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Estimation of the stability of skeletal muscle myoglobin of chilled pork treated with brine activated by low-frequency high-intensity ultrasound

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ABSTRACT

We studied the effect of ultrasonic activation of brine (3%) during salting on the degree of stability of colour parameters of pork with normal (NOR) and abnormal course of autolysis in the CIE Lab colour space. The mechanism of stabilisation of the colour of meat is attributed to donor–acceptor bonds of metmyoglobin (MetMb). The accumulation of excessive number of free electrons in the medium are capable of activating MetMb. This reduces the activity of meat, when the native participants of the metmyoglobin reductase system and their own antioxidant systems of meat are depleted.

Based on the additive calculation of deviations (increase / decrease) by the coordinates L*, a*, b* in the CIE Lab system, and the total colour difference (ΔE) in control and experimental samples, recommendations were developed. To optimize the colour characteristics of all types of meat, both on the surface and in the thickness of the meat, the preliminary activation of a 3% brine in a low-frequency submersible ultrasonic unit is recommended. Moreover, preliminary cavitation activation of a 3% is more preferable to stabilise the colour of PSE – meat (pale, soft, exudative (watery),) brine in a flow-through installation.

1. Introduction

A key indicator of quality and safety of meat products, along with taste, aroma and texture, is appearance which is primarily determined by colour. Visual appearance and colour of meat product not only helps in presentation, but also helps to identify staling and deterioration during biochemical processes occurring in post-slaughter period. Currently, there are three types of deterioration in the Russian food market during autolysis [1]: PSE – meat (pale, soft, exudative (watery), RSE – meat (red, soft (sagging), exudative,) and DFD – meat (dark, firm (hard), dry). It is known that the share of exudative meat (including those with RSE defect) arriving processing units in the Russian Federation ranges from 40 to 47%, which is on average 7% higher than the market share of DFD – meat. This exacerbates the processes involved in identification of raw materials as wells as ensuring the specified quality of meat products are maintained [2,3].

At biochemical level, colour of meat is determined by the concentration of main pigment of myocardium and skeletal muscle – myoglobin (hereinafter referred to as Mb), consisting of a protein component (globin), a prosthetic component (gem) and the ratio of its redox forms in muscle tissue [4]. By its nature, Mb is a myofibrillar protein capable of actively binding oxygen. The main function of Mb is to form an oxygen reserve in muscle tissue, which is consumed during a temporary lack of oxygen [5].The saturation of Mb with oxygen allows in achieving a bright pink colour to pork due to the formation of oxymyoglobin (hereinafter – OMb). At the same time, the transition of iron in haem from Fe^{+2} to Fe^{+3} causes the loss of consumer-friendly colour in muscle tissue and is accompanied by the formation of a dark brown Mb form – metmyoglobin (hereinafter – MetMb) [4,5].

Due to the action of metmyoglobin reduction system and the

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oxidation of meat's own antioxidant systems (vitamin B_{12}, ascorbic acid, glutathione, etc.), free electrons are present in meat. Due to this, it is possible to restore Fe^{+3} to Fe^{+2}, that is, to convert MetMb to Mb, with a more acceptable colour [6]. However, over time, the substrates that ensure the functioning of these two mechanisms naturally become depleted and metmyoglobin-reducing activity (hereinafter – MPA) becomes insufficient for the transformation of MetMb into Mb. In addition, there is a presence of non-traditional meat raw materials with malformations of autolysis, in an amount of about 50% of the total volume sold in the territory of the Russian Federation of raw materials [3].

In a general sense, the colour of chilled meat that is natural and acceptable for a consumer is limited by a set of factors such as sex and age, intravital diet, hormonal background, and the level of physical activity [7]. Meat obtained from males has a higher concentration of Mb in muscle tissue than meat obtained from females [7]. Within one species, it is known that meat obtained from mature individuals has a darker colour than meat obtained from young individuals [5]. A particular influence on the colour of the meat is provided by the breed of the animal and the type of muscle most used during life. The muscles used by the animals for movement usually contain a higher concentration of Mb than the muscles that perform the supporting function, which is due to the need to saturate the former with increased oxygen concentrations necessary for energy production [8]. The intravital level of an animal's physical activity also determines the colour of the meat received from it; for example, less active animals raised on a feedlot provide meat with a lower concentration of Mb than more active animals grown on a pasture [7].

The appearance of the animal has a key effect on the colour of the meat; thus, red muscle fibres, as a rule, have a higher Mb concentration than white. This is due to the fact that they have predominantly aerobic metabolism and require high concentrations of oxygen, which is carried by Mb, while white fibres are predominantly anaerobic [8]. The colour intensity of meat also depends on the pH and morphology of muscle tissue. The pH of meat is affected by both premortem and post-mortem factors. The negative effect of low pH on the colour characteristics of meat can be justified through several mechanisms [7]. A rapid premortem or post-mortem pH decrease caused by the accumulation of the final metabolic product during anaerobic digestion of glucose and glycogen -lactic acid – at high carcass temperatures causes partial or complete denaturation of sarcoplasmic and myofibrillar proteins, which include Mb due to the presence of the globin part [5]. In this case, low pH starts the transition of the easily oxidised Mb fraction into MetMb, which has a low colour intensity and a brownish-grey colour [7]. From the point of view of the structural and mechanical features of meat, any deviations from the norm in terms of pH affect the charge of the proteins that make up the muscle tissue. This results in change in the distance between the meat fibres (fibrils) and affects how the light incident on the surface meat is reflected and absorbed. Meat with a low pH will be characterized by a more open structure of muscle tissue, which will scatter light and therefore appear pale in colour [5]. Meat with a high pH is characterized by a more closed structure – muscle fibres swell and are tightly packed together, forming a barrier for oxygen diffusion. This interferes with the oxygenation process, hence the absorption and reflection of light. In addition, a high pH level prevents oxygen binding to Mb, resulting in inhibition of the formation of red OMb [8].

The salt pickling is one of the most important technological operations in meat processing where water and sodium chloride are traditionally used. However, there are several problems associated with the use of sodium chloride, due to its destructive (oxidative) effect on Mb [9]. Sodium chloride has two mechanisms to stimulate oxidation; the first is that the saline solution reduces the buffering ability of meat, at which an uncontrolled change in pH occurs. This reduction in buffering capacity of meat negatively affects the colour of the meat. The second mechanism involves sodium chloride reducing the ability of the muscle to absorb oxygen, which contributes to the appearance of depleted oxygen stresses, leading to Mb oxidation [10].

Table 1
Experimental runs details.

№ п/п	Group	Type of meat	Concentration of brine (NaCl), %	The presence of cavitation effects on the brine	
				Immersion method	Flow method
1	**Control**	**NOR**	**(–)**	**(–)**	**(–)**
2	Experiment	NOR	3	(–)	(–)
3			3	(+)	(–)
4			3	(–)	(+)
5	**Control**	**PSE**	**(–)**	**(–)**	**(–)**
6	Experiment	PSE	3	(–)	(–)
7			3	(+)	(–)
8			3	(–)	(+)
9	**Control**	**DFD**	**(–)**	**(–)**	**(–)**
10	Experiment	DFD	3	(–)	(–)
11			3	(+)	(–)
12			3	(–)	(+)

There is a traditionally established solution for compensation of the negative effects of sodium chloride during the process of salt pickling in meat industry, the addition of nitrite salt to the salting mixture, which acts as a colour fixer. However, the addition of extra salts contradicts the worldwide emerging concept of switching to the production of clean label. As a result, the problem of stabilising and preserving the colour of raw meat during the shelf life by using safe technological methods of exposure is relevant. Safe and clean methods development and implementation by using innovative solutions in this area is required.

Currently, there are research and development efforts in searching for the alternative ways to preserve the colour characteristics of meat raw materials through the usage of packaging like modified gas media – HIOX MAP and CO MAP, vacuum packaging and skin – packaging [11–14]; intermediate products of the tricarboxylic acid cycle (lactate, succinate, pyruvate, malate) [15,16]; nitrite films [17,18]; natural and synthetic antioxidants [19,20]; physical methods (excessive hydrostatic pressure [21], atmospheric cold plasma [22], ultrasound [23–26], etc.).

Sonochemical-processing based on ultrasonic technologies are particularly interesting for the preservation of the colour of meat systems. There are contradictory findings in literature about direct or indirect effects of ultrasonication on the colour of meat systems. There are research results confirming the positive effect [26] of ultrasound on the pigment system of chilled meat, but there are also opposite results of research, indicating a neutral [23] and even negative effect [24] of ultrasonic cavitation. In addition, in most studies on the effects of ultrasound on the colour of meat, cattle meat was used, while the effects of sonochemical processing on the colour of muscle tissue of meat obtained from other animal species, including pork, were not fully investigated. In order to fill the existing gap in the literature, we aimed to evaluate the indirect effect of ultrasound (due to the preliminary activation of weakly saturated brines based on sodium chloride) on the colour stability of chilled NOR pork, as well as pork with deterioration during autolysis (PSE and DFD).

2. Materials and methods

2.1. Sample preparation

We used cylindrical meat samples with diameter and height of 50 mm and weight of 0.1 kg, formed from a whole muscle piece of meat of the longest muscle of the back (*L. dorsi*) of pork of 3 types: NOR, PSE and DFD whose compositions are shown below: NOR (% protein content = 20.7 ± 1.6, fat = 5.2 ± 0.8, moisture = 75.4 ± 7.5, pH = 5.7), as well as – DFD (% protein content = 19.8 ± 3, fat = 2.0 ± 0.3, moisture = 65.4 ± 7.1, pH = 6.7) and PSE (% protein content = 17.78 ± 2.54, fat = 3,43 ± 0.9, moisture = 79.2 ± 6.4, pH = 4,13). For each type, 2 groups of samples were created – control and experimental (Table 1). Samples of the control group (No. 1, 5, 9) were not exposed to brine and, therefore,

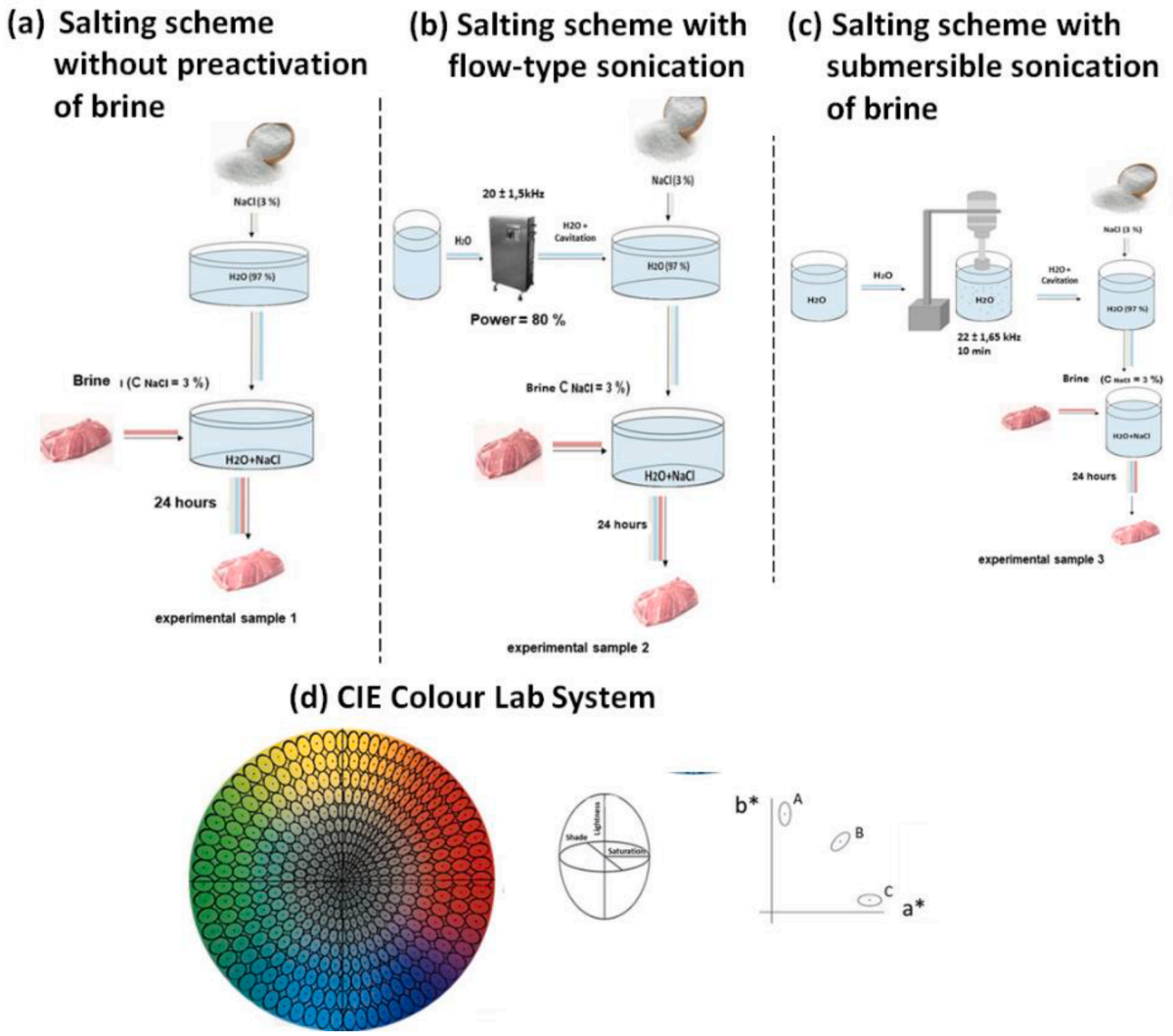


Fig. 1. The geometric meaning of colour in the CIE system.

cavitation treatment (marked as (–) in Table 1). Samples of the experimental group NOR – meat – No. 2, PSE – meat – No. 6 and DFD – meat No. 10 were placed for 1 day in a 3% brine not activated by cavitation (in Table 1 is marked as (–)). Samples of the experimental group NOR –meat No. 3, PSE –meat No. 7 and DFD –meat No. 11 were placed for 1 day in a 3% brine previously activated in a low-frequency submersible ultrasonic unit (marked as (+)). Samples of the experimental group NOR – meat – No. 4, PSE – meat – No. 8 and DFD – meat – No. 12 were placed for 1 day in 3% brine, previously activated in a low-frequency flow-through ultrasonic unit (marked as (+)).

2.2. Low frequency ultrasonic brine activation

When preparing the brine, tap water was used in accordance with GOST R 51232-98 and food salt in accordance with GOST R 51574-2018. The concentration of NaCl in brine is 3%. Ultrasonic exposure time, depending on the type of installation (immersion or flow through type method as schematically shown in Fig. 1 a and b), is from 8 to 10 min.

For ultrasonic treatment of brine, the two submersible devices of the type Volna and the type RKU – 0.63 were used. The Volna device (model UZTA-0.4 / 22-OM) has the following technical characteristics: frequency of mechanical vibrations – 22 ± 1.65 kHz, maximum power consumption – 400 W, power control range – from 30 to 100%. The power parameters used in this experiment (180 W and the exposure duration – 5 min), were chosen due to the proven optimality [27] of such types of mode in work.

The flow-type cavitation reactor RKU was used under the following exposure modes: amplitude of sound pressure – 0.20 MPa, intensity – 20 KHz, productivity – 5 L per minute; they were considered optimal in a dissertation research [28].

2.3. Spectrocolorimetric method

To record changes in the colour of muscle tissue of meat in the present study, we used the generally accepted CIE colour space model – L*a*b* and the spectrocolorimetric method for assessing small colour differences in the CIE equal-contrast system (Fig. 1). The colour of muscle tissue was determined on a spectrocolorimeter (Spectroton, Russia), which includes a measuring unit and a sensor, which were connected by an electron-optical cable. A personal computer was connected to the spectrocolorimeter. The PC contained Windows-based software that allowed us to control the process of measuring and performing calculations of the colour characteristics of meat samples.

The principle of operation of the Spectroton instrument is to simultaneously measure the reflection coefficients of the studied meat samples at twenty-four fixed wavelengths, which are located in the visible part of the spectrum (from 380 to 720 nm) at a distance of 13 nm from each other. Due to the measurement of the reflection coefficients of the samples by means of a microprocessor controller, an integrated

measuring unit, mathematical processing of the measurement results is carried out. The device fixes the colour coordinates CIE L*a*b* – where L* (lightness), a* (redness), b*(yellowness).

2.4. The assessment of the stability of the colour characteristics of meat systems

To assess colour stability, we used colorimetric measurement of the surface and internal cut in the CIE Lab model system (Fig. 1). The ability of the meat system to maintain the original colour characteristics (L*, a*, b*) under the action of ultrasonic exposure – was calculated from formulas 1– 3 proposed in [29].

$$YL = \left(1 - \frac{|L1 - L2|}{L1}\right)*100\%, \quad (1)$$

where L_1 – the degree of lightness of the control sample,%; L_2 – the degree of lightness of the prototype, %

$$Ya = \left(1 - \frac{|a1 - a2|}{a1}\right)*100\%, \quad (2)$$

where: a_1 – is the degree of redness of the control sample,%; a_2 – degree of redness of the prototype,%

$$Yb = (1 - \frac{|b1 - b2|}{b1})*100\% \quad (3)$$

where: b_1 – the yellowness of the control sample,%; b_2 – the yellowness of the prototype, %

However, during the analysis of the experimental results, it was found that the values of the criteria a* and b* can take negative values, as well as values less than 1, which is true from their physical meaning, but not typical for applying the existing assessment methodology of colour stability (Y) of meat and meat products.

In the CIE Lab system, the L* coordinate means lightness, a* is the ratio between red and green, b* is the ratio between blue and yellow. The axis –a*/+a* goes from left to right, the movement of colour along the axis, in the positive direction shows the colour shift towards red, in the negative direction – towards green. The axis –b*/ +b* goes from bottom to top, the colour movement is along the axis. The colour shifts to the yellow side in the positive direction shows and to the blue side in the negative direction. The axis 0/L* is perpendicular to the plane formed due to the intersection of the axes -a* / +a* and –b*/ +b* and is located at the intersection of these axes. At the same time, at the point of intersection of two axes, L* is 0, that means the presence of black colour at this point (complete absorption). There are shades of grey between the maximally possible levels of L* and the level at which L* is 0. Therefore, going beyond the positive values according to the criteria – a* and b* is permissible.

According to the theory of stability and interval parameter estimates, if a value falls below the unit in the interval under study, then the parameter is considered unstable, and the known method for determining stability is not correctly applied to such a parameter [29], as a result, it is necessary to use other estimation methods with high resolution [30]. In addition, when assessing colour stability, it is important to consider the geometric meaning of the concept of colour and the nonlinearity of the dependencies between the coordinates L*, a*, b*.

To assess the colour deviation of the sample exposed to brine (including previously activated by cavitation), we used the colour tolerance or colour difference (ΔE) to assess the colour stability of meat systems [31,32]. The calculation allows to accurately establish the consistency between the visual assessment and the instrumentally measured colour difference. We calculated ΔE, the difference between the two data sets: the coordinates L*, a* and b* of the control sample and the coordinates L*, a* and b* of the test sample (subjected to technological influence, in particular, the indirect influence of ultrasound through the brine medium).

Under the above conditions, the set of coordinates L*, a* and b* of a control sample was taken as the standard colour value. The ΔE calculation describes the boundaries of the tolerance ellipsoid around the point of the standard colour value. The axes of the tolerance ellipsoid (ellipsoid measurements) correspond to hue (S_H), saturation (S_C) and lightness (S_L). The tolerance ellipsoid is the amount of tolerances (tolerances / colour differences) that can vary in size and shape depending on the position in the CIE L*a*b* colour space (Fig. 1).

Thus, the calculation result ΔE is the determination of a mathematically calculated ellipsoid with three semi-axes (hue, saturation, lightness), which occurs around each experimentally measured colour value, composed of the coordinates L*, a* and b*. The size of the ellipsoids is uneven – the ellipsoids of the orange zone are longer and thinner than the ellipsoids of the green region, which are wider and rounded. With increasing colour saturation the size and shape of the ellipsoids changes.

The calculation of colour differences was calculated by the formula 4:

$$\Delta E = \sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2 + \left(\frac{\Delta C}{K_C S_C}\right)^2 + \left(\frac{\Delta H}{K_H S_H}\right)^2}, \quad (4)$$

where ΔL, ΔC, ΔH is the difference between meat samples before and after treatment with cavitation activated brine in terms of lightness, saturation and colour tone, respectively; K_L, K_C, K_H – weighting factors (equated to 1); S_L, S_C, S_H – weight functions (lengths of semi-axes of an ellipsoid).

The calculation of the difference between the lightness of colour of the meat images before and after treatment with activated brine is carried out according to formula 5.

$$\Delta L = L1 - L2, \quad (5)$$

where: L_1 is the lightness of the colour of the meat sample before processing it with cavitation activated brine; L_2 is the lightness of the colour of the meat sample after processing it with cavitation activated brine.

The calculation of the colour saturation of the samples before and after treatment with cavitation activated brine is carried out according to formulas 6 and 7.

$$C_1 = \sqrt{a_1^2 + b_1^2}, \quad (6)$$

where: a_1 is the ratio of red and green colours in the colour of the meat sample before treatment with cavitation activated brine; b_1 is the ratio of red and green colours in the colour of the meat sample treated with cavitation activated brine.

$$C_2 = \sqrt{a_2^2 + b_2^2}, \quad (7)$$

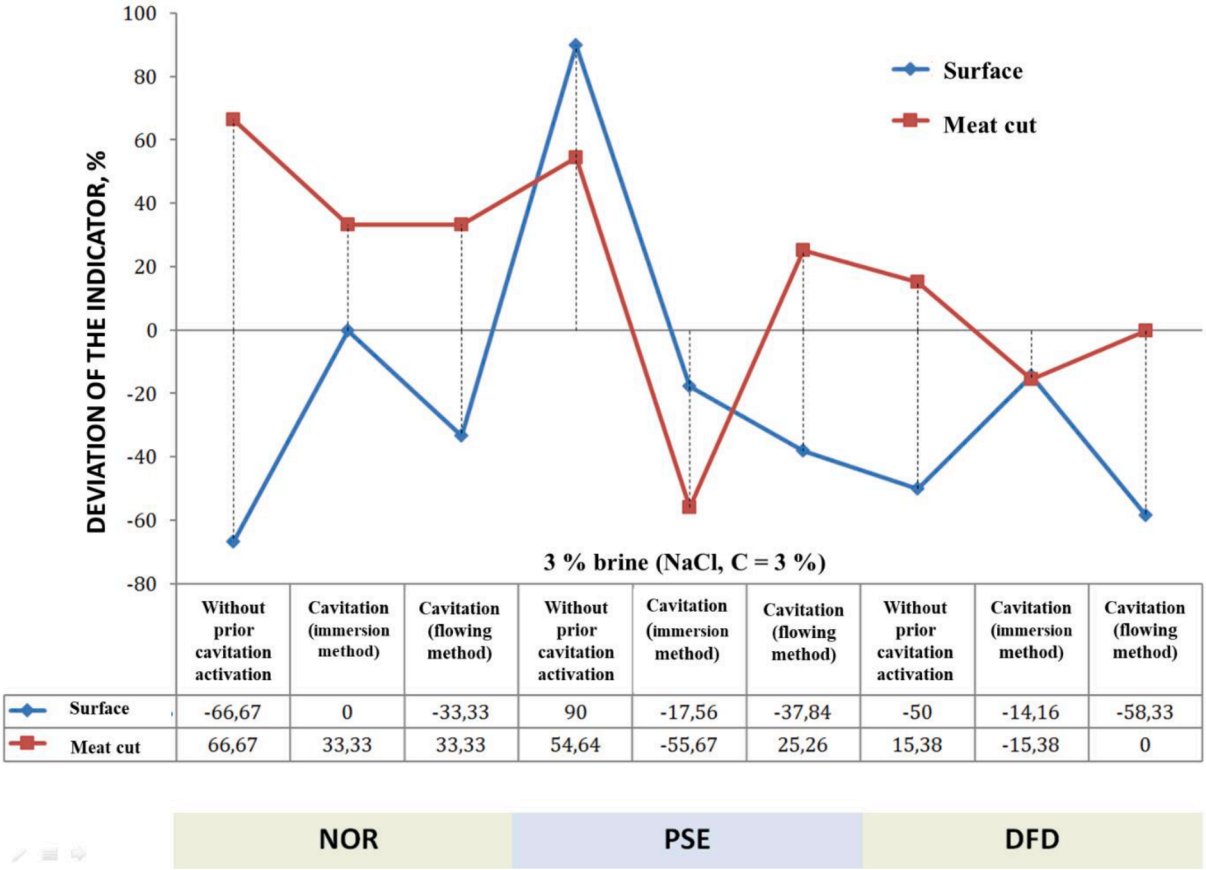
where: a_2 is the ratio of red and green colours in the colour of the meat sample before treatment with cavitation activated brine; b_2 is the ratio of red and green colours in the colour of the meat sample treated with cavitation activated brine.

The calculation of the difference between the colour saturation of meat samples before and after treatment with cavitation activated brine is carried out according to formula 8.

$$\Delta C = C_1 - C_2, \quad (8)$$

where C_1 is the colour saturation of the meat sample before treatment with cavitation activated brine; C_2 is the colour saturation of the meat sample treated with cavitation activated brine.

Determination of the colour tone of the samples before and after treatment with cavitation activated brine is carried out according to formulas 9 and 10.



	Without prior cavitation activation	Cavitation (immersion method)	Cavitation (flowing method)	Without prior cavitation activation	Cavitation (immersion method)	Cavitation (flowing method)	Without prior cavitation activation	Cavitation (immersion method)	Cavitation (flowing method)
Surface	-66,67	0	-33,33	90	-17,56	-37,84	-50	-14,16	-58,33
Meat cut	66,67	33,33	33,33	54,64	-55,67	25,26	15,38	-15,38	0

Fig. 2. Deviation of the value of the indicator a (degree of redness in the CIE Lab system) of test samples of NOR–, PSE–, DFD– pork from a (degree of redness in the CIE Lab system) of the corresponding control samples.

$$H_1 = \text{arctg}\frac{b_1}{a_1}, \tag{9}$$

where b_1 – component b* of the colour of the meat sample before processing cavitation activated brine; a_1 – component a* of the colour of the meat sample before treatment with cavitation activated brine.

$$H_2 = \text{arctg}\frac{b_2}{a_2}, \tag{10}$$

where b_2 – component b* of the meat sample treated with cavitation activated brine; a_2 – component a* of a meat sample treated with cavitation activated brine.

The determination of the colour tone change of the samples before and after the test (treatment with cavitation activated brine) is carried out according to formula 11.

$$\Delta H = 2\sqrt{C_1 C_2}\sin\left(\frac{H_1 - H_2}{2}\right), \tag{11}$$

where H_1 and H_2 are the colour tone of the meat sample before and after the test, respectively; C_1 and C_2 are the colour saturation of meat samples before and after treatment with cavitation activated brine, respectively.

Using formula 12, the average lightness value is calculated before and after treatment with cavitation activated brine.

$$L_{12} = \frac{L_1 + L_2}{2}, \tag{12}$$

where L_1 – component L of the meat sample before treatment with cavitation activated brine; L_2 – component L of the meat sample after treatment with cavitation activated brine.

The first weight function SL is calculated according to the formula 13.

$$S_L = 1 + \frac{0,014(L_{12} - 50)^2}{\sqrt{20 + (L_{12} - 50)^2}}, \tag{13}$$

The average value of the saturation of the samples before and after treatment with cavitation activated brine is determined by the formula 14.

$$C_{12} = \frac{C_1 + C_2}{2}, \tag{14}$$

where C_1 and C_2 – colour saturation of meat samples before and after treatment with cavitation activated brine, respectively.

According to the formula 15, the first weight function S_C is calculated.

$$S_C = 1 + 0,048C_{12}, \tag{15}$$

Determination of the average colour tone of meat samples before and after their treatment with cavitation activated brine was carried out according to formula 16.

$$H_{12} = \frac{H_1 + H_2}{2}, \tag{16}$$

where H_1 and H_2 – colour tone of meat samples before and after treatment with cavitation activated brine, respectively.

The determination of the weight function was carried out according to formulas 17 and 18.

Table 2
Colour study for NOR – meat [33].

Number of samples	Colour indicator	Meaning	Deviation (increase / decrease), %	Colour permanence, (Y_L/ Y_a/ Y_b), %
1	**On the surface**			
Control	Lightness L*	57,51	– *	
	Redness a*	3,52		
	Yellowness b*	9,86		
	On the internal cut			
	Lightness L*	58,02	– *	
	Redness a*	3,48		
	Yellowness b*	10,12		
2	**On the surface**			
3% brine	Lightness L*	62,72	**(↑) 8.77**	(↑)
	Redness a*	1,88	(↓)66.67	33.33
	Yellowness b*	5,66	(↓) 44.44	55.56
	On the internal cut			
	Lightness L*	52,16	(↓)10.34	89,66
	Redness a*	5,86	**(↑) 66.67**	(↑)
	Yellowness b*	8,48	(↓)20.00	80.00
3	**On the surface**			
3% brine + cavitation (submersible device)	Lightness L*	57,93	(0)	**100.00**
	Redness a*	3,75	(0)	**100.00**
	Yellowness b*	11,12	**(↑) 22.22**	(↑)
	On the internal cut			
	Lightness L*	60,02	**(↑)3.45**	(↑)
	Redness a*	4,25	**(↑)33.33**	(↑)
	Yellowness b*	10,96	(0)	(↑)
4	**On the surface**			
3% brine + кав. (flow device)	Lightness L*	63,20	**(↑) 10.53**	(↑)
	Redness a*	2,21	(↓)33.33	66.67
	Yellowness b*	5,21	(↓)44.44	55.56
	On the internal cut			
	Lightness L*	55,08	(↓)5.17	94.83
	Redness a*	4,63	**(↑)33.33**	(↑)
	Yellowness b*	7,91	(↓)30.00	70.00

A control sample of NOR – pork, relative to which is considered the deviation of the indicator a (degree of redness in the CIELab system) in experimental samples.

$$T = 1 - 0,17\cos(H_{12} - 30^{\circ}) + 0,24\cos(2H_{12}) + 0,32\cos(2H_{12} + 6^{\circ}) - 0,2\cos(4H_{12} - 64^{\circ}) \quad (17)$$

$$S_H = 1 + 0,014C_{12}T \quad (18)$$

where C_{12} is the average value of the saturation of the samples before and after treatment with cavitation activated brine.

After calculating the colour difference (ΔE) for each prototype, the level of deviations that occur during the exposure to ultrasonic cavitation-treated brine is determined. To assess stability, three levels are accepted [31,32]:

(1) minimum discernible colour difference ($\Delta E < 2$);
(2) acceptable discernible colour difference ($\Delta E = 2$–6);
(3) a noticeable difference between the colours of the control and the prototype ($\Delta E > 6$).

3. Results and discussion

In the first part of the study, a mathematical assessment [29] of the colour stability of meat systems with normal and abnormal autolysis was carried out for the meat sample with ultrasound-activated saturated brine. The degree of redness (a*) of chilled meat can be associated with

Table 3
The results of the study of colour characteristics for PSE – meat [33]

Number of sample	Colour indicator	Meaning	Deviation (increase / decrease), %	colour permanence (Y_L/ Y_a/ Y_b), %
5	**On the surface**			
Control	Lightness L*	63,57	– *	
	Redness a*	0,74		
	Yellowness b*	10,16		
	On the internal cut			
	Lightness L*	63,08	– *	
	Redness a*	1,94		
	Yellowness b*	10,87		
6	**On the surface**			
3% brine	Lightness L*	68,92	**(↑) 8.42**	(↑)
	Redness a*	1,91	**(↑)–**	(↑)
	Yellowness b*	5,93	(↓) 41.63	58,37
	On the internal cut			
	Lightness L*	59,86	(↓)5.10	94,90
	Redness a*	3,00	**(↑)54.64**	(↑)
	Yellowness b*	10,64	(↓) 2.11	97,88
7	**On the surface**			
3% brine + cavitation (submersible device)	Lightness L*	68,21	**(↑) 7.30**	(↑)
	Redness a*	0,61	(↓)17.56	82,44
	Yellowness b*	4,66	(↓) 54.13	45,87
	On the internal cut			
	Lightness L*	60,16	(↓) 4.63	95,37
	Redness a*	0,86	(↓)55.67	44,33
	Yellowness b*	9,70	(↓)10.76	89,24
8	**On the surface**			
3% brine + cavitation (flow installation)	Lightness L*	70,59	**(↑) 11.04**	(↑)
	Redness a*	0,46	(↓) 37.84	62,16
	Yellowness b*	5,00	(↓) 50.78	49,22
	On the internal cut			
	Lightness L*	62,88	(↓) 0.30	**100.00**
	Redness a*	2,43	**(↑) 25.26**	(↑)
	Yellowness b*	10,77	(↓) 0.92	99,08

* A control sample of PSE – pork, relative to which is considered the deviation of the indicator a* (degree of redness in the CIELab system) in the experimental samples.

the concentration of reduced Mb or OMb. Therefore, the indicator a*, degree of redness is of the greatest interest in the analysis [33]. Fig. 2 shows the deviation (%) in indicator a* (degree of redness in the CIELab system) for test samples NOR–, PSE– and DFD– of pork from control samples.

As shown in Table 2 and Fig. 2, it was observed that the impact of 3% brine solution on NOR-pork for 24 h negatively affects the value of the indicator a* (degree of redness) of its surface and positively in the thickness of the meat cut where the degree of redness (a*) decreased by 67% on the surface and increased by 67% on the cut compared with the values of a* of the samples of the control group. Pre-cavitation activation of a 3% brine solution in an immersion-type ultrasonic device maintains the degree of redness on the surface of the sample same as for the control sample whereas it increased in the cut by 33%. However, in the case of flow-type ultrasonic device, on the surface of the pork the redness increased by 33% compared to control sample without prior activation. This is not characteristic of degree of redness on the sample section (cut) NOR– pork [33].

The effects observed in meat with PSE defect indicate that the

Table 4
The results of the study of color characteristics for DFD – meat [33].

Number of sample	Colour indicator	Meaning	Deviation (increase/ decrease), %	colour permanence ($У_L$/ $У_a$/ $У_b$), %
9	**On the surface**			
Control	Lightness L*	44,58	– *	
	Redness a*	12,55		
	Yellowness b*	9,28		
	On the internal cut			
	Lightness L*	46,92	– *	
	Redness a*	13,67		
	Yellowness b*	9,84		
10	**On the surface**			
3% brine	Lightness L*	56,8	**(↑) 27.27**	**(↑)**
	Redness a*	6,48	(↓)50.00	50.00
	Yellowness b*	10,7	**(↑)11.11**	**(↑)**
	On the internal cut			
	Lightness L*	45,78	(↓)2.17	97.73
	Redness a*	15,45	**(↑)15.38**	**(↑)**
	Yellowness b*	10,73	**(↑) 11.11**	**(↑)**
11	**On the surface**			
3% brine + cavitation (submersible device))	Lightness L*	43.51	(↓)2.27	97,73
	Redness a*	10.3	(↓)14.16	85.84
	Yellowness b*	8.14	(↓) 09.55	90,45
	On the internal cut			
	Lightness L*	42,44	(↓) 8.70	91.30
	Redness a*	11,90	(↓)15.38	84,62
	Yellowness b*	8,45	(↓) 11.11	88.89
12	**On the surface**			
3% brine + cavitation (flow device)	Lightness L*	53,77	**(↑)20.45**	**(↑)**
	Redness a*	5,40	(↓) 58.33	41,67
	Yellowness b*	8,57	(↓) 11.11	88.89
	On the internal cut			
	Lightness L*	42,89	(↓) 8.70	91,30
	Redness a*	13,88	(0)	**100.00**
	Yellowness b*	09,60	(0)	**100.00**

The control sample DFD – pork, the relative which is considered the deviation of the indicator a (degree of redness in the CIELab system) in the experimental samples.

preliminary activation of a 3% brine in a low-frequency submersible type negatively affects the state of pigments of the PSE myoglobin group – pork both on the surface and in the thickness of the meat – on the surface a* decreased by 18%, at the cut – by 56% compared to the sample that was not treated with brine (Table 3, Fig. 2). Moreover, the preliminary activation of a 3% brine in a flow-through installation causes surface discoloration of PSE – pork where the value of a* decreased on the surface by 38% and by 25% the control sample that was not treated with brine [33]. Therefore, pre-activation of the brine in a submersible-type installation provides 20% higher colour stability of samples than the stability of samples when using a flow-through installation. From Fig. 2, it can be concluded that for PSE pork, the best values for a* (redness) in the CIE Lab system are recorded on the surface and on the slice for samples whose salting was carried out with 3% brine, that was not activated in a cavitation way.

Considering that the DFD defect, in comparison with the defect PSE, initially provides a dark red colour that is not typical for pork, for this type of raw material the task is to slow down the transition of the valence of the iron atom from Fe^{+2} to Fe^{+3}, leading to transformation of OMb into MetMb and accompanied by further darkening of muscle tissue. The preliminary cavitation activation of the 3% brine in a low-frequency submersible-type installation results in the reduction of a* of DFD pork on the surface and on the slice by 14% and 15%, respectively, to the control sample (untreated with brine) (Table 4, Fig. 2). The preliminary cavitation activation of the 3% brine in a flow-through installation results in the reduction of a* of DFD – pork on the surface by 58% compared to the control sample. In the thickness of the meat, a* (degree of redness) did not change compared to control.

At the second stage, an extended mathematical assessment of the stability of the colour characteristics of meat systems under the indirect influence of ultrasound (through a brine medium) was carried out, based on the calculation of the colour difference (ΔE). As shown in Fig. 3 and Table 5, to stabilize the colour of pork with the normal course of autolysis in the process of salting (3% brine) preliminary cavitation activation of the brine in a low-frequency submersible type can be used. The sonochemical treated 3% brine used for salting the meat results in achieving a colour almost identical to the colour of meat not subjected to salting, both on the surface (ΔE = 0.983) and on the slice (meat cut) (ΔE = 1,983) indicating that it can reduce the negative of effect of sodium chloride on meat.

Cavitation activation of a 3% brine in a flow-type reactor is less effective for preserving the colour of NOR – pork, due to a more significant loss of colour on the surface (ΔE = 6.359), however, the degree of preservation of colour in the thickness of the meat is also quite high (ΔE = 3.021), compared with samples that were not treated with activated brines. When considering meat with autolytic deviations, it was found that the best preservation of Mb is demonstrated by PSE samples – pork processed with 3% brine, both pre-activated and non-activated cavitation – the colour differences of all the indicated samples were in approximately the same range (average ΔE from 3.52 to 4.14) (Table 6, Fig. 4). However, the smallest colour difference with respect to the control sample was experienced with a 3% brine activated in a flow-through cavitation unit (average ΔE = 3.52), which is ensured by the smallest colour difference in the section among all samples (ΔE = 0.171) (Fig. 4). The best colour retention effect is achieved when processing DFD samples – meat with a 3% brine activated in a submersible type (the smallest average colour difference was recorded in relation to the control sample – ΔE = 3.2) (Table 7, Fig. 5). Moreover, with the selected technological mode of processing the brine, the minimum deviation of the colour of pork is fixed on the surface (ΔE = 1.925), while a more significant but acceptable colour change is observed on the slice compared to the control sample (ΔE = 4.521).

In relation to the samples for which salting was not used preliminary cavitation activation, all samples treated with brine activated by ultrasound have the smallest colour deviation in colour from the control samples. Thus, based on the analysis of the results obtained, it can be accepted as a debatable working hypothesis that the preliminary sonochemical treatment of the brine acts as a thread in relation to the Mb pigment system during oxidative processes occurring under the influence of high and low concentrations of sodium chloride during salting.

As is known, the state of Mb is closely related to the functional capabilities of mitochondria [34], whose role in ensuring the colour stability of meat is associated with the fact that during life in animals Mb is a carrier of oxygen to mitochondria in muscles, and after the death of an animal, mitochondria continue to metabolize oxygen in the post-mortem state is still a period of time [35]. Relations between the Mb molecule and mitochondria are competitive. Mitochondria can affect colour stability through oxygen uptake and decreased OMb levels. As a result, factors affecting mitochondrial activity can also affect the colour of meat. Mitochondrial respiration affects the colour of meat by reducing the partial pressure of oxygen, and mitochondria can also interact with OMb, causing the transfer of haem-linked oxygen from OMb to mitochondria. The low oxygen partial pressure resulting from an increase in mitochondrial activity maintains Mb in a deoxygenated state, while the meat does not form a characteristic bright pink colour. Radicals generated by lipid oxidation can promote the accumulation of MetMb, which can be reduced to the oxygen-binding form of OMb under normal reducing activity, but in a degrading oxidizing environment of muscles with free radicals formed during lipid oxidation, NADH is

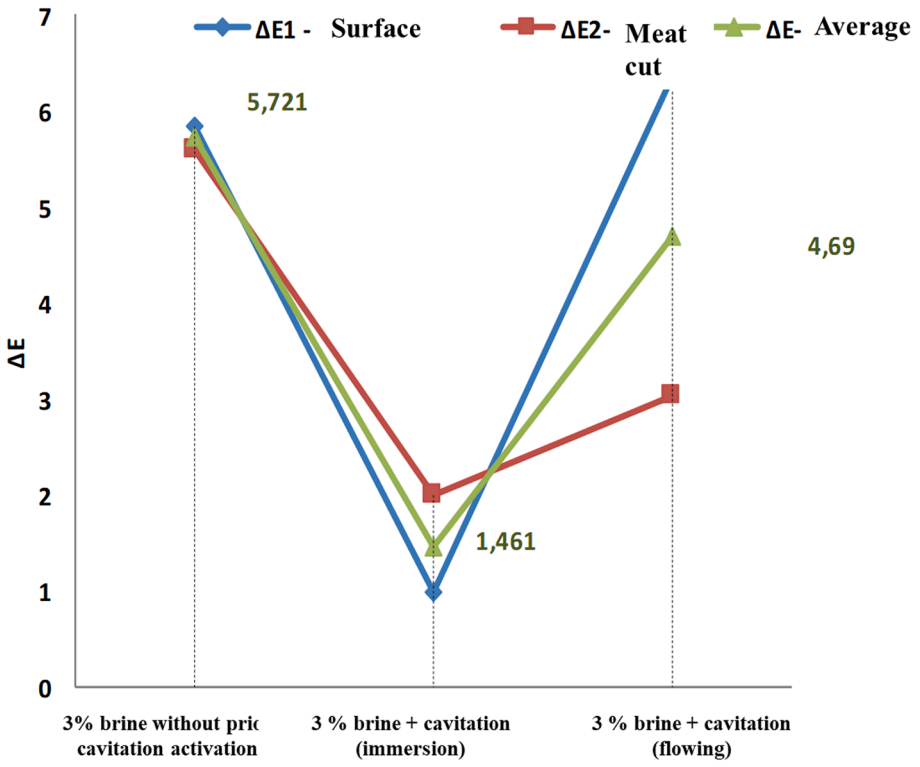


Fig. 3. The distribution of samples of NOR – meat in terms of colour difference (ΔE).

Table 5
The results of the mathematical evaluation of the color characteristics of the prototypes of NOR – meat.

NOR Short description	The level before Ultrasound		The level after ultrasound		Change in lightness	Average lightness value and weight function		Change in saturation	Average saturation value and weight function		Change hue	Average colour tone and weight function		Colour difference	General colour difference
3% brine without prior cavitation activation	**SURFACE**				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	**ΔE_{12}**
	L1	57.51	**L2**	62.72	–5.210	60.115	1.130	4.505	8.217	1.394	1.684	1.121	1.078	5.844	**5.721**
	a1	3.52	**a2**	1.88											
	b1	9.86	**b2**	5.66											
	MEAT CUT				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	
	L1	58.02	**L2**	52.16	5.860	55.090	1.054	0.394	10.505	1.504	0.611	1.210	1.087	5.597	
	a1	3.48	**a2**	5.86											
	b1	10.12	**b2**	8.48											
3% brine + cavitation (immersion)	**SURFACE**				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	**1.461**
	L1	57.51	**L2**	57.93	–0.420	57.720	1.094	–1.266	11.102	1.533	–0.403	1.246	1.087	0.983	
	a1	3.52	**a2**	3.75											
	b1	9.86	**b2**	11.12											
	MEAT CUT				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	
	L1	58.02	**L2**	60.02	–2.00	59.020	1.113	–1.054	11.228	1.539	–0.266	1.251	1.087	1.938	
	a1	3.48	**a2**	4.25											
	b1	10.12	**b2**	10.96											
3% brine + cavitation (flowing)	**SURFACE**				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	**4.690**
	L1	57.51	**L2**	63.2	–5.690	60.355	1.133	4.810	8.064	1.387	1.929	1.102	1.079	6.359	
	a1	3.52	**a2**	2.21											
	b1	9.86	**b2**	5.21											
	MEAT CUT				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	
	L1	58.02	**L2**	55.08	2.940	56.550	1.076	1.536	9.934	1.477	0.824	1.198	1.084	3.021	
	a1	3.48	**a2**	4.63											
	b1	10.12	**b2**	7,91											

depleted faster, reducing the reducing activity of Mb and leading to the accumulation of MetMb. As is known, mitochondrial oxygen consumption contributes to the formation of an anaerobic environment in which there is a decrease in MetMb. Mitochondrial-mediated reduction of MetMb occurs through the transfer of available electrons to MetMb using a cytochrome system. The reduction of MetMb to Mb occurs due to the metmioglobin reductase system, which consists of cytochrome b5, cytochrome b5 reductase (flavoprotein), hydrogen protons (H^+), the donor of which is NADH2, which is formed during glycolysis as a standard [6].

Initially, cytochrome b5 reduces Fe^{+3} MetMb to Fe^{+2} Mb according to reaction 19.

Table 6
The results of the mathematical evaluation of the colour characteristics of prototypes of PSE – meat.

PSE Short description	The level before Ultrasound		The level after ultrasound		Change in lightness	Average lightness value and weight function		Change in saturation	Average saturation value and weight function		Change hue	Average colour tone and weight function		Colour difference	General colour difference
3% brine without prior cavitation activation	**SURFACE**				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	**ΔE_{12}**
	L_1	63.57	**L_2**	68.92	–5,350	66,245	1,219	3,957	8,208	1,394	0.410	1.472	1.038	5.241	**4.02**
	a_1	0.74	**a_2**	1.91											
	b_1	10.16	**b_2**	5.93											
	MEAT CUT				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	
	L_1	63.08	**L_2**	59.86	3.220	61.470	1.150	–0.013	11.048	1.530	0.041	1.392	**1.063**	2.801	
	a_1	1.94	**a_2**	3.00											
	b_1	10.87	**b_2**	10.64											
3% brine + cavitation (immersion)	**SURFACE**				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	**4.14**
	L_1	63.57	**L_2**	68.21	–4.640	65.890	1.214	5.487	7.443	1.357	0.586	1.456	1.036	5.592	
	a_1	0.74	**a_2**	0.61											
	b_1	10.16	**b_2**	4.66											
	MEAT CUT				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	
	L_1	63.08	**L_2**	60.16	2.920	61.620	1.152	1.304	10.390	1.499	0.215	1.384	1.060	2.688	
	a_1	1.94	**a_2**	0.86											
	b_1	10.87	**b_2**	9.70											
3% brine + cavitation (flowing)	**SURFACE**				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	**3.52**
	L_1	63.57	**L_2**	70.59	–7.020	67.080	1.231	5.166	7.604	1.365	0.531	1.461	1.037	6.862	
	a_1	0.74	**a_2**	0.46											
	b_1	10.16	**b_2**	5.00											
	MEAT CUT				**ΔL**	**L_{12}**	**S_L**	**ΔC**	**C_{12}**	**S_C**	**ΔH**	**H_{12}**	**S_H**	**ΔE**	
	L_1	63.08	**L_2**	62.88	0.200	62.980	1.172	0.001	11.041	1.530	0.018	1.393	1.063	0.171	
	a_1	1.94	**a_2**	2.43											
	b_1	10.87	**b_2**	10.77											

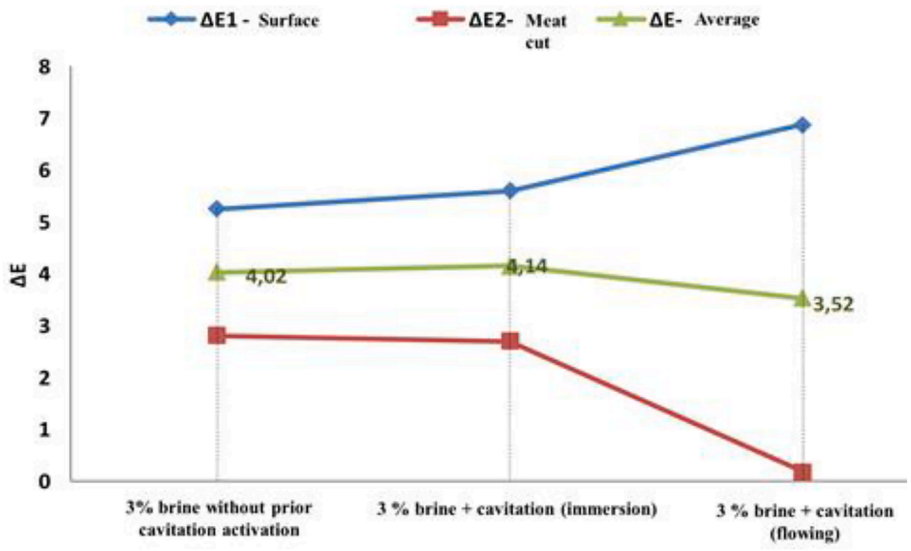


Fig. 4. The distribution of samples of meat with a defect PSE in terms of colour difference (ΔE).

$$\text{MetMb } (Fe^{+3}) + \text{cytochr.b5 (reduced)} \rightarrow \text{Mb } (Fe^{+2} + \text{cytochr. b5 (oxidized)} \quad (19)$$

Oxidized cytochrome b5 is reduced by cytochrome b5 reductase with the release of free electrons (reaction 20).

$$\text{MetMb } (Fe^{+3}) + NADH_2 \rightarrow \text{cytochr.b5 (reduced)} + NAD^+ \quad (20)$$

However, the presence of defects in the structure and in the functional ability of enzymes, as well as in case of a malfunction of the metmyoglobin reductase system, the restoration of MetMb may not go enzymatically – due to its own antioxidant systems – vitamin B_{12}, glutathione, ascorbic acid and others that are found in fresh meat [36].

The oxidation of their own antioxidant systems is considered using glutathione as an example and occurs according to reaction 21 [37].

$$GSH \rightarrow GSSG + 2H+ + 2\ e^- \quad (21)$$

During the reaction, free electrons and hydrogen ions are released, which cause a decrease in the pH of muscle tissue. In this case, free electrons are sent to restore MetMb to Mb.

The reduction reaction of Fe^{+2} in Fe^{+3} proceeds according to reaction 22.

$$Fe^{+3} + e^- \rightarrow Fe^{+2} \quad (22)$$

Acoustic cavitation that occurs in liquid food media (brine) gives rise to a large number of redox reactions that result in the breaking of chemical bonds and the release of energy in the form of free electrons. Thus, cavitation-activated brine acts as a powerful electron donor that can be used to reduce MetMb to Mb. Consequently, the resulting MetMb will be the acceptor of free electrons, and the cavitation activated brine will be their donor.

Table 7
The results of the mathematical evaluation of the color characteristics of prototypes DFD – meat.

DFD = 6,60 Short description	The level before Ultrasound	The level after ultrasound	ИзМенение светлоты	Average lightness value and weight function	Change in saturation	Average saturation value and weight function	Change hue	Average colour tone and weight function	Colour difference	General colour difference
3% brine without prior cavitation activation	**SURFACE**		**ΔL**	L_{12}	S_L	**ΔC**	C_{12}	S_C	**ΔH**	H_{12}	S_H	**ΔE**	**ΔE_{12}**
	L_1 44.58	L_2 56.8	–12.220	50.690	1.001	3.099	14.059	1.675	–0.968	0.671	1.182	12.369	**7.00**
	a_1 12.0.55	a_2 6.48											
	b_1 9.28	b_2 10.7											
	MEAT CUT		**ΔL**	L_{12}	S_L	**ΔC**	C_{12}	S_C	**ΔH**	H_{12}	S_H	**ΔE**	
	L_1 46.92	L_2 45.78	1.140	46.350	1.032	–1.967	17.827	1.856	–0.740	0.645	1.233	1.644	
	a_1 13.67	a_2 15.45											
	b_1 9.84	b_2 10.73											
3% brine + cavitation (flowing)	**SURFACE**		**ΔL**	L_{12}	S_L	**ΔC**	C_{12}	S_C	**ΔH**	H_{12}	S_H	**ΔE**	**6.8**
	L_1 44.58	L_2 53.77	–9.190	49.175	1.002	5.479	12.869	1.618	0.472	0.618	1.169	9.785	
	a_1 12.55	a_2 5.4											
	b_1 9.28	b_2 8.57											
	MEAT CUT		**ΔL**	L_{12}	S_L	**ΔC**	C_{12}	S_C	**ΔH**	H_{12}	S_H	**ΔE**	
	L_1 46.92	L_2 42.89	4.030	44.905	1.054	0.295	16.696	1.801	0.687	0.603	1.221	3.870	
	a_1 13.67	a_2 13.88											
	b_1 9.84	b_2 9.01											
3% brine + cavitation (immersion)	**SURFACE**		**ΔL**	L_{12}	S_L	**ΔC**	C_{12}	S_C	**ΔH**	H_{12}	S_H	**ΔE**	**3.2**
	L_1 44.58	L_2 43.51	1.070	44.045	1.067	2.480	14.368	1.690	0.878	0.606	1.190	1.925	
	a_1 12.55	a_2 10.3											
	b_1 9.28	b_2 8.14											
	MEAT CUT		**ΔL**	L_{12}	S_L	**ΔC**	C_{12}	S_C	**ΔH**	H_{12}	S_H	**ΔE**	
	L_1 46.92	L_2 42.44	4.480	44.680	1.057	2.248	15.719	1.755	1.101	0.589	1.209	4.521	
	a_1 13.67	a_2 11.9											
	b_1 9.84	b_2 8.45											

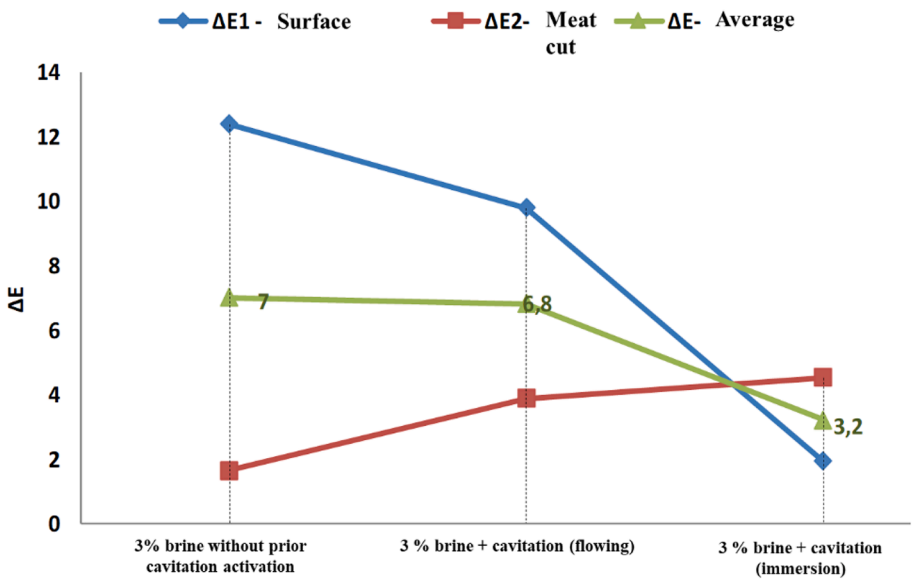


Fig. 5. The distribution of meat samples with DFD defect in terms of colour difference (ΔE).

4. Conclusions

The use of low-frequency cavitation for the activation (20 kHz) of liquid salting media makes it possible to level the oxidizing effect of sodium chloride during salting and to stabilize the colour characteristics of meat systems due to the activation of metmioglobin-reducing activity of meat (MPA). This supports the MetMb transformation process in Mb. MRA activation occurs due to the appearance of a donor – acceptor meat system between the activated brine and MetMb, in which the cavitation activated brine is the donor, and MetMb is the acceptor. Recommendations have been developed for surface and intramuscular stabilization of the colour of pork with the normal course of autolysis by means of cavitation activation of liquid salting media based on sodium chloride.

According to the results of stability assessment of the colour of meat, based on an additive calculation of deviations (increase / decrease) in the coordinates L*, a*, b* in the CIE Lab system, it was shown that the most effective is the use of preliminary cavitation activation of 3% brine in an immersion-type ultrasonic installation. This a makes it possible to maintain the value of a* on the surface of the NOR-pork sample same as the control sample and to achieve an increase in this indicator on the meat cut by 33% compared to the control sample. This is consistent with the calculation of total colour difference (ΔE) – for a given sample on the surface, ΔE was 0.983, on the slice ΔE – 1.983, which indicates a minimally distinguishable difference in the colour of the experiment, compared to the control.

The preliminary activation of the brine in a flow-through installation caused surface discoloration of PSE – pork according to the a* indicator (redness of meat) – the value of a* decreased by 38% on the surface and by 25% in the cut with respect to the a* of the control sample. The additive deviation calculation technique based on the CIE Lab model showed the negative indirect effect of ultrasound on the colour of meat with PSE defect. However, when calculating ΔE, it was noted that the prototype treated with brine activated in the flow-through installation had the smallest average colour deviation from the control, which was ensured by the smallest colour difference at the slice among all the experimental samples (ΔE = 0.171).

Based on the calculations of the colour stability of the components L*, a* and b*, for the stabilization of the colour of DFD pork, the preliminary cavitation activation of the brine in both flow and submersible types was recognized as the most effective due to the possibility of reducing the a* values of DFD pork on the surface compared with the control (for flow-through installation – by 58%, for submersible installation – by 14%) and in the thickness of meat (for submersible installation – by 15%, for flow-through installation – no reduction was found). According to the total colour difference, for DFD pork, in order to optimize its colour parameters, the pre-activation of the brine in a submersible type turned out to be the most effective; the smallest average colour deviation of the prototype with respect to the control sample was recorded (on the surface ΔE –1.925, on the cut ΔE – 4.521).

Thus, the method for calculating the stability of deviations of individual values of the coordinates L*, a* and b* in the CIE model system has shown its insolvency, which necessitates the parallel use of more detailed and selective calculation methods similar to the method for calculating colour differences (ΔE).

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105363.

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