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ORIGINAL PAPER

The influence of ultrasound treatment on the composition and food properties in traditional organic pork-beef meat products*

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Abstract

One of the core assumptions of traditional food production is to manufacture natural products following recipes passed from generation to generation, containing as few food additives, contaminants and residues of plant protection agents as possible. In consumers' opinion, traditional food is characterized by unique sensory properties and high quality. Producers of organic meat products made according to traditional methods try to satisfy the buyer's needs; therefore, they use new technologies to guarantee the expected quality of these products. One of the new preservation methods is ultrasound treatment. The aim of this study was to investigate differences in the composition of traditional organic meat products, such as salami and sausages, treated with ultrasounds at various frequencies. The experimental material consisted of samples of salami and sausages with various beef additions. The results showed that the products ultrasonicated at a higher frequency contained significantly more protein and pigments. It was found that sonication reduced nitrate and nitrite content in products, which significantly improved the health quality of the assessed products. No significant effect of the different ultrasound frequencies on the content of chlorinated hydrocarbons was found; however, the content of these compounds was lower than the threshold levels contained in the regulation of the European Commission.

Keywords: ultrasound treatment, organic meat products, meat composition, meat quality

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INTRODUCTION

Due to growing consumer demands, the food market is developing in terms of both novel food products and innovative technologies (Antunes--Rohling et al. 2018, Ambadgatti et al. 2020, Bhargava et al. 2021). Consumer interest in these products stems mainly from the recent promotion of a healthy lifestyle, involving changes in eating habits and increased physical activity. Nutritional deficiencies and excessive food consumption may lead to the development of chronic non-communicable diseases, such as obesity, diabetes, hypertension, or cancer. For this reason, consumers search the market for natural products offering a high nutritional value. To meet their demands, the food industry has introduced, *inter alia*, gentle processing technologies of raw materials that help preserve their natural features (Caleb et al. 2013, Smoluk-Sikorska 2022). Therefore, the food preservation methods are enriched with innovative technologies, including microwave, ultraviolet, high hydrostatic pressure, and ultrasound treatments (Dang et al. 2018, Chávez-Martínez et al. 2020).

Acoustic waves with frequencies above 20 kHz (kilohertz), i.e., ultrasounds (ultrasound sonication), are increasingly used in the food industry, among others, for grinding the particles of the dispersed phase of emulsions and suspensions, for example in dairy processing and in food processing and preservation (Kapturowska et al. 2011, Kang et al. 2017, Gallo et al. 2018). Food technology uses mainly ultrasonic waves of high power and low frequency (from 20 to 100 kHz) to induce the cavitation effect, which affects the biochemical and physicochemical properties of food products, in particular it causes the disintegration of their cellular structures (Konopacka et al. 2015). The ultrasounds are sound waves having the character of a mechanical wave, with frequencies between 16 kHz and 1 gHz (gigahertz), i.e., beyond the human hearing range (Maksymiec et al. 2016). Ultrasounds are widely used in many scientific specialties and technical fields. Recently, they have also been used in the food industry, especially in combination with traditional techniques (Konopacka et al. 2015). In the food industry, many researchers distinguish their two functions: testing food products and direct aid at the production and processing stage (Kang et al. 2017, Ambadgatti et al. 2020). Moreover, supersonic waves are used to effectively destroy microorganisms during the preservation of food products (Krzysztofik et al. 2015).

Microorganisms are especially responsible for spoilage and poisoning of food; therefore, food preservation is aimed at their eradication. The currently used methods of food preservation are based on both growth inhibition and complete inactivation of microorganisms. The safest method is heat treatment, but it causes unwanted effects regarding the sensory, nutritional, and functional values of food products. These drawbacks (as well as consumer demands for foods that are as closest to the fresh food as possible) have promoted the development of alternative methods, such as the use of ultrasounds. Sound inactivation of bacterial cells has been proven and described. Most authors agree that the cavitation reaction is responsible for the lethal effects of ultrasound to cells (Kapturowska et al. 2011, Miano et al. 2016, Ojha et al. 2017). Sonication is therefore an alternative technology to pasteurization and sterilization, allowing for a significant increase in the microbiological purity of the processed raw material while having a little impact on the nutritional value and quality of the final product (Zhang et al. 2008, Maksymiec et al. 2016). Scientific research also confirms the positive effect of sonication on the technological properties of meat, such as increasing tenderness and shortening the cooking time (Miano et al. 2016).

Salami and dried sausage are traditional meat products manufactured in many countries. Their shelf life is long due to the long drying and maturation processes. Traditional food products include only those of the highest quality, the uniqueness of which is due to the traditional production method (successfully used for over 25 years). Moreover, they contribute to the identity of the local community, being an element of the cultural heritage of the region of origin. Production methods and product features do not have to be inevitably tied to a specific place (Rudawska 2014, Smoluk-Sikorska 2022).

Growing consumer awareness of healthy nutrition and healthy food prompts producers to constantly improve the quality of their products. Currently, there is an increasing interest in high-quality health-promoting food products, containing the desired nutrients, with as few additives (including preservatives) as possible, convenient and easy to store. Of great importance are also their functional value and their nutritional value preservation during processing. This motivates food producers to continuous technological progress and to searching for novel technologies. Therefore, the aim of this study was to determine whether preservation with ultrasounds of various frequencies affects the quality of traditional organic meat products, based on the evaluation of their selected quality parameters. The study also attempted to evaluate possible differences in the composition of traditional organic meat products, such as salami and dried sausage, treated with ultrasound at different frequencies.

MATERIALS AND METHODS

Materials

The experimental material included traditional organic pork-beef meat products (salami and dried sausage) with different beef contents, treated with ultrasounds of various frequencies:

– 40% and 60% beef content, samples treated with ultrasounds having the following frequencies: control sample (0 kHz), 40 kHz, 60 kHz, and 100 kHz for 15 min. Samples were taken from five different production batches and treated with ultrasounds using Biobase UC-ST series.

Product samples were obtained from a local cured meat producer certified for organic production. Samples were homogenized and treatments were performed in three replications for each sample.

Analytical methods

Fat and fatty acids analysis

A 5-g portion of comminuted meat was mixed with 30 mL of methanol, then 60 mL of chloroform were added, and the mixture was again mixed for 2 minutes. Afterward, it was filtered, and the residue was transferred back to the flask. Then, 20 mL of methanol and 40 mL of chloroform were added to the residue, which was again mixed for 3 min, and filtrated. The filtrated residue was rinsed with chloroform and methanol. Then, 0.88% sodium chloride was added to the filtrate in the amount of ¼ of filtrate volume, the mixture was stirred, and left for 12 h for phase separation. Next, the chloroform layer was transferred to a distillation flask and distilled in a rotary evaporator at a temp. of 50°C (Folch et al 1957, Peisker 1964). The fatty acid profile was determined under conditions of the chromatographic separation. The extracted lipids were methylated at a temp. of 80°C for 2 h to obtain methyl esters. Then, 2 mL of a methylating mixture (100 mL of chloroform, 100 mL of methanol, and 1 mL of sulfuric acid) were added to 40-60 mg of lipids. The fatty acid methyl esters were separated using a 7890A gas chromatograph (Agilent Technologies) with a flame-ionization detector. The separation settings: capillary column (30 m x 0.32 mm); stationary phase: Supelcowax 10; film thickness: 0.25 μm; temp.: detector 250°C, injector 230°C, column 190°C; carrier has: helium; flow rate: 1.5 ml min^{-1}, injection split ratio: 50:1.

Protein content

A weighted portion of the sample (0.4-0.5 g) was transferred to a analytical flask, to which 10 mL of sulfuric (VI) acid, ca. 4 g of potassium sulfate (VI), and ca. 4 g of copper (II) sulfate (VI) were added. The flask was then placed under the fume hood in the incineration set and gently heated. Then, ammonia was distilled off and titrated with 0.1M hydrochloric acid. Based on the amount of acid used for titration, nitrogen content was calculated and multiplied by the protein conversion factor (6.25) to obtain the percentage protein content (ISO 2023).

Total pigment content

A meat sample (5 g) was weighed into a 50 mL measuring flask, to which ca. 20 mL of the extraction mixture (acetone: concentrated HCl: distilled water, 80:2:18) were added. The solution was stirred, filled up with the mixture, and left for 2 h in a dark and cold room. Afterward, it was centrifuged at 5,000 rpm for 10 min and filtered. The solution was filtered, then its

4 mL sample was collected to a cuvette, and the absorbance was measured at wavelengths of 512 and 640 mm. The extraction solution served as the blank sample. The total content of pigments in the meat products analyzed was determined based on the absorbance values obtained (Kędzior 2003).

Extraction and determination of organochlorine (OC) pesticides

The meat samples were determined for the contents of γ-HCH (gamma isomer of hexachlorocyclohexane), DDT (dichlorodiphenyltrichloroethane) and its metabolites (DDD – dichlorodiphenyldichloroethane and DDE – dichloroenyltrichloroethane). The experimental material was finely comminuted. Then, 5 g portions of each type of meat material were weighed, homogenized in 200 mL of *n*-hexane, filtered, and rinsed with 100 mL of *n*-hexane. The extract was flushed with a saturated sodium chloride solution. The concentrated hexane layer was treated with sulfuric acid, separated, and passed through an activated Florisil column (Fluka) – Wieczorek et al. 2010. The separation and determinations of the contents of organochlorine pesticide residues were performed using capillary gas chromatography with electron capture detection (GC-ECD).

Content of nitrates (III) and (V)

A sample of homogenized meat (10 g) was transferred to a 500 mL beaker, followed by 60 mL of water and 3 mL of a 25% sodium carbonate solution. The sample was incubated at a temp. of 80°C for 30 min. Then, it was mixed and filtered through a fluted filter paper, and the filtrate was collected to a 100 mL measuring flask. Next, nitrates (V) were reduced to nitrates (III) by the addition of metallic cadmium. The filtrate obtained was thoroughly mixed and filtrated. The resulting solution was transferred to a 100 mL measuring flask, which was filled up with distilled water. Then, 20 mL of the solution was collected to a 50 mL measuring flask, 5 mL of Griess I reagent were added, the solution was mixed, and left in a dark place for 5 minutes. Afterward, 1 mL of Griess II reagent was added, and the solution was left in a dark place for 10 minutes. Next, the flask was filled up with distilled water and left for 20 minutes. Absorbance was measured at the wavelength of 523 nm, and the absorbance values obtained allowed determining contents of nitrates (III) and (V) (ISO 1975).

Dry matter, salt content, pH

The dry matter content of the meat products was determined using a reference method according to the Polish Standard PN-ISO 1442:2000. The salt content was determined according to the Polish Standard PN-A-82112:1973/Az1:2002, whereas the pH value was determined following the Polish Standard PN-ISO 2917:2001.

The results obtained were subjected to statistical and mathematical calculations. In order to determine the statistical significance of the effect of sonication on selected characteristics of the quality of the meat products, the test of significance of differences between the means was used. In the calculations, one-factor analysis of variance was used (ANOVA), and the significance of differences between the means was determined by the Fisher's NIR test, with a significance level of $p \leq 0.05$. A null hypothesis was formulated for the purposes of this study (H_0): The use of ultrasound at different frequencies has no statistically significant effect on selected quality attributes of meat products, according to the equation:

$$H_0: \mu Qf1 = \mu Qf2 = \ldots = \mu Qfn$$

where:

f	–	ultrasound frequency,
Q	–	quality attribute,
Qf1,Qf2,... Qfn	–	average values of the quality attribute at different frequencies f1,f2,...,fn.

RESULTS AND DISCUSSION

Table 1 presents the results of analyses of selected quality attributes of the meat products studied. The analyses conducted and results obtained indicate that the protein content of the samples was significantly affected by the frequency of ultrasounds applied during sonication.

The highest protein content was determined in the samples sonicated at 100 kHz, regardless of their beef content (Table 1). In the case of salami with 40% beef content, the protein content increased by 6.79%, and in that with 60% beef content – by 7.53%, compared to the control sample. In the dried sausage samples with 40% beef content, the content of protein increased by 1.81%, whereas in these with 60% beef content – by 1.78%. The statistical analysis demonstrated that these differences were significant (Table 1). A similar observation was made by Garbowska (2019), who noted an increase in the protein content of homogenized meat products treated with ultrasounds of various frequencies. In her study, the sonication at 100 kHz contributed to the protein content increase by 3.6% in the products with 20% beef content, by 5.08% in those with 40% beef content, and by 5.35% in those with 60% beef content, compared to the control sample. According to Kapturowska et al. (2011), ultrasounds affected protein release from cells of *Saccharomyces crevisiae* yeast strain. Also, Velasco-Argano and Ordóñez-Santos (2020) reported that supersonication (45 kHz) increased the hydrophobic surface of myofibrillar proteins in pork. Considering the above, it can be concluded that the use of ultrasounds in food production contributes to the improvement of food quality by increasing protein content. During pro-

Table 1

Quality indicators of salami and dried sausages with different beef content (mean±SD)

U.F.	Protein (wt.%)	d.m. (wt.%)	Salt (wt.%)	pH	Pigment (ppm haematin)
Salami 40% beef					
0 kHz	28.45^{a}±0.79	55.25^{a}±0,23	3.55^{a}±0.09	5.30^{a} ±0.11	133.90^{a}±2.45
40 kHz	31.15^{b}±0.25	54.38^{a}±0,19	4.01^{a}±0.21	5.35^{a} ±0.09	179.39^{b}±7.13
60 kHz	32.87^{b}±0.35	55.15^{a}±0,37	3.85^{a}±0.19	5.41^{a} ±0.10	211.73^{c}±8.35
100 kHz	35.24^{c}±0.87	54.45^{a}±0,15	3.66^{a}±0.25	5.49^{a} ±0.15	225.97^{d}±9.87
ANOVA					
F	1.25	1.83	3.91	8.15	63.41
p	<0.001	0.47	0.59	0.80	<0.001
Salami 60% beef					
0 kHz	30.45^{a}±0.21	56.00^{a}±0.55	3.77^{a}±0.15	5.22^{a}±0.09	158.93^{a}±5.76
40 kHz	32.63^{b}±0.09	55.88^{a}±0.63	3.86^{a}±0.21	5.50^{a}±0.11	161.84^{a}±4.73
60 kHz	35.27^{c}±0.15	56.28^{a}±0.39	3.70^{a}±0.22	5.36^{a}±0.12	172.63^{b}±5.18
100 kHz	37.98^{d}±0.22	56.16^{a}±0.35	3.60^{a}±0.17	5.55^{a}±0.15	248.50^{c}±6.91
ANOVA					
F	1.35	2.72	2.53	1.35	22.75
p	0.02	0.69	0.67	0.77	<0.001
Dried sausage 40% beef					
0 kHz	24.13^{a}±0.07	45.45^{a}±0.23	2.68^{a}±0.07	5.35^{a}±0.01	129.71^{a}±2.35
40 kHz	24.91^{b}±0.11	45.78^{a}±0.21	2.65^{a}±0.05	5.33^{a}±0.02	155.14^{b}±3.59
60 kHz	25.01^{b}±0.15	45.91^{a}±0.35	2.69^{a}±0.04	5.42^{a}±0.05	167.34^{c}±2.95
100 kHz	25.94^{c}±0.09	45.13^{a}±0.29	2.66^{a}±0.11	5.43^{a}±0.10	185.43^{d}±4.63
ANOVA					
F	2.25	2.35	4.15	7.25	45.21
p	<0.001	0.51	0.89	0.91	<0.001
Dried sausage 60% beef					
0 kHz	25.37^{a}±0.02	53.00^{a}±0.55	2.69^{a}±0.05	5.69^{a}±0.05	165.56^{a}±3.67
40 kHz	25.93^{b}±0.04	52.08^{a}±0.49	2.71^{a}±0.15	5.66^{a}±0.09	171.23^{a}±4.21
60 kHz	26.45^{c}±0.05	51.68^{a}±0.63	2.70^{a}±0.35	5.67^{a}±0.06	184.13^{b}±4.02
100 kHz	27.15^{d}±0.05	52.76^{a}±0.54	2.67^{a}±0.18	5.71^{a}±0.08	195.68^{c}±3.75
ANOVA					
F	1.98	2.15	3.15	2.45	35.47
P	0.01	0.75	0.87	0.81	<0.001

U.F. – ultrasound frequency, d.m. – dry matter, SD – standard deviation, ^{a,b,c,d} – statistically significant differences $p \leq 0.05$

cessing, proteins and lipids contained in products can react as a result of temperature or the additives used. Protein oxidizing substances can be peroxides introduced during technological processes, active radicals formed by the action of light or enzymes and, finally, active oxidation products of lipids and polyphenols. Considering the chemical aspects of the reaction of protein with fat as a result of the technological process and the raw materials used in the formulation, covalent bonds are formed between the protein and the fat and its transformation products contained in the product (Šopík et al 2022, Lazárková et al 2023). A study by Yang et al (2024) showed that sonication at high power can break covalent bonds during protein extraction. The results showed that the use of ultrasound can significantly affect the conformation and structure of proteins due to the cavitation effect, resulting in improved solubility, interfacial properties, viscosity, gelation and flavor binding of proteins. When meat is processed using this type of treatment, it can modify the structure and thus improve the functional properties of myofibrillar protein (MP), leading to improved quality, the development of low-fat and/or low-salt products, and an extended shelf life (Sun et al 2023). Sonication improves myoglobin stability and also alters the structure of myoglobin-associated proteins. In the case of pigments, their total content was significantly influenced by the frequency of ultrasounds, and increased along with the frequency increase. The highest total content of pigments was determined in the samples sonicated at 100 kHz, and statistically significant differences were demonstrated in both types of meat products depending on the ultrasound frequency (Table 1). Sonication for 20 min at 12°C increased the level of pigments in meat products. This US condition also yielded higher red color indices and lower yellow color indices (Leães et al. 2023). Ultrasound affects many properties of meat products, including the content of pigments such as myoglobin and its derivatives (oxymyoglobin, metmyoglobin and deoxymyoglobin), which determine the color of the meat. It improves the stability of myoglobin and intensifies the red color of the meat through better oxidation to oxymyoglobin. It also changes the structure of myoglobin-related proteins, affecting their solubility and dye stability. Ultrasound is used to improve the absorption of curing salts, which can affect the color stability of cured meat products.

Another quality attribute of meat products is the pH value. As perceived by consumers, meat and meat products with a low pH value seem to be more salty and, therefore, more tasty, compared to the products with a high pH value (Gaoliang et al. 2022). In the present study, the lowest pH value was measured in the control sample of salami with 60% beef content and in the dried sausage sample with 40% beef content treated with ultrasounds having the frequency of 40 kHz. The ultrasound treatment of both types of meat products caused a slight decrease in the their acidity; however, the differences noted were statistically insignificant (Table 1). The research conducted by Gaoliang et al. (2022) showed that pH value decreased as the ultrasonic power increased. Simitar results were obtained by Guo et al. (2021).

The use of ultrasounds of various frequencies had no statistically significant effect on salt content of the meat products (the level of which met requirements for food products of this type) and their dry matter content (Table 1). Only slight fluctuations were observed in both salt and dry matter contents, which may indicate that they were not affected by sonication frequency. Inguglia et al. (2021) obtained similar results. These authors observed no differences (p>0.05) between the level of sodium in the control and sonicated samples (Inguglia et al. 2021).

Tables 2 (saturated fatty acids) and 3 (unsaturated fatty acids) present the results of analyses of the lipid content and fatty acid profile of traditional organic meat products. The contents of all fatty acids determined in the meat samples tested were similar, regardless of the ultrasound frequency. The highest content was determined for oleic acid (C18:1 cis 9), and the lowest one for lauric acid (C12, ca. 0.11%). The major saturated fatty acids of salami samples turned out to be palmitic acid (C16) and stearic acid (C18). The average content of palmitic acid was ca. 29% and its highest content was determined in the salami sample with 60% beef sonicated at 100 kHz. The highest content of stearic acid was also found in the same sample and reached 16.15%. Similar observations were made for sausage samples (Table 2). The mentioned fatty acids are the major SFAs (saturated fatty acids) of beef (Velasco-Argano, Ordóñez-Santos 2020), hence their percentage content was the highest in the analyzed meat products with the highest beef content. In the case of myristic acid (C14), statistically significant differences were demonstrated only in the meat product with 60% beef content.

The content of oleic acid (C18:1 cis 9) increased in the meat products treated with ultrasounds, and differences observed were statistically significant (Table 3). A similar observation was made for linoleic acid (C18:2) – Table 3.

A study conducted by Jung et al. (2005) on poultry meat showed increased contents of C18:0 and C18:2 acids in the sonicated samples. In turn, Zhang et al. (2008) demonstrated only a minimal effect of sonication on the fatty acid profile of food products analyzed in their study, whereas Ojha et al. (2017) showed that the ultrasound treatment caused no significant increase in the contents of fatty acids in dried beef, except for C16:1 and C18:2. However, some investigations have emphasized degradation of certain fatty acids upon ultrasound treatment, resulting in the formation of volatile fatty acids, which trigger changes in the taste of food products. In addition, cavitation induced by ultrasounds may cause damage to cellular membranes rich in unsaturated fatty acids, thereby contributing to the oxidation of fatty acids and changes in their contents (Cheng et al. 2015). Gaoliang at al. (2022) showed that the SFA content increased significantly with the increase in ultrasonic power (p<0.05). In particular, ultrasound treatment could increase C18:0 contents significantly (P<0.05), whereas those of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) decreased (Gaoliang at al. 2022). Wang et al. (2017) reported similar results. Kang et al. (2016) also found that ultrasound treatment greatly promotes lipid oxidation.

The production of traditional meat products entails the use of multiple additives, including the synthetic ones. Consumers express serious concerns over the addition of nitrates (III) and (V) to meat products, which are used in

Table 2

Fat content and profile of saturated fatty acids in pork-beef products (%±SD)

U.F.	Fat	Fatty acids							ΣSFA
		C12	C14	C15	C16	C17	C18	C20	
		Salami 40% beef							
0 kHz	15.5^{a}±0.3	0.11^{a}±0.01	1.95^{a}±0.09	0.14^{a}±0.06	25.79^{a}±0.35	0.35^{a}±0.05	15.65^{a}±1.33	0.27^{a}±0.09	44.26±1.98
40 kHz	15.6^{a}±0.2	0.13^{a}±0.03	1.81^{a}±0.07	0.13^{a}±0.09	26.62^{a}±0.22	0.42^{b}±0.09	14.79^{a}±3.21	0.29^{a}±0.06	44.19±3.77
60 kHz	15.3^{a}±0.2	0.12^{a}±0.03	1.83^{a}±0.11	0.15^{a}±0.16	27.15^{b}±1.15	0.44^{b}±0.14	14.35^{a}±2.11	0.32^{a}±0.08	44.36±3.78
100 kHz	14.5^{b}±0.1	0.11^{a}±0.02	1.81^{a}±0.12	0.13^{a}±0.08	28.93^{c}±0.37	0.49^{b}±0.17	12.93^{b}±0.87	0.31^{a}±0.11	44.71±1.74
ANOVA									
F	2.47	1.27	3.65	25.1	63.65	4.07	2.27	0.73	
p	0.03	0.42	0.83	0.13	<0.01	0.03	0.03	0.11	
		Salami 60% beef							
0 kHz	14.9^{a}±0.2	0.11^{a}±0.05	2.26^{a}±0.05	0.17^{a}±0.04	28.58^{a}±0.21	0.53^{a}±0.09	16.05^{a}±0.19	0.27^{a}±0.02	47.97±0.65
40 kHz	14.7^{a}±0.1	0.13^{a}±0.04	2.44^{b}±0.03	0.19^{a}±0.05	29.13^{b}±0,.15	0.51^{a}±0.05	15.27^{b}±0.09	0.32^{b}±0.01	47.99±0.42
60 kHz	14.6^{a}±0.2	0.12^{a}±0.03	2.21^{a}±0.03	0.18^{a}±0.07	29.11^{b}±0.12	0.54^{a}±0.06	15.91^{a}±0.21	0.33^{b}±0.02	48.14±0.54
100 kHz	14.1^{b}±0.1	0.12^{a}±0.04	2.45^{b}±0.04	0.21^{a}±0.08	29.95^{c}±0.15	0.57^{a}±0.06	13.15^{c}±0.17	0.36^{c}±0.03	46.81±0.57
ANOVA									
F	45.47	2.12	24.3	12.9	23.51	2.79	15.89	2.95	
p	0.02	0.45	0.01	0.11	0.01	0.29	0.01	0.00	
		Dried sausage 40% beef							
0 kHz	18.7^{a}±0.09	0.09^{a}±0.01	2.01^{a}±0.12	0.11^{a}±0.09	23.69^{a}±0.35	0.31^{a}±0.03	16.01^{a}±0.35	0.29^{a}±0.09	42.51±1.04
40 kHz	18.6^{a} ±0.10	0.10^{a}±0.09	1.98^{a}±0.11	0.12^{a}±0.08	24.55^{a}±0.65	0.36^{ab}±0.02	15.49^{a}±0.41	0.28^{a}±0.05	42.88±1.41
60 kHz	18.3^{a} ±0.12	0.10^{a}±0.03	1.95^{a}±0.11	0.13^{a}±0.09	26.13^{b}±0.15	0.39^{b}±0.02	15.15^{a}±0.35	0.30^{a}±0.06	44.15±0.81
100 kHz	17.5^{b} ±0.07	0.11^{a}±0.08	1.91^{a}±0.12	0.12^{a}±0.04	26.12^{b}±0.32	0.42^{bc}±0.03	13.95^{b}±0.37	0.29^{a}±0.09	42.92±1.05
ANOVA									
F	3.15	1.35	4.25	1.98	51.43	5.32	3.17	0.95	
p	0.02	0.93	0.81	0.93	0.01	0.04	0.02	0.83	
		Dried sausage 60% beef							
0 kHz	17.7^{a}±0.09	0.10^{a}±0.05	2.47^{a}±0.04	0.15^{a}±0.07	26.15^{a}±0.25	0.47^{a}±0.11	15.45^{a}±0.47	0.28^{a}±0.04	45.07±1.03
40 kHz	17.6^{a}±0.05	0.12^{a}±0.04	2.49^{a}±0.09	0.16^{a}±0.06	27.05^{b}±0.64	0.46^{a}±0.09	14.97^{a}±0.65	0.31^{a}±0.09	45.56±1.66
60 kHz	17.6^{a}±0.03	0.11^{a}±0.07	2.11^{b}±0.03	0.17^{a}±0.06	27.33^{b}±0.46	0.48^{a}±0.14	14.15^{b}±0.32	0.34^{ab}±0.04	44.49±1.12
100 kHz	17.1^{b}±0.01	0.12^{a}±0.03	2.15^{b}±0.05	0.20^{a}±0.09	26.09^{a}±0.39	0.51^{a}±0.16	15.01^{a}±0.31	0.37^{b}±0.06	44.45±1.09
ANOVA									
F	5.47	4.15	32.1	13.8	27.54	3.75	12.43	3.17	
p	0.02	0.95	0.02	0.22	0.01	0.11	0.02	0.00	

U.F. – ultrasound frequency, ^{a,b} – statistically significant differences p≤0.05, SFA – saturated fatty acids

the meat curing process. The health risk posed by nitrites contained in cured products is associated with the formation of toxic compounds called nitrosamines. Due to the potentially adverse indirect effect of nitrates and the direct harmful effect of nitrites on the human body, in accordance with the recommendations of the FAO/WHO (Food Agriculture Organization/World

Table 3

Profile of unsaturated fatty acids in pork-beef products (%±SD)

U.F.	Fatty acids								ΣMUFA	ΣPUFA
	C16:1	C17:1	C18:1cis9	C18:1cis11	C18:2	C18:3	C20:1	C20:2		
Salami 40% beef										
0 kHz	3.19^{a}±0.11	0.45^{a}±0.12	38.45^{a}±0.21	3.32^{a}±0.15	3.01^{a}±0.09	0.31^{a}±0.11	0.75^{a}±0.12	1.75^{a}±0.09	46.16±0.71	5.07±0.29
40 kHz	3.27^{a}±0.15	0.47^{a}±0.09	41.12^{b}±0.35	3.21^{a}±0.11	3.44^{b}±0.35	0.37^{a}±0.09	0.79^{a}±0.09	2.85^{b}±0.12	48.86±0.79	6.66±0.56
60 kHz	3.31^{a}±0.21	0.48^{a}±0.12	41.24^{b}±0.13	2.95^{b}±0.09	3.52^{b}±0.27	0.39^{a}±0.15	0.77^{a}±0.11	3,.01^{b}±0.32	48.75±0.66	6.92±0.74
100 kHz	3.33^{a}±0.35	0.49^{a}±0.17	40.50^{b}±0.21	2.75^{b}±0.11	3.50^{b}±0.36	0.38^{a}±0.07	0.79^{a}±0.15	3.15^{b}±0.27	47.86±0.99	7.03±0.70
ANOVA										
F	5.53	2.19	1.13	2.56	3.63	1.44	13.5	11.7		
p	0.11	0.32	<0.01	0.01	0.01	0.79	0.7	0.10		
Salami 60% beef										
0 kHz	3.85^{a}±0.04	0.47^{a}±0.05	37,.39^{a}±0.73	3.17^{a}±0.45	3.54^{a}±0.19	0.60^{a}±0.05	0.60^{a}±0.05	3.35^{a}±0.11	44.48±1.32	7.49±0.35
40 kHz	3.97^{b}±0.03	0.46^{a}±0.06	38.26^{b}±0.53	3.11^{a}±0.23	4.15^{b}±0.22	0.41^{b}±0.07	0.66^{a}±0.04	2.27^{b}±0.15	46.46±0.89	6.83±0.44
60 kHz	3.99^{b}±0.05	0.43^{a}±0.05	38.75^{b}±0.37	3.13^{a}±0.12	4.19^{b}±0.14	0.38^{b}±0.08	0.69^{ab}±0.04	2.31^{b}±0.12	46.99±0.63	6.88±0.34
100 kHz	4.11^{c}±0.02	0.42^{a}±0.07	40.17^{b}±0.47	3.24^{a}±0.56	4.21^{b}±0.21	0.45^{b}±0.04	0.72^{b}±0.03	2.69^{c}±0.09	48.94±1.15	7.35±0.34
ANOVA										
F	16.1	1.78	12.55	3.31	3.15	5.23	14.8	14.8		
p	0.00	0.81	0.01	0.17	0.01	0.00	0.01	0.00		
Dried sausage 40% beef										
0 kHz	3.01^{a}±0.13	0.47^{a}±0.22	37.45^{a}±0.93	3.55^{a}±0.14	4.89^{a}±0.21	0.33^{a}±0.09	0.71^{a}±0.15	2.95^{a}±0.35	44.48±1.57	9.17±0.65
40 kHz	2.99a±0.11	0.48^{a}±0.11	39.12^{b}±0.45	3.36^{a}±0.08	5.02^{a}±0.34	0.35^{a}±0.10	0.75^{a}±0.11	3.47^{a}±0.21	46.70±0.86	9.52±0.65
60 kHz	3.15^{a}±0.19	0.47^{a}±0.12	39.02^{b}±0.54	3.01^{b}±0.12	5.32^{a}±0.17	0.36^{a}±0.06	0.74^{a}±0.15	4.15^{b}±0.17	46.39±1.12	9.83±0.40
100 kHz	3.11^{a}±0.17	0.46^{a}±0.09	39.42^{b}±0.41	2.83^{b}±0.13	5.47^{a}±0.25	0.37^{a}±0.11	0.73^{a}±0.19	4.65^{b}±0.37	46.55±1.36	10.49±0.73
ANOVA										
F	4.89	6.76	2.13	3.15	3.25	1.11	19.8	139		
p	0.23	0.78	0.01	0.02	0.07	0.85	0.35	0.01		
Dried sausage 60% beef										
0 kHz	3.81^{a}±0.35	0.49^{a}±0.09	38.15^{a}±1.75	3.15^{a}±0.35	4.85^{a}±0.11	0.71^{a}±0.11	0.65^{a}±0.05	3.25^{a}±0.13	46.25±2.59	8.81±0.35
40 kHz	4.01^{a}±0.18	0.50^{a}±0.11	38.05^{a}±2.01	3.19^{a}±0.28	5.19^{b}±0.09	0.63^{a}±0.12	0.74^{b}±0.07	2.49^{b}±0,.11	46.49±2.65	8.31±0.38
60 Hz	3.95^{a}±0.21	0.52^{a}±0.08	38.37^{a}±1.37	3.88^{a}±0.19	5.23^{b}±0.16	0.48^{a}±0.15	0.78^{b}±0.06	2.61^{b}±0.12	47.50±1.91	8.32±0.43
100 kHz	4.01^{a}±0.26	0.52^{a}±0.10	38.95^{a}±1.28	2.92^{a}±0.15	5.37^{b}±0.28	0.51^{a}±0.07	0.82^{b}±0.09	2.75^{b}±0.15	47.22±1.88	8.53±0.50
ANOVA										
F	25.3	2.38	13.75	4.89	4.85	7.91	15.5	16.8		
p	0.11	0.41	0.12	0.25	0.02	0.09	0.01	0.00		

U.F. – ultrasound frequency, ^{a,b} – statistically significant differences p≤0.05, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

Health Organization) Expert Committee on Food Additives, their Acceptable Daily Intake (ADI) was set at max. 5 mg of nitrates per kg body weight and 0.2 mg of nitrites per kg body weight. According to Commission Regulation (EU) No. 1129 (2011), the content of nitrates in traditional products of this type should not exceed 300 mg kg^{-1} of product and that of nitrites 180 mg kg^{-1} of the product. Therefore, the content of these compounds in meat products is one of the distinguishing features that determine their quality.

Figure 1 shows the content of the sum of nitrates (III) and (V) in the examined salami and sausage samples. In all tested products, the sum of these compounds was significantly lower than the permissible maximum concentrations specified in the Regulation.

The total content of these compounds decreased significantly compared to the control sample, depending on the increase in the frequency used for ultrasound preservation (ANOVA: salami: 40% beef content F=7.15, p<0.001;

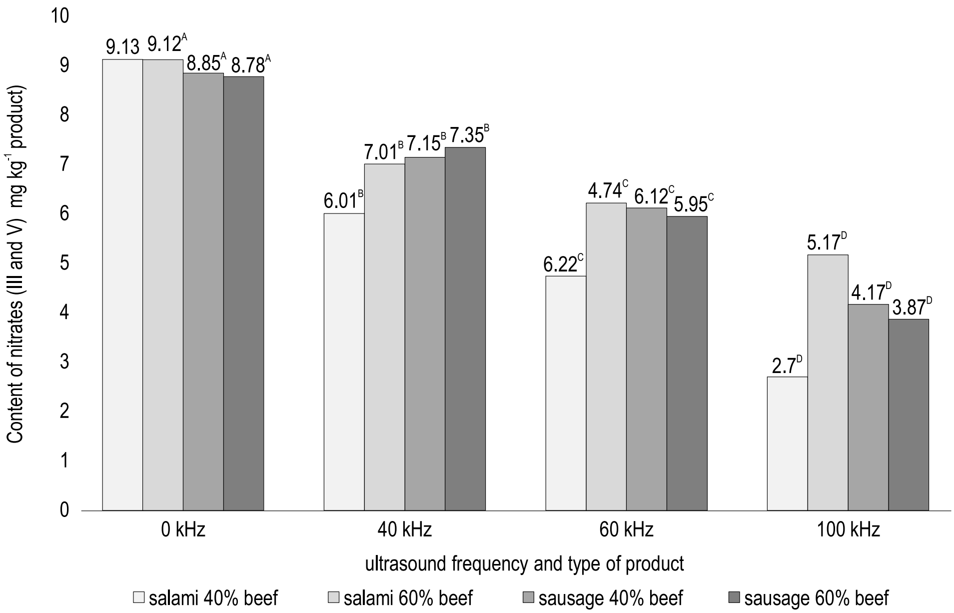
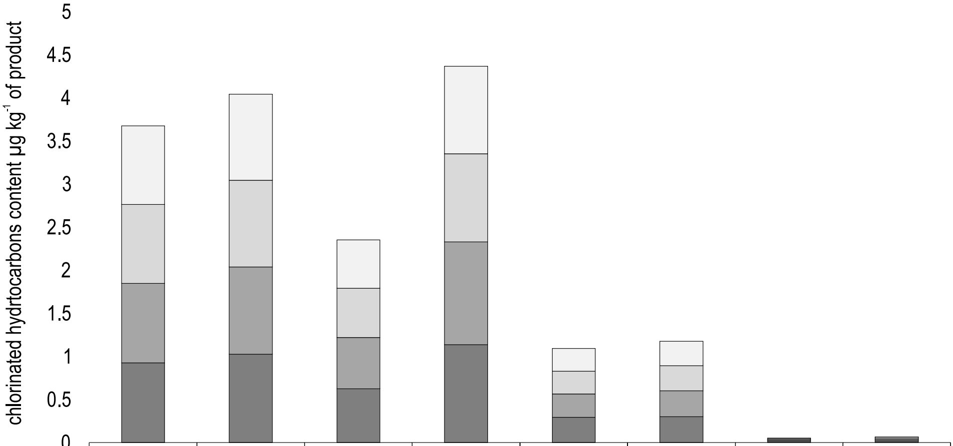


Fig. 1. Content of the sum of nitrates (III) and (V) in the examined salami and sausage samples (mg kg^{-1}): ^{A,B,C,D} – statistically significant differences $p \leq 0.05$

60% beef content F=5.12, p<0.001; sausage: 40% beef content F=6.41, p<0.001, 60% beef content F=8.15, p<0.001), and the differences observed were statistically significant (Figure 1). High ultrasound intensity can cause the breakdown of nitrate and nitrite molecules, leading to their degradation. In an environment rich in free radicals (generated by ultrasound), non-enzymatic nitrate reduction can occur. The use of high frequencies can lead to the degradation of nitrates, reducing their content in the final product. The appropriate use of ultrasound can also reduce the risk of nitrosamine formation. Garbowska (2019) reported similar results, i.e. almost two-fold lower content of total nitrates in the homogenized meat products with 60% beef content sonicated at 100 kHz, compared to the control sample. In the study by Guo et al. (2021), the content of nitrates (III) in meat products reached 1.735 mg g^{-1}. In turn, Kalaycıoğlu and Erim (2019) demonstrated the average nitrate content at 294 mg kg^{-1} and nitrite content at 90 mg kg^{-1} product in traditional meat products, whereas Velasco-Arango et al. (2020) showed the average nitrite content at 150 mg kg^{-1} product in beef burgers. In meat products, nitrites (NO_2^-), formed, among other things, as a result of the reduction of NO_3^-, can react with myoglobin, haemoglobin and other proteins, leading to the formation of stable complexes. This produces nitrosylated myoglobin (Mb-NO), which is responsible for the characteristic pink colour of processed meat products. In this case, nitrogen from NO_3^- is 'bound' in the form of protein-nitrosyl complexes and is not detected as free nitrate (Xiangxiang et al. 2023).

Chlorinated hydrocarbons are compounds whose levels in food products are regulated by law (Commission Regulation (EC) No 149/2008). Figures 2 and 3 present contents of organochlorine insecticides in the examined salami and sausage samples. According to the Regulation of the European Commission (2008) on the maximum permissible concentrations of pesticide residues in food, the concentration of ΣDDT should not exceed 0.05 mg kg^{-1} of the product, and the concentration of γ-HCH should be less than 0.01 μg kg^{-1} of the product. The highest concentration of ΣDDT, reaching 1.509 μg kg^{-1} of the product on average, was found in salami samples with 60% beef content



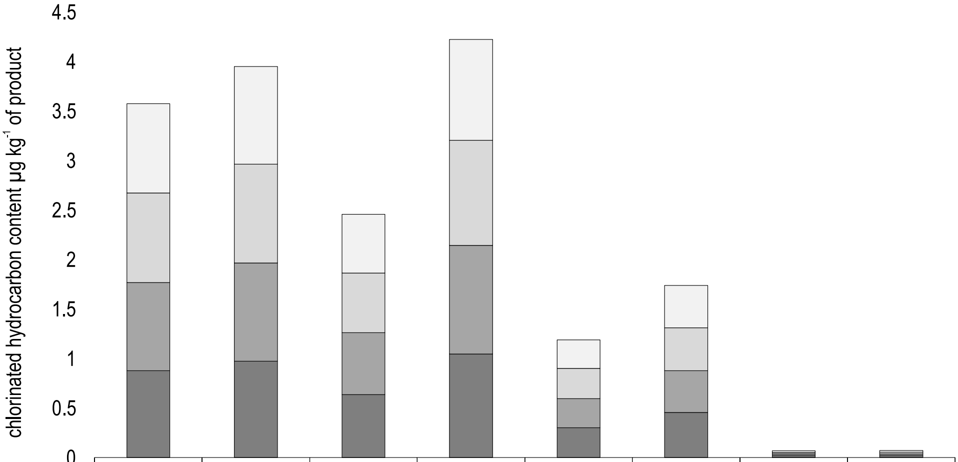
	γ-HCH		DDT		DDD		DDE	
	40% beef	60% beef	40% beef	60% beef	40% beef	60% beef	40% beef	60% beef
□ 100 kHz	0.911	1.001	0.561	1.017	0.261	0.283	0.012	0.014
60 kHz	0.915	1.005	0.571	1.022	0.265	0.291	0.014	0.016
40 kHz	0.923	1.012	0.593	1.192	0.271	0.299	0.013	0.018
■ 0 kHz	0.925	1.025	0.625	1.135	0.293	0.301	0.015	0.019

ultrasound frequency and type of salami

Fig. 2. Content of chlorinated hydrocarbons in salami samples (μg kg^{-1} of product)

sonicated at 40 kHz, whereas the lowest one, 0.834 μg kg^{-1}, was detected in the salami samples with 40% beef content sonicated at 100 kHz (Figure 2). Statistical analysis demonstrated no significant differences, in salami samples, between the contents of pesticide residues and ultrasound frequency (ANOVA: γ-HCH: 40% beef content F=7.15, p=0.385; 60% beef content F=5.56, p=0.451; DDT: 40% beef content F=5.16, p=0.744; 60% beef content F=6.23, p=0.786; DDD: 40% beef content F=8,32, p=0.548; 60% beef content F=7.36, p=0.745; DDE: 40% beef content F=9.45, p=0.321; 60% beef content F=15.76, p=0.658).

In the case of dried sausage samples, the highest concentrations of ΣDDT and γ-HCH were determined in the samples with 60% beef content and reached 1.538 μg kg^{-1} product and 0.998 μg kg^{-1} product, respectively (Figure 3).



	40% beef	60% beef	40% beef	60% beef	40% beef	60% beef	40%beef	60% beef
	γ-HCH		DDT		DDD		DDE	
100 kHz	0.901	0.987	0.595	1.019	0.289	0.427	0.017	0.016
60 kHz	0.905	0.998	0.601	1.061	0.303	0.435	0.016	0.017
40 kHz	0.891	0.991	0.627	1.098	0.295	0.421	0.017	0.019
0 kHz	0.876	0.974	0.635	1.045	0.301	0.455	0.019	0.018

ultrasound frequency a type of sausage

Fig. 3. Content of chlorinated hydrocarbons in dried sausage samples (µg kg^{-1} of product)

The statistical analysis of the results demonstrated that the ultrasound treatment caused no changes in the contents of pesticide residues in the examined meat products. There were no significant differences in the contents of both ΣDDT and its components (ANOVA: γ-HCH: 40% beef content F=4.14, p=0.365; 60% beef content F=2.25, p=0.865; DDT: 40% beef content F=3.39, p=0.528; 60% beef content F=3,49, p=0.318; DDD: 40% beef content F=3,89, p=0.42; 60% beef content F=6.59, p=0.861; DDE 40% beef content F=5.16, p=0.317, 60% beef content F=9,34, p=0.795).

CONCLUSIONS

Ultrasound is an innovative technology used in the meat industry, bringing numerous benefits in terms of both product quality and production process efficiency. Thanks to its properties, ultrasound can improve texture, lengthen product shelf life and reduce the use of chemical food additives. This makes it possible to achieve a more stable meat color, better texture and increase its shelf life without the need for large amounts of preservatives. Ultrasound can also affect the stability of meat coloring agents. When used with the right parameters, it can increase the oxymyoglobin content, which is responsible for the intense red color of meat, thus extending the shelf life of products and their visual appeal. In addition, ultrasound can reduce the oxidation of dyes, which prevents the formation of a brown color in meat. Ultrasonic technology is also used to reduce chemical additives. By improving the efficiency of processing, it is possible to reduce the amount of preservatives, phosphates and salts used, which is in line with health trends and consumer expectations. The result is more natural, high-quality products. In summary, ultrasound in the meat industry offers numerous benefits, such as shorter production times, improved product quality, stabilization of colorants, reduction of chemical additives and increased microbiological safety. Thanks to further research and optimization of processes, this technology can be expected to become even more widespread, contributing to the sustainable development of the meat industry. Ultrasounds is the alternative method preserving food products, their coupled application with any other type of treatment can significantly improve the quality of the finished product. In the case of meat products with different beef contents, a higher content of available protein was found in the sonicated samples. These ultrasound-treated samples had also a higher content of pigments, which may positively influence the color of the finished product, and thus ensure better consumer acceptance. In addition, the sonicated products had reduced contents of nitrates and nitrites, which is beneficial from the perspective of health safety of the finished products.

CONFLICTS OF INTEREST

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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