Fourier Transform (FFT) technique, having the following equation: $h(t) = FFT^{-1}[FFTf(t) \cdot FFTg(\tau)]$. The sought TOF corresponds to the maximum of the determined cross-correlation function $h(t)$.

The resolution of the TOF determined from the cross-correlation function $h(t)$ applied to digitized signals $f(t)$, $g(t)$ is not better than the sampling rate of the digitizer used, which, in our case, was 16 ns. A significant improvement in the resolution of the sought TOF was achieved by employing the cubic-spline interpolation in the vicinity of the cross-correlation maximum. In our case, we were able to increase the time resolution of the TOF to ~ 1 ps.

The Hilbert transform was used to calculate the envelope of ultrasonic signals, enabling for a more user-friendly graphical user interface (GUI) of the system.

Appendix A.3. Software Development Tools Used in Development of the New Ultrasonic System

The software controlling the new ultrasonic system was created using the following software development tools:

1. Microsoft Visual Studio (version 8.0) software development environment;
2. National Instruments Measurements Studio (version 8.1.2);
3. Microsoft Foundation Class (MFC) Library (version 8.0);
4. Microsoft Object-Oriented C++ programming (version 8.0) language.

Microsoft Visual Studio provides an integrated development environment (IDE) enabling integration of a large number of different software tools in one development environment, such as the National Instrument Measurements Studio (graphical user interface); Microsoft Foundation Class (MFC) Library, providing an object-oriented layer over the internal Windows system; and a few high-level programming languages, such as C++, Java, and Basic.

Since the industrial PC computer in the developed ultrasonic system works under the Windows operating system, the software controlling the system was written using the Microsoft Visual C++ (version 8.0) object-oriented programming language. Visual C++ is a general-purpose programming language, which is not limited to hardware control and instrumentation, in contrast to some specialized software packages, such as LabVIEW (version 2014). In principle, using the Visual C++ programming language, we can implement any conceivable software functionality including website development, data base creation, a graphical user interface (GUI) and scientific calculations. Visual C++ compiles the source code into a native executable file without invoking any intermediate virtual machine layer. Another advantage of the Visual C++ is a very high speed of the generated software, comparable to that achieved with the older C language.

Since a programmer has full control over the resulting code, the programs written in Visual C++ can operate much faster (10–100 times) than those written, for example, with the LabVIEW package. Moreover, the resulting executable files are very slim (a few MB in size) and do not carry redundant overheads even for very complicated programs such as those including simultaneous hardware control, signal processing procedures, visual presentation, data saving and other digital functionalities.

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