

EFFECT OF SELECTED FUNCTIONAL ADDITIVES
ON THE PROPERTIES OF PSE PORK MEAT

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Abstract. The effect of the chosen additional substances, microbial transglutaminase and sodium caseinate on the functional properties of PSE meat, resulting from the changes in fraction of cytoskeletal proteins, was examined. The research material included meat from the following ham muscles: normal (RFN) and watery meat (PSE) and meat batter after tumbling and model hams produced from RFN and PSE meat with the studied functional additives and without them. It was found that transglutaminase as added to PSE meat, participated in the binding of released proteins from myofibrils and in the generation of protein aggregates, modifying its functional properties. It had a significant influence on the improvement of consistency. At the same time, a deterioration of the water binding ability of the product took place. Sodium caseinate, as added to PSE meat enhanced aggregation of the proteins, released from myofibrils and significantly improved water binding, causing a weakening of the structure and in the consistency of the final product. None of the functional additives employed, used as a single substance, decreased significantly the unfavorable effects of PSE meat processing to a satisfactory degree and only their joint application should bring positive results.

Key words: pork PSE meat, microbial transglutaminase, cytoskeletal protein fraction, washed myofibrils, centrifugal drip

INTRODUCTION

One of the quality defects of pork meat most frequently occurring is its watery meat. Such meat is defined as PSE (pale, soft, exuding) meat; this differs from normal meat by a lighter and less stable color, a softer consistency, inherent and non-elastic structure and considerable wateriness which results from its small water-binding capacity; water may thus easily drip from the meat [23].

The basic functional properties of meat are, first of all, its tenderness, water binding capacity and gel forming and emulsifying properties as determined by myosin – the main myofibril protein of muscle tissue and its reactions with actin. Studies conducted during recent years have contributed to the discovery of new cytoskeletal proteins and have attempted to determine the role of these proteins in processes of water-binding capacity and meat tenderness. Among the newly discovered cytoskeletal proteins, titin is found in the greatest quantities. As studies have shown, it plays a significant role in the post-slaughter changes of meat, and the degree of its degradation may be related to the improvement of meat tenderness [1,10,19,28], or to the incidence of watery meat [2,20].

PSE meat is characterized by its lowered culinary and processing value. The quality of products, manufactured from this meat is worse and is characterized by a lack of any appropriate consistency and juiciness, a deterioration of texture, the weak binding ability of the meat 'en bloc' and excessive quantity of thermal drip.

The unfavorable consequences of processing meat with PSE properties may be relieved, to a certain degree, by the application of substances, which bind water in a stable way, such as e.g. functional proteins. Phosphates and carbonates, as added to the meat stuffing, play a similar function [24]. During recent years, transglutaminase, as a functional additive, has raised much interest [16,9,12,13]. This enzyme forms stable covalent cross-bonds between the particles of proteins and peptides and causes far-reaching modifications in their structure. It may have an effect on the change in the water-holding capacity of the muscle tissue and an improvement of the texture of the final product.

The aim of the present study was to determine the effect of the following additives: microbiological-origin transglutaminase and sodium caseinate on the functional properties of PSE meat, resulting from the changes in the fraction of cytoskeletal proteins.

RESEARCH MATERIALS

The experimental material included:

- meat from selected ham muscles: *m. semimembranosus* (top round, topside) and *m. biceps femoris* (lower side) being classified in two quality classes:
 - normal (RFN) meat, characterized by pH₂ value in the limits of 5.7 - 6.3 24 hours after slaughter and electric conductivity below 10 mS,
 - watery (PSE) meat – characterized by pH₂ value below 5.7 24 hours after slaughter and conductivity above 10 mS;

- meat paste after tumbling - obtained after tumbling disintegrated ham muscles in brine and the studied functional additives (2 preparations of transglutaminase and sodium caseinate) or without it;
- a model ham – the selected muscles from the chosen quality group (RFN, PSE) were minced in a mincer, supplied with a kidney plate and a two-part knife; then, they were covered in brine of the following composition: water – 86.88%, salt – 11.3%, sugar – 1.10%, sodium ascorbate – 0.22%, sodium glutamate – 0.22%, protein hydrolysate – 0.22% and sodium nitrate – 0.055%. The brine was used in the quantity of 30% in relation to the weight of disintegrated meat. Before the mixing of meat mass of variants with the addition of microbiological transglutaminase (TG), the preparations “Activa WM” or “Activa EB” in the quantity of 0.1 % in relation to the meat weight were added to curing brine. The commercial preparations of microbiological transglutaminase, offered by the Ajinomoto Company, contained: 1.0% of active enzyme, suspended in a maltodextrin carrier in the case of “Activa WM” or 0.5% of this enzyme in the case of the “Activa EB”. The meat mass thus prepared was tumbled in a “Stephan” tumbler (10 rotations/min) according to the following tumbling cycles:
 - tumbling I – 30 minutes – lowered pressure – 200 hPa, break – 3 hours
 - tumbling II - 3 times in 25 minutes, lowered pressure – 400 hPa, break equal to 10 minutes between the work cycles – relaxation. after the last cycle of work – relaxation – 16 hours.
 - tumbling III – 40 minutes, lowered pressure, 400 hPa and relaxation 1 hour.

After completion of tumbling, some of the meat mass was sent for testing of the protein fraction (including centrifugal drip tests). The remainder of the meat mass was packed into 450 g cylindrical tins with a diameter of \varnothing of 102 and a height of 63 mm) and pasteurized at a temperature of 75°C until the temperature was 72°C in the middle of the meat. The tins were then intensively cooled to room temperature and transported to a refrigeration room where they were stored at about 2-4°C until the studies began.

Four variants of model pork hams were produced. The differentiating factors included the type of meat (RFN or PSE) and the lack of (0.0%) or the 0.1% addition of the microbiological transglutaminase preparation, with the commercial name “Active WM” (TG). No model hams were manufactured from the samples of meat mass containing the “Activa EB” preparation. They were used only in the studies on the myofibrillar proteins.

The experiment was performed in three repetitions. The statistical evaluation of the results was conducted using the Statgraphic for Windows program ver. 3.1.

METHODS

In the raw meat, the following determinations were performed:

- measurement of the pH of the meat - this was conducted using a mobile pH-meter of "WTW" Company, model pH 320/set-Z, supplied with the electrode, integrated with the temperature compensation;
- measurement of electric conductivity- this was conducted, using a device for measuring meat quality PQM I of the INTEK GmbH Company, with a head for measuring electric conductivity;
- basic composition – total protein content by the Kjeldahl method according to PN-A-04018 1975, using the Kjeltex Analyzer 1026; the water content by the dryer method according to PN-ISO 1442:2000 and the fat content by the Soxhlet method according to PN-ISO 1444:2000;
- the centrifugal drip from the raw meat and from the meat after tumbling (the samples for testing the protein fractions only) – this was determined by the separation method, using a Beckman centrifuge - type J2-21 - and the conditions of centrifugation were: 30 000 G for 15 min., temperature of -1°C [15];
- free water content – in the RFN and PSE meat, determined by the filter paper method, developed by Wolovinska and Kelman [31], modified by Gracz and Dolata [6], and then, by Makała and Olkiewicz [15];
- electrophoretic separation of proteins – extraction of proteins from myofibrils was performed by the method according to Fritz et al. [4], using buffer rigor with $\text{pH} = 7$; electrophoresis was conducted in a vertical system on a 2-layer polyacrylamide SDS gel using instruments of the Hoefer Scientific Instruments Company - type SE 250 - according to the method developed by Fritz et al. [4], with later modification of Pospiech et al. [25], consisting in the use of an 8M urea addition in the separating layer of gel.

In the final product, the following tests were carried out:

- thermal drip from the preserved meat block – the test performed by the weight method according to own methodology [18],
- slice strength –UTM Zwick model 1445 MOPS was used; the measurement of maximum force, breaking 3 mm thick and 60 mm – wide slices of the product, calculated into resistance of the slices to breaking was performed according to the procedure developed at the Meat and Fats Research Institute [29],
- rheological characteristics – by the CASRA method (Continuously Stress-Relax Analysis) – the test was performed in a Zwick instrument, model 1445 MOPS; penetrometric measurement of the sample's deformation, using the time-variable stress and relaxation program, allowing the characteristic rheograms to be obtained and graphically determine the plasticity (P), elasticity (E) and fluidity (F) limits [30].

DISCUSSION OF RESULTS

The results of the physico-chemical analyses, characterizing the raw meat, used in the experiment are presented in table 1. PSE meat, as compared to normal meat was characterized by a significantly lower pH value and significantly higher electric conductivity. The total water content in the PSE meat was only slightly lower than in the normal meat – on average by 1.3%. On the other hand, the free water content was almost double and this is based solely on the evidence of the PSE meat. Free water easily exudes from the product, as affected by various external factors, because it is not – as is most of water present in the muscle tissue – connected with a spatial tissue structure via hydrogen bridges and electrostatic forces, or adsorption-like related to polar ions of proteins and saccharides. The significantly higher content of total protein and the higher fat content in the PSE meat were caused by the dripping of water from this meat causing a relative increase in the content of the remaining components.

Table 1. Characteristics of pork meat used in tests

Discriminants	RFN meat	PSE meat	LSD
Water content (%)	75.6	74.3	1.9
Protein content (%)	19.8 ^a	21.7 ^b	1.6
Fat content (%)	2.6	2.8	1.1
pH	5.89 ^b	5.56 ^a	0.22
Electrical conductivity (mS)	8.0 ^a	15.3 ^b	7.3
Centrifugal drip (%)	5.6 ^a	9.0 ^b	2.2
Protein content in centrifugal drip (%)	10.2	10.0	1.5
Free water content (%)	13.9 ^a	22.6 ^b	4.6

^{a, b} – means in lines with different superscript are significantly different ($P < 0.05$)

RFN – meat of normal quality, PSE – watery meat, LSD – Least Significant Difference.

The electrophoretic separation of proteins of fraction of washed myofibrils and fraction of centrifugal drips, as obtained from meat of different qualities has been presented in figure 1 and 2 and the percentages of the selected proteins of these fractions have been shown in tables 2 and 3.

The separations obtained as a result of the electrophoresis of proteins, isolated from PSE meat were characterized by a somewhat higher number of bands as compared to those from the meat of normal quality. In the first case, 30-36 bands were obtained while in the second, 27-30 bands. This may be connected with the

more rapid changes of proteins occurring in PSE meat directly after slaughter - especially in the range below 42 kDa.

It is well known that in the case of watery, raw PSE meat, the extraction of proteins is weaker [3,11,14,27] and the bands of high-molecular proteins with MW above 200 kDa are considerably more pronounced. The percentage of native titin (T1) is somewhat higher in the case of myofibrils from PSE meat as compared to RFN meat which is indicated by the data presented in table 1. In the normal meat, double the amount of titin-degraded products was found which is evidence of the more rapid degradation of cytoskeletal proteins. The slower degradation of the cytoskeletal proteins in watery muscles is a typical picture, being found not only in pig meat [2] but also in turkey meat [21, 22,26].

Relatively small differences between the meat of both quality groups appeared in the case of troponin T (Tn-T) although the recent study by Pospiech *et al.* [24] indicated that in the case of fast glycolysis, a quicker degradation of troponin complex proteins was found, including mainly Tn-T. The probable reason for these small differences could be the fact that the samples used in the tests were not collected directly after slaughter. The results from the study of Pospiech *et al* [26] showed that the greatest differences in the proteins of watery (PSE) and normal (RFN) meat appear soon after slaughter.

It is much easier to observe the liberation of myofibril proteins from muscle tissue based on the analysis of the centrifugal drip from the meat [7]. If we find the presence of proteins in the drip, which, as a rule, should not be present, this may indicate evidence of protein degradation and its shifting from the muscle fibers to the ambient environment.

The data, contained in table 2 shows that the samples of the centrifugal drip from the PSE meat as compared to the drip from the normal (RFN) meat, contained less titin (T1) and products of its degradation (T2) and smaller chains of myosin with a molecular weight of ca. 200 kDa. These differences resulted from the increased liberation of myosin from the myofibrillar structure and the quicker degradation of the cytoskeletal proteins in the normal meat. According to other authors [1,10,19,28], these differences may be evidence of the better tenderness and water-binding capacity of normal meat.

The addition of substances to PSE meat which should improve its technological properties showed that the electrophoretic separation of proteins of the centrifugal drip, obtained from these samples, was distinctly different when compared with the separation of proteins of the centrifugal drip from RFN or PSE meat. Proteins were found in these drips (samples PSE1 and PSE2), which did not appear in the drips from PSE and RFN meat, or were present in smaller quantities.

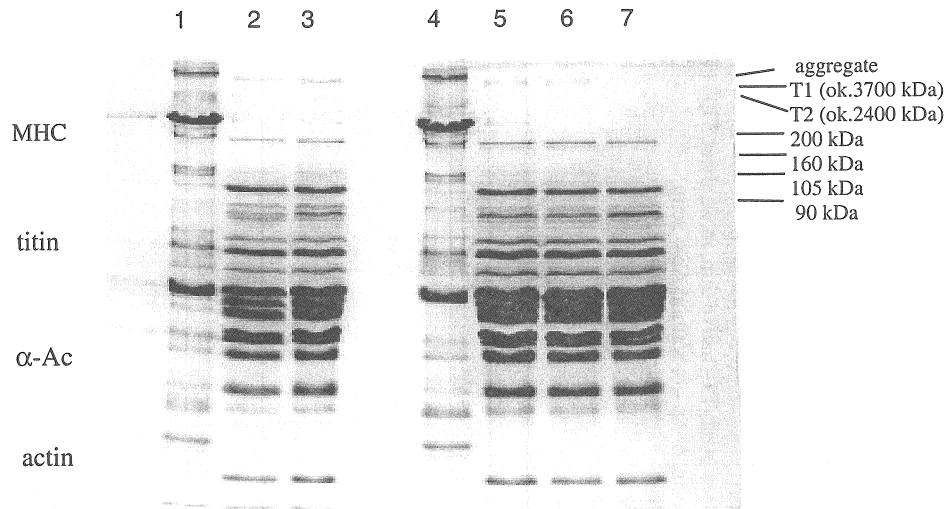


Fig. 1. Protein separation of the washed myofibrils fraction and the fraction of centrifugal drips obtained from diverse quality meat. Band: 1 – washed myofibrils from meat of normal quality, 2,3 – centrifugal drips from meat of normal quality, 4 – washed myofibrils from watery meat, 5,6,7 – centrifugal drips from watery meat

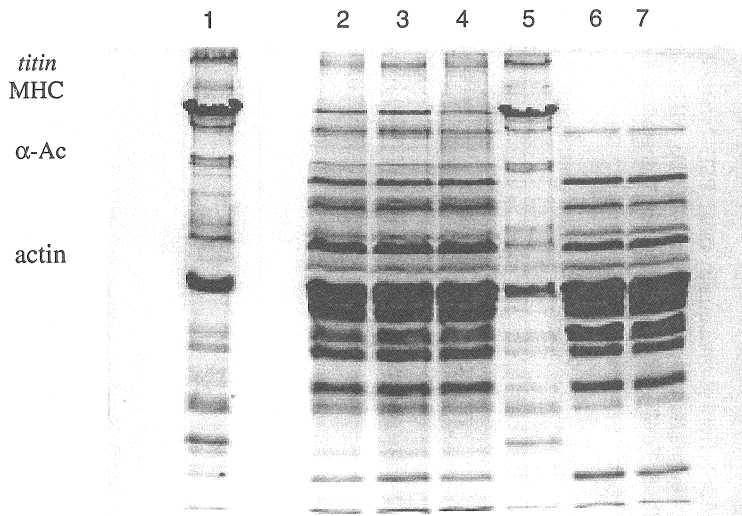


Fig. 2. Protein separation of the washed myofibrils fraction and the fraction of centrifugal drips obtained from diverse quality meat. Band: 1 – washed myofibrils from watery meat, 2 – centrifugal drips from watery meat with 0.1% „Activa EB” preparation (PSE1), 3 – centrifugal drips from watery meat with 0.1% „Activa WM” preparation (PSE2), 4 – centrifugal drips from watery meat with 2% sodium caseinate preparation (PSE3), 5 – washed myofibrils from meat of normal quality (RFN), 6 – centrifugal drips from meat of normal quality (RFN), 7 – centrifugal drips from watery meat (PSE).

Table 2. Percentage of the selected proteins of fraction washed myofibrils of ham meat

Type of meat	Protein fraction						
	T1 3700 kDa	T2 2400 kDa	MHC 200 kDa	α -Ac 105 kDa	Ac 42 kDa	Tn-T	Prod. Tn-T
RFN	5.41	1.94	20.12	6.25	20.83	2.58	5.72
PSE	6.05	0.92	22.37	4.47	19.75	2.41	5.56

RFN – washed myofibrils from normal quality meat, PSE – washed myofibrils from watery meat, T1 – native titin, m.w. about 3700 kDa
 T2 – product of titin degradation T1, m.w. about 2400kDa, MHC – heavy chains of myosin, α -Ac – α -actinin, Ac – actin,
 Tn-T – troponin T, Prod. Tn-T – product of troponin T degradation (about 28 – 30kDa)

Table 3. Percentage of the selected proteins of fraction centrifugate drip of ham meat

Experiment	Test	Protein fraction							
		aggregate	3700 kDa	2400 kDa	200 kDa	160 kDa	105 kDa	90 kDa	
1	RFN	0.46	0.11	0.20	0.32	1.30	0.53	6.54	
	PSE	0.43	0.13	0.17	0.26	1.48	0.34	5.97	
	PSE1	trace	1.57	0.48	2.89	3.51	1.45	5.17	
	PSE2	0.30	2.13	0.69	3.03	4.56	1.46	5.19	
	PSE3	0.69	1.14	1.49	4.96	3.75	2.39	4.83	
2	RFN	0.30	1.29	0.34	0.57	1.55	1.13	7.67	
	PSE	0.28	0.69	0.14	0.35	1.65	0.55	6.25	

RFN – drip from raw meat of normal quality,
 PSE – drip from raw watery meat,
 PSE1 – drip from raw watery meat after tumbling with 0.1 % „Activa EB” preparation,
 PSE2 – drip from raw watery meat after tumbling with 0.1 % „Activa WM” preparation,
 PSE3 – drip from raw watery meat after tumbling with 2.0 % sodium caseinate preparation.

The content of proteins with a molecular weight of 3700 kDa (probably mainly the T1) was increased by more than 10 times and the quantity of proteins with molecular weight of 2400 kDa and 105 kDa was 2-3 times higher than in the PSE and RFN meat drips. The above changes are probably a consequence of the total effect of the curing and tumbling processes and also, the influence of transglutaminase. During these treatments, the effect of which was evaluated in total, the ionic strength of the brine and the effect of the mechanical plastification of the meat caused the release of myofibrillar proteins from the structure and also, the generation of new proteins modifying the meat's properties. This has been confirmed by the results of the test on 'slice strength' and the rheological characteristics of model hams (tab. 4). The desirable technological effect of the transglutaminase (TG) action was illustrated by the significant improvement of the slice strength: from value 1.50 N cm^{-2} in the samples from PSE meat without transglutaminase, to 1.80 N cm^{-2} in the sample with the addition of the enzyme. An improvement in the rheological properties of the model hams, (as expressed in the significant rise of their plasticity from $6.55 \times 10^5 \text{ N m}^{-3}$ in the samples without transglutaminase to $7.72 \times 10^5 \text{ N m}^{-2}$ in the sample with TG preparation and in a lowering of elasticity from $6.63 \times 10^{-6} \text{ m}^2 \text{ N}^{-1}$ to $5.56 \times 10^{-6} \text{ m}^2 \text{ N}^{-1}$ and fluidity from $5.56 \times 10^{-8} \text{ m}^2 \text{ N}^{-1} \text{ s}^{-1}$ to $5.05 \times 10^{-8} \text{ m}^2 \text{ N}^{-1} \text{ s}^{-1}$), was also found.

When evaluating the effect of transglutaminase on the water-binding capacity of PSE meat, we should stress that the process observed of releasing the proteins and generation of new aggregates, including aggregates with transglutaminase, affected unfavorably the water-binding capacity which is supported by the results of the tests with the thermal drip (tab. 3). After the addition of the transglutaminase, the drip increased when compared to the sample without this additive, by 5.6% on average. The reason for this could be the participation of transglutaminase in the formation of strong covalent cross-bonds between the particles of proteins and peptides which caused such a strengthening of protein structures that an additional, mechanical squeezing of water from the product, containing PSE meat, took place.

The separations of proteins of the centrifugal drip obtained indicate a similar effect of the both transglutaminase preparations, as being added to the meat during its tumbling process (see fig. 2 and tab. 2). On the other hand, the addition of sodium caseinate to the meat caused the appearance of one band more in the electrophoretic picture, which was not present in the previous separations. This was a band with a molecular weight of more than 3700 kDa. The studies of Fritz et al. [5] and Grześ [8] showed that proteins of such a weight might constitute the

products of aggregation. In the case of both the studies cited, titin was present in the composition of the aggregates. Additionally, the study of Grześ [8] demonstrated that the aggregates also contained myosin. The role of these aggregates in the formation of the functional properties of meat and its structure is not fully explained. The studies of Grześ [8] and Fritz *et al.* [5] showed that together with the prolongation of the tumbling and heating, the incidence of aggregates was more intensive. These results indicate that milk proteins may stimulate the generation of aggregates because the percentage content of this band in the separation of proteins was almost twice as high compared to the remaining samples. Besides, the addition of caseinate caused a decrease of the band responding to T1 by half, almost triple the increase of the content of band 2400 kDa (band T2) and an increase, by more than half of the proteins with a molecular weight of 200 kDa (tab. 2).

Table 4. Binding ability and rheological characteristics of model hams made of RFN and PSE meat

Test	Binding ability		Rheological characteristics		
	Slice strength (N cm ⁻²)	Thermal drip (%)	Plasticity (x 10 ⁵ N m ⁻²)	Elasticity (x10 ⁻⁶ m ² N ⁻¹)	Fluidity (x10 ⁻⁸ m ² N ⁻¹ s ⁻¹)
RFN	2.65 ^c +/-0.21	11.3 ^a +/-0.7	6.87 ^{ab} +/-0.47	6.10 ^{ab} +/-0.88	4.34 ^a +/-0.17
PSE	1.50 ^a +/-0.04	19.1 ^c +/-0.8	6.55 ^a +/-0.84	6.63 ^b +/-0.82	5.05 ^a +/-0.59
PSE 2	1.80 ^b +/-0.07	24.7 ^d +/-1.7	7.72 ^{bc} +/-0.50	5.56 ^{ab} +/-0.26	5.56 ^b +/-0.26
PSE 3	1.34 ^a +/-0.07	15.4 ^b +/-1.39	6.46 ^a +/-0.21	5.65 ^{ab} +/-0.19	4.73 ^a +/-0.30
NIR	0.22	2.4	0.96	1.12	1.12

a, b, – means in lines with different superscript are significantly different (P<0.05)

LSD – Least Significant Difference,

RFN – raw meat of normal quality,

PSE – raw watery meat,

PSE1 – raw watery meat after tumbling with 0.1 % „Activa EB” preparation,

PSE2 – raw watery meat after tumbling with 0.1 % „Activa WM” preparation,

PSE3 – raw watery meat after tumbling with 2.0 % sodium caseinate preparation.

The changes observed in the proteins resulting from the addition of sodium caseinate to the PSE meat favorably affected water binding which is confirmed by the comparison of the size of the thermal drip (tab. 4). The addition of sodium

caseinate to the ham produced from the PSE meat decreased the thermal drip by 3.7% in comparison to the ham manufactured from PSE meat without the caseinate. Clarification of whether the water was retained by the generated protein aggregates, or whether it was bound by the caseinate alone, requires further study.

The retention of a great quantity of water in the hams caused a lowering of the resistance of the slices to breaking by 0.46 N cm^{-2} as compared to the PSE meat sample without caseinate. Also, a change in the rheological characteristics took place. Plasticity was lowered from $6.55 \times 10^5 \text{ N m}^{-2}$ to $6.46 \times 10^5 \text{ N m}^{-2}$, elasticity from $6.63 \times 10^{-6} \text{ m}^2 \text{ N}^{-1}$ to $5.65 \times 10^{-6} \text{ m}^2 \text{ N}^{-1}$ and fluidity from $5.56 \times 10^{-8} \text{ m}^2 \text{ N}^{-1} \text{ s}^{-1}$ to $4.73 \times 10^{-8} \text{ m}^2 \text{ N}^{-1} \text{ s}^{-1}$.

The study conducted suggests that the single addition of any of the functional additives employed did not decrease the unfavorable consequences of PSE meat to any satisfactory degree and only their total application should bring a positive effect.

CONCLUSIONS

1. Transglutaminase, when added to PSE meat participated in the binding of released proteins from myofibrils and in creating protein aggregates, modifies its functional properties. It affected significantly the improvement of consistency. At the same time, the product's water binding deteriorated.
2. Sodium caseinate when added to PSE meat intensified the aggregation of proteins, released from the myofibrils and improved water binding significantly, causing a weakening of the structure and consistency of the final product.
3. None of the functional additives employed, as a single substance, decreased the unfavorable effects of PSE meat processing to any satisfactory degree and only their total application could bring positive results.

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WPLYW WYBRANYCH DODATKÓW NA WŁAŚCIWOŚCI FUNKCJONALNE WIEPRZOWEGO MIĘSA PSE

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Streszczenie. Badano wpływ wybranych substancji dodatkowych: mikrobiologicznej transglutaminazy i kazeinianu sodu na właściwości funkcjonalne mięsa PSE wynikające ze zmian we frakcji białek cytoszkieletowych. Materiałem badawczym było mięso z wybranych mięśni szynkowych: normalne (RFN) i wadliwe (PSE) oraz farsz mięsny po masowaniu i szynki modelowe z mięsa normalnego oraz PSE z badanymi dodatkami funkcjonalnymi lub bez. Stwierdzono, że transglutaminaza dodana do mięsa PSE uczestnicząc w tworzeniu agregatów białkowych modyfikowała jego właściwości funkcjonalne. Wpływała istotnie na poprawę konsystencji. Jednocześnie pogarszało się wiązanie wody przez produkt. Kazeinian sodu dodany do mięsa PSE wzmagając agregację białek uwolnionych z miofibryli istotnie poprawiał wiązanie wody powodując tym samym osłabienie struktury i konsystencji gotowego produktu. Żaden z zastosowanych pojedynczo dodatków funkcjonalnych nie zminimalizował niekorzystnych skutków przetwarzania mięsa PSE w dostatecznym stopniu i dopiero łączne ich zastosowanie powinno przynieść pozytywny efekt.

Słowa kluczowe: wieprzowe mięso PSE, mikrobiologiczna transglutaminaza, frakcja białek cytoszkieletowych, przemyte miofibryle, wyciek wymuszony

