

Effect of Transglutaminase and Bacterial Concentrates on the Development of Functional and Technological Properties of Minced Meat

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Key words: minced meat, transglutaminase, bacterial concentrate, technological properties, rheological properties

The results of the effects of transglutaminase (TG) and bacterial concentrates (BC) of different strains of microorganisms on the functional and technological properties of minced meat obtained from beef trimming (80/20) are presented. A control and seven experimental samples of minced meat were obtained – three samples with the addition of 10% BC: Bifilact-Pro, Bifilact-AD, Bifilact-Kefir (S-Pro, S-AD, SK), a sample with the addition of 0.2% TG (S-TG) and three samples with simultaneous addition of 5% BC: Bifilact-Pro, Bifilact-AD, Bifilact-Kefir and 0.1% TG (S-Pro + TG, S-AD + TG, S-K + TG). The control and experimental samples were analyzed for: pH, water binding capacity (WBC), water holding capacity (WHC), cooking loss, as well as physicochemical and rheological properties. The highest WHC was observed in the S-TG samples during the 6–12 h of ripening, whereas in the S-Pro+TG, S-AD+TG and S-K+TG samples during the ripening period of 12–18 h. The data obtained correlated with an increase in the pH value in the samples during the indicated periods. Significant differences were noted in the changes in WBC and WHC when BCs were added to the minced meat, as dependent on the composition of the microorganism cultures. The lowest cooking losses were determined in the S-TG sample, *i.e.* 19.03–19.99% ($p < 0.05$), whereas in the S-AD+TG and S-K+TG samples they reached 17.91–18.61% ($p < 0.05$) and 18.96–19.58% ($p < 0.05$), respectively. The most pronounced elastic properties were found in the samples with TG. Thus, the results showed that the combination of BC and TG demonstrates a synergistic effect on the functional and technological properties of meat systems.

ABBREVIATIONS

TG – Transglutaminase; BC – Bacterial concentrate; WBC – Water binding capacity; WHC – Water holding capacity; EPS – Exopolysaccharides; LAB – Lactic acid bacteria.

INTRODUCTION

The rising cost of beef products has led to the need for the rational use of low-value meat cuts and beef trimmings as restructured meat, as prepared from small cuts of meat. This approach allows for an increase in the yield of marketable products and enterprises to gain additional profits [Sorapukdee & Tangwatcharin, 2018].

In recent years, enzymatic processing of raw materials has become particularly popular with the intensive development of biotechnology [Cheng *et al.*, 2009; Liu *et al.*, 2012; Rawdkuen & Benjakul, 2012]. Enzymatic modification of food components is more acceptable to the food industry than the more commonly used chemical methods [Kieliszek & Misiewicz, 2014]. The experience of the practical use of enzymes for the processing of raw meat, as accumulated in many countries, suggests that this method of processing

is very effective at improving the properties of raw materials and increasing the productive yield of meat products [Rivier, 2007; Rawdkuen & Benjakul, 2012; Uran & Yilmaz, 2018]. Transglutaminase (TG) plays an important role among these enzymes [Uran & Yilmaz, 2018], with its potential to improve the firmness, viscosity, elasticity, and water binding capacity of food products [Kieliszek & Misiewicz, 2014].

The use of TG in the meat industry improves the functional properties of proteins in meat products, contributes to the strong cohesion of a block of meat without the further need for the addition of salt or phosphates, and also has a positive effect on the texture of the final product, which results in an increase in its hardness [Kieliszek & Misiewicz, 2014; Atilgan & Kilic, 2017; Uran & Yilmaz, 2018]. Meat products contain a high level of protein, including the myofibrillar proteins: actin and myosin, which affect the development of product texture and at the same time serve as good substrates for transglutaminase [Tseng *et al.*, 2002].

TG helps solving technological problems in the production of both emulsified sausages and fine and coarse-minced sausages. The ability to bind proteins of different origins can be used in the production of combined meat products, allowing for the inclusion of lower-quality raw materials and ingredients, such as mechanically deboned meat, skimmed milk powder, soy or wheat flour. The use of TG allows for the production of lower fat content meat products which do not dif-

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fer in organoleptic properties from the conventional products [Kieliszek & Misiewicz, 2014].

The results obtained by Sorapukdee & Tangwatcharin [2018] and Baugreet *et al.* [2018] showed that pieces of restructured beef processed with microbial TG could be bound together *via* cross-linking of myosin chains and actin.

Many researchers point to the widespread use of bacterial preparations and starter microorganisms to improve the properties of raw meat [Campbell-Platt, 1995; Hammes & Hertel, 1998; Ruiz-Moyano *et al.*, 2008]. Many of these bacterial preparations include bifidobacteria and propionic acid bacteria [Ruiz-Moyano *et al.*, 2008; Danylenko *et al.*, 2014].

Exopolysaccharides (EPS) have an immense commercial value because of their industrially useful physico-chemical properties [Patel *et al.*, 2012]. The polysaccharides excreted by lactic acid bacteria (LAB) during the fermentation processes result in the particular viscosity of certain foods [Sanni *et al.*, 2002].

EPS are complex carbohydrates located outside the cell. Many strains of LAB produce extracellular polysaccharides which may be tightly associated with the bacterial cell wall as capsules or otherwise liberated into the growth medium as a loose slime [Cerning, 1995]. Some Bifidobacterium strains, as well as many other microorganisms, are able to produce these polymers [Ruas-Madiedo *et al.*, 2007]. Dairy propionibacteria are also capable of producing EPS [Cerning, 1995].

EPS-producing lactic acid bacteria (EPS-producing cultures) are used to improve dairy product functionality by binding free water. EPS have the ability to bind water and to increase moisture retention by water binding or entrapment within their three-dimensional networks [El Soda, 2014]. EPS derived from LAB play a crucial role in improving the rheology, texture, and mouth feel of fermented food formulations and conferring beneficial physiological effects to human health [Doleyres *et al.*, 2005].

However, the influence of TG in combination with bacterial concentrates (BC) on physicochemical and technological properties of meat products has not been reported to date in the relevant literature. Thus, the aim of this research was to determine the effect of transglutaminase and bacterial concentrates composed of different strains of microorganisms on the functional and technological properties of minced meat during the ripening process.

MATERIALS AND METHODS

Materials

Beef was collected 48 h after slaughtering from a breeding plant “Dubrovsky” (Chelyabinsk, Russia). Fresh beef trimmings (80/20) (66.42 g/100 g moisture, 16.10 g/100 g protein, and 17.64 g/100 g fat) were obtained from rib and round portions. Beef trimmings were transported in a refrigerator at 4°C to the laboratory of the Food and Biotechnology Department (South Ural State University, Chelyabinsk, Russia) within 2 h for further processing. Then, beef trimming was analyzed for pH using a portable pH-meter (HANNA HI83141, Woonsocket, RI, USA). An electrode was inserted to a depth of 5 ± 1 cm, where the values obtained ranged from 5.56 to 5.62.

Bacterial concentrates (BCs): Bifilact-Pro, Bifilact-AD and BK-Kefir, were produced by the Federal State Unitary

Enterprise “An Experimental Biofactory” of the Russian Agricultural Academy (Uglich, Russian Federation):

- concentrate Bifilact-Pro consists of *Lactococcus lactis* subsp. *diacetylactis*, *Streptococcus thermophilus*, *Propionibacterium freudenreichii*, *Bifidobacterium Bifidum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis* species;
- concentrate Bifilact-AD consists of *Lactococcus lactis* subsp. *diacetylactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium Bifidum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis* species;
- concentrate BK-Kefir consists of *Lactococcus lactis* subsp. *Lactis*, *Lac. lactis* subsp. *Cremoris*, *Lac. lactis* subsp. *Diacetylactis*, *Leuconostoc mesenteroides* subsp. *Cremoris*, *Streptococcus thermophilus*, *Lactobacillus plantarum*, *Lbc. fermentum*, *L. casei*, *Acetobacter aceti*.

Transglutaminase is an enzyme preparation produced by cultivation of *Streptomyces mobaraensis* (BioBond Shanghai's Kinry Pharmaceutical Co., Ltd. Shanghai, China).

Preparation of minced meat samples

The beef trimmings (80/20) were passed through a grinder (Fimar 32/RS Unger, Italy) fitted with a plate with 6 mm diameter holes.

BCs were activated in 1 L of sterilized skimmed milk at a temperature of 37 ± 1°C. Microbial TG was added to the meat in a dry form, and water was added to activate it. For the research, control and test samples were made – experimental samples of minced meat with the introduction of 10% BCs by weight of raw materials (S-Pro – sample with concentrate Bifilact-Pro, S-AD – sample with concentrate Bifilact-AD, S-K – sample with concentrate BK-Kefir), experimental samples of minced meat with the addition of 0.2% TG by weight of raw materials (S-TG), and experimental samples

TABLE 1. Formulation of samples.

Samples	Components (kg per 100 kg of minced meat)		Biotechnological additives (kg per 100 kg of minced meat)	
	beef trimmings (80/20)	water	TG	bacterial concentrate
S-C	90	10	0	0
S-Pro	90	0	0	10
S-AD	90	0	0	10
S-K	90	0	0	10
S-TG	90	10	0.2	0
S-Pro+TG	90	5	0.1	5
S-AD+TG	90	5	0.1	5
S-K+TG	90	5	0.1	5

Note: S-C – control sample; S-Pro, S-AD, S-K – samples with the addition of 10% concentrate Bifilact-Pro, Bifilact-AD, Bifilact-Kefir; S-TG – samples with the addition of 0.2% transglutaminase; S-Pro+TG, S-AD+TG, S-K+TG – samples with the addition of 0.1% transglutaminase and 5% Bifilact-Pro, Bifilact-AD, Bifilact-Kefir.

with simultaneous addition of 0.1% enzyme TG and 5% BC by weight of raw materials (S-Pro+TG – sample with concentrate Bifilact-Pro and TG, S-AD+TG – sample with concentrate Bifilact-AD and TG, S-K+TG – sample with concentrate BK-Kefir and TG). A sample of the minced meat from the trimmings was taken as the control sample (S-C). Thus, a total of eight formulations were taken (Table 1), where five samples of minced meat were made for each formulation.

Prepared minced meat samples were kept for ripening at a temperature of $2\pm 2^\circ\text{C}$ for 24 h, and parts of the samples were taken to determine indicators characterizing the functional and technological properties of minced meat (pH, water binding capacity (WBC), water holding capacity (WHC), cooking loss and rheological properties) every 6 h (0, 6, 12, 18, 24 h).

Technological properties measurements

Water binding capacity

The WBC was determined *via* the filter paper method [Grau & Hamm, 1956], which is based on determining the amount of separated moisture after lightly pressing and calculating the spot area formed on a piece of filter paper using a planimeter. The mass fraction of the bound moisture in a given sample was calculated using the following formula:

$$X = (A - 8.4 \times B) \times 100 / A,$$

where: X – mass fraction of bound water in a sample (% total moisture), A – total mass of moisture in a sample (mg), and B – area of the wet spot (mm^2).

Water holding capacity

The WHC was determined gravimetrically as described by Zhang *et al.* [2018]. Samples were placed in sealed centrifuge tubes and centrifuged at $70 \times g$ for 15 min at 4°C (Laboratory centrifuge “OKA”, Sibagroprom, Novosibirsk, Russia). After heating at 75°C , the supernatant was decanted, and samples were weighed. The samples were then stored at 4°C for 24 h and centrifuged at $110 \times g$ for 10 min at 4°C (Laboratory centrifuge “OKA”, Sibagroprom, Novosibirsk, Russia). The resulting supernatant was removed using filter paper and samples were weighed. Water holding capacity (%) was expressed using the following formula:

$$\text{WHC}(\%) = [(W_2 - W) / (W_1 - W)] \times 100\%$$

where: W – mass of the sample (g), W_1 – mass of the sample after heating and decanting the supernatant (g), and W_2 – mass of the sample after centrifuging and removal of resulting supernatant (g).

Cooking loss

Weight loss of minced meat during heat treatment was determined using the gravimetric method, based on measuring the mass of a sample before and after heat treatment. To determine cooking loss, the minced meat was formed into meatballs weighing 11 ± 1 g each, which were weighed before heating at 75°C in the air-o-steam (Rational AG, Landsberg

am Lech, Germany) for 10 min. Cooking loss was calculated using the following formula:

$$\text{Cooking loss}(\%) = [(\text{weight of raw sample (g)} - \text{weight of cooked sample (g)}) / \text{weight of raw sample (g)}] \times 100.$$

pH

To determine pH values, 5 g of minced meat sample was blended with 20 mL of distilled water for 1 min using a chopper [Bosch MMR 0801, Gerlingen, Germany]. The pH was measured with a stationary pH meter (model HANNA HI 2210 (PH/T), Woonsocket, RI, USA) equipped with a pH and temperature electrode HI 1131B and HI 7662 (HANNA) at 20°C . Before measuring pH, the detector was calibrated with pH 4.01 and pH 6.86 buffers.

Rheological measurements

Rheological measurements were conducted using a texture analyzer “Structurometer ST2” (LAB, Quality Laboratory, Moscow, Russia) by compressing it with an indenter “Cylinder Ø36” (duralumin, mass 42.5 g). The analysis of the mechanical tension induced on a cylindrical indenter during its introduction into the product was carried out under the following loading mode: contact force ($F_c = 7$ g), strain rate ($V_d = 0.5$ mm/s), and the introduction of the indenter continued until the effort $F_{\text{max}} = 500$ g. Total, plastic, and elastic deformations were determined. Measurements were carried out at a sample temperature of $2\pm 2^\circ\text{C}$.

Physicochemical analyses

Chemical analyses of the minced meat samples were performed 24 h after their preparation. Total nitrogen content was assayed using the Kjeldahl method [ISO 937:1978]. Nitrogen was converted to equivalent protein content using a factor of 6.25. Moisture was determined according to ISO standard [ISO 1442:1997]. Total fat was determined by the Soxhlet method according to ISO standard [ISO 1444:2000].

Statistical analysis

The experiment was carried out in five replicates and each analysis was performed in three repetitions. All measurements were conducted in triplicate. Results were expressed as mean values of five replicates \pm standard deviation. Probability values $p \leq 0.05$ were taken to indicate statistical significance. The data was analyzed *via* one-way ANOVA and the Tukey test using the free web-based software offered by Assaad *et al.* [2014].

RESULTS AND DISCUSSION

pH, WBC, WHC and cooking loss

TG maintains its maximal enzymatic activity at temperatures close to 0°C [Yokoyama *et al.*, 2004], allowing it to be used at the low temperatures required for meat ripening.

The research results (Table 2) demonstrated that the pH of minced meat changed depending on the concentration of biotechnological components and length of the ripening period. In the control sample, a gradual increase in the pH to 5.82 by the end of the ripening period was observed, which

TABLE 2. The pH value of minced meat samples during ripening.

Samples	0 h	6 h	12 h	18 h	24 h
S-C	5.53±0.004 ^{eA}	5.56±0.005 ^{dD}	5.66±0.004 ^{cE}	5.76±0.005 ^{bD}	5.82±0.005 ^{aC}
S-Pro	5.46±0.005 ^{bcB}	5.48±0.007 ^{bE}	5.50±0.004 ^{aF}	5.46±0.005 ^{cF}	5.34±0.004 ^{dE}
S-AD	5.45±0.004 ^{dB}	5.49±0.004 ^{cE}	5.52±0.005 ^{bF}	5.56±0.004 ^{aE}	5.46±0.004 ^{dD}
S-K	5.45±0.004 ^{bb}	5.50±0.004 ^{aE}	5.44±0.003 ^{bG}	5.40±0.005 ^{cG}	5.37±0.003 ^{dE}
S-TG	5.51±0.004 ^{cA}	5.87±0.005 ^{bA}	5.93±0.004 ^{aC}	5.88±0.007 ^{bC}	5.86±0.004 ^{bB}
S-Pro+TG	5.45±0.002 ^{dB}	5.65±0.005 ^{cC}	5.88±0.005 ^{aD}	5.86±0.005 ^{bC}	5.84±0.004 ^{bBC}
S-AD+TG	5.45±0.004 ^{eB}	5.69±0.005 ^{dC}	5.97±0.004 ^{aB}	5.95±0.004 ^{bB}	5.90±0.007 ^{cA}
S-K+TG	5.46±0.005 ^{dB}	5.76±0.004 ^{cB}	6.02±0.004 ^{aA}	6.00±0.007 ^{aA}	5.94±0.006 ^{bA}

Note: data are the mean values of five replicates ± standard deviation (n=5). Means in a row without a common superscript letter a, b, c – are statistically significantly different ($p<0.05$). Means in a column without a common superscript letter A, B, C – are statistically significantly different ($p<0.05$) as analyzed by one-way ANOVA and the TUKEY test.

S-C – control sample; S-Pro, S-AD, S-K – samples with the addition of 10% concentrate Bifilact–Pro, Bifilact–AD, Bifilact–Kefir; S-TG – samples with the addition of 0.2% transglutaminase; S-Pro+TG, S-AD+TG, S-K+TG – samples with the addition of 0.1% transglutaminase and 5% Bifilact–Pro, Bifilact–AD, Bifilact–Kefir.

is typical of a meat system that does not contain technological additives.

The pH values of minced systems varied from 5.45 to 5.53 before the start of the ripening process. From results presented in Table 2, it can be concluded that the introduction of BC in a 10% concentration led to a successive increase in the acidity of the meat system and a decrease in pH to 5.34–5.46 after 24 h of fermentation. This effect is due to the accumulation of acidic metabolic products of bacteria that will reduce the pH as a fact of their acidity [Mejri *et al.*, 2017]. Moreover, the accumulation of the acidic products of microbial activity in minced meat was irregular throughout the ripening period; in the samples containing BC – S-AD and S-Pro (10%), there was a significant increase in pH to 5.50–5.56 ($p<0.05$) after 12–18 h of ripening, followed by a successive decrease.

There was a significant increase in pH in the samples containing TG compared with the control samples after 12–18 h of ripening. The maximum pH level in the S-TG samples was observed during the 6–12 h interval (5.87–5.93), and for the samples containing a complex of TG and BC (S-AD+TG and S-K+TG) at 12–18 h (5.97–6.02). Meiyong *et al.* [2002] demonstrated that the pH of meat batter treated with TG was higher than that of meat without TG treatment. The pH value established in the samples of minced meat with TG was remote from the isoelectric point of muscle proteins, which significantly affected their functional properties, ensuring a high level of WBC, WHC, and low weight loss during heat treatment. Hughes *et al.* [2014] reported that loss of water from muscles and the degree of changes in their properties depends on the pH which muscle proteins are sensitive to.

Significant differences in the dynamics of WBC were noted depending on the composition of microorganisms in experimental samples with BC (Table 3). In sample S-K, WBC increased slightly and reached its maximum during 6–12 h of the ripening period. There was a rapid increase in WBC, which reached a maximum by 12–18 h of ripening, when a starter containing acidophilic and propionic acid cul-

tures (samples S-AD and S-Pro) was added to the minced meat. It should be emphasized that the addition of BC Bifilact–Pro and Bifilact–AD significantly improved the water binding capacity of the meat system after 12 h of maturation to 51.8–52.1% ($p<0.05$) compared to the control. Cerning [1995] and Patel *et al.* [2012] proved that lactic acid bacteria and propionic bacteria are capable of producing EPS at low temperatures and pH levels close to 5.5. It can be assumed that the improvement in water binding capacity observed for S-Pro and S-AD samples is due to the water-absorbing properties of EPS, which accumulate during the vital activity of these types of bacteria. However, there was a considerable decrease in WBC of minced meat with bacterial starters to 39.9–46.3% ($p<0.05$) by the end of the ripening process (24 h), which was apparently due to the accumulation of lactic acid and the consequent decrease in pH to that close to the isoelectric point of muscle proteins. Mauriello *et al.* [2004] noted that WBC decrease is related to pH decrease; when pH is close to the isoelectric point of the protein, the functional properties of the meat proteins are reduced. Puolanne *et al.* [2013] researched the WHC of meat without added salt, which was found to have a distinct minimum at pH 5.0, which is the average isoelectric pH of meat structural proteins.

It should be emphasized that the combination of BC and TG has a synergistic effect on the functional and technological properties of minced systems. Thus, when TG and BC are added to minced meat, the protein matrix is rapidly formed during the first 6–12 h of exposure; the technological properties of meat are enhanced as a result of TG activity. This has been confirmed by the research results. The highest WBC was observed in the S-TG samples during the first 6–12 h of maturation, whereas in the S-Pro+TG, S-AD+TG, and S-K+TG samples – in 12–18 h of the maturation period. The data obtained correlate with an increase in pH of the meat system during the indicated periods. In addition, in the samples containing the complex of TG and bacterial cultures, the WBC increased considerably to 58.6–65.0% ($p<0.05$) as compared

TABLE 3. The functional and technological properties of minced meat samples.

Samples	0 h	6 h	12 h	18 h	24 h
<i>WBC (%)</i>					
S-C	39.70±0.014 ^{eH}	42.03±0.062 ^{dF}	49.45±0.017 ^{cG}	50.24±0.057 ^{bE}	50.83±0.03 ^{aE}
S-Pro	44.53±0.014 ^{dC}	46.62±0.015 ^{cD}	52.10±0.013 ^{aE}	49.91±0.015 ^{bF}	39.90±0.016 ^{eH}
S-AD	41.29±0.012 ^{eG}	42.80±0.013 ^{dE}	51.75±0.014 ^{aF}	50.50±0.013 ^{bD}	46.35±0.019 ^{eF}
S-K	44.61±0.012 ^{eA}	46.60±0.013 ^{aD}	45.11±0.012 ^{bH}	44.34±0.012 ^{dG}	43.93±0.016 ^{eG}
S-TG	45.44±0.011 ^{eB}	59.56±0.015 ^{aA}	58.49±0.063 ^{bD}	55.10±0.017 ^{cB}	54.80±0.015 ^{dC}
S-Pro+TG	41.52±0.015 ^{eF}	46.66±0.013 ^{dD}	61.58±0.015 ^{aC}	57.04±0.086 ^{bC}	53.46±0.016 ^{eA}
S-AD+TG	43.17±0.013 ^{eE}	48.42±0.015 ^{dC}	64.68±0.014 ^{aB}	58.66±0.015 ^{bA}	55.91±0.016 ^{eB}
S-K+TG	43.53±0.018 ^{eD}	55.57±0.012 ^{cB}	65.03±0.023 ^{aA}	58.77±0.013 ^{bA}	54.37±0.014 ^{dD}
<i>Cooking loss (%)</i>					
S-C	27.35±0.14 ^{aBC}	26.08±0.16 ^{bB}	25.40±0.08 ^{cB}	24.34±0.15 ^{dC}	24.03±0.08 ^{dD}
S-Pro	27.91±0.15 ^{aA}	27.18±0.09 ^{bA}	24.96±0.08 ^{dB}	25.58±0.09 ^{eB}	28.09±0.08 ^{aA}
S-AD	27.75±0.14 ^{aAB}	26.20±0.1 ^{bb}	23.54±0.17 ^{dC}	23.05±0.09 ^{dD}	24.94±0.05 ^{cC}
S-K	28.02±0.1 ^{aA}	26.76±0.09 ^{eA}	26.17±0.11 ^{dA}	27.75±0.13 ^{abA}	27.32±0.12 ^{bbB}
S-TG	26.45±0.05 ^{aE}	19.99±0.14 ^{dE}	19.03±0.07 ^{eE}	21.4±0.03 ^{eE}	23.68±0.04 ^{bE}
S-Pro+TG	27.62±0.06 ^{aAB}	25.25±0.05 ^{bC}	20.27±0.2 ^{eD}	21.71±0.1 ^{dE}	22.35±0.05 ^{eF}
S-AD+TG	26.96±0.05 ^{aCD}	24.78±0.07 ^{bD}	17.91±0.06 ^{eF}	18.61±0.09 ^{dG}	20.92±0.04 ^{eH}
S-K+TG	26.68±0.04 ^{aDE}	24.33±0.04 ^{bD}	18.96±0.04 ^{eE}	19.58±0.09 ^{dF}	21.60±0.03 ^{cG}
<i>WHC (%)</i>					
S-C	35.36±0.179 ^{bA}	38.72±0.168 ^{aD}	38.84±0.201 ^{aC}	39.12±0.252 ^{aD}	39.33±0.256 ^{aD}
S-Pro	33.83±0.195 ^{eB}	35.94±0.246 ^{dE}	37.2±0.259 ^{eD}	36.7±0.214 ^{abE}	35.9±0.272 ^{aE}
S-AD	35.2±0.199 ^{eA}	36.48±0.227 ^{dE}	37.86±0.24 ^{cD}	37.3±0.259 ^{abE}	36.2±0.251 ^{aE}
S-K	34.99±0.156 ^{dA}	36.54±0.242 ^{eE}	38.14±0.22 ^{bCD}	37.9±0.182 ^{abDE}	37.2±0.283 ^{bcE}
S-TG	34.62±0.23 ^{dAB}	48.12±0.177 ^{abA}	48.62±0.188 ^{aA}	47.34±0.28 ^{bcC}	46.84±0.298 ^{cC}
S-Pro+TG	34.87±0.161 ^{dA}	43.24±0.232 ^{cC}	45.9±0.228 ^{bB}	48.16±0.31 ^{abC}	49.08±0.334 ^{aB}
S-AD+TG	35.06±0.171 ^{dA}	44.88±0.159 ^{eB}	46.58±0.304 ^{bB}	49.28±0.36 ^{aAB}	49.84±0.317 ^{aB}
S-K+TG	33.94±0.144 ^{dB}	44.19±0.25 ^{eBC}	46.34±0.273 ^{bb}	50.16±0.296 ^{aA}	51.23±0.328 ^{aA}

Note: data are the mean values of five replicates ± standard deviation (n=5). Means in a row without a common superscript letter a, b, c – are statistically significantly different ($p < 0.05$). Means in a column without a common superscript letter A, B, C – are statistically significantly different ($p < 0.05$) as analyzed by one-way ANOVA and the TUKEY test.

S-C – control sample; S-Pro, S-AD, S-K – samples with the addition of 10% concentrate Bifilact–Pro, Bifilact–AD, Bifilact–Kefir; S-TG – samples with the addition of 0.2% transglutaminase; S-Pro+TG, S-AD+TG, S-K+TG – samples with the addition of 0.1% transglutaminase and 5% Bifilact–Pro, Bifilact–AD, Bifilact–Kefir; WBC – water binding capacity; WHC – water holding capacity.

with samples containing only the TG in 12–18 h of the ripening period. A slight decrease in WBC to 53.5–55.9% ($p < 0.05$) was observed in these samples during the last exposure period, which can be associated with the rising proteolytic activity of the microorganisms and which leads to partial destruction of the protein matrix.

In the literature, there is contradictory information about the influence of TG on WBC and cooking loss, respectively. Some studies have found that TG does not significantly affect product loss [Uran *et al.*, 2013], while others point to

the negative impact of TG on cooking loss [Atilgan & Kilic, 2017]. Atilgan & Kilic [2017] demonstrated that the use of microbial TG had a negative effect on WBC and caused increased cooking loss in muscle-based foods. Aaslyng *et al.* [2003] noted the correlation between the WHC of raw pork muscle and cooking loss which was dependent on cooking temperature.

The results of our research showed that decreasing WHC and WBC was correlated to increased cooking loss. Significant differences in weight loss compared to the control sample

TABLE 4. Contents of major components of minced meat samples.

Samples	Fat (g/100 g)	Protein (g/100 g)	Moisture (g/100 g)
S-C	17.82±0.13 ^a	16.36±0.10 ^{ab}	65.16±0.26 ^d
S-Pro	16.56±0.07 ^{bc}	15.58±0.09 ^{cd}	67.08±0.29 ^{abc}
S-AD	16.50±0.17 ^c	15.18±0.11 ^d	67.40±0.26 ^{ab}
S-K	16.22±0.11 ^c	15.44±0.12 ^d	67.68±0.32 ^a
S-TG	17.22±0.27 ^{ab}	16.58±0.16 ^a	66.00±0.23 ^{cd}
S-Pro+TG	16.64±0.14 ^{bc}	16.20±0.07 ^{abc}	66.42±0.22 ^{ad}
S-AD+TG	16.90±0.17 ^{bc}	15.88±0.19 ^{ad}	66.26±0.18 ^{bd}
S-K+TG	16.90±0.12 ^{bc}	15.78±0.14 ^{bd}	66.56±0.13 ^{abc}

Note: data are the mean values of five replicates ± standard deviation (n=5). Means in a column without a common superscript letter a, b, c – are statistically significantly different ($p < 0.05$) as analyzed by one-way ANOVA and the TUKEY test.

S-C – control sample; S-Pro, S-AD, S-K – samples with the addition of 10% concentrate Bifilact–Pro, Bifilact–AD, Bifilact–Kefir; S-TG – samples with the addition of 0.2% transglutaminase; S-Pro+TG, S-AD+TG, S-K+TG – samples with the addition of 0.1% transglutaminase and 5% Bifilact–Pro, Bifilact–AD, Bifilact–Kefir.

were found only in the S-TG and S-K+TG samples at the initial stage of maturation (0 h) (Table 3), while in the 18–24 h period, a considerable reduction in cooking loss in comparison to the control was established for all samples containing TG enzyme (0.1 and 0.2%). Thus, the greatest mass loss was observed in the samples without TG (S-C, S-Pro, S-AD, S-K), and this tendency was found to continue from 6 h until the end of the maturation process. The lowest cooking loss was observed in samples with high WBC and WHC. Thus, in the S-TG sample, the lowest cooking losses were established between 6–12 h and reached 19.03–19.99% ($p < 0.05$), whilst in the S-AD+TG and S-K+TG samples the lowest weight loss was found in the 12–18 h period and amounted to 17.91–18.61% ($p < 0.05$) and 18.96–19.58% ($p < 0.05$), respectively.

Pietrasik *et al.* [2007] noted that TG increases the WHC of meat products and decreases cooking loss, which is in agreement with the results reported above. Zeng *et al.* [2017] determined that the degradation of myofibrils as a result of enzyme proteolytic activity led to improved water holding in myofibrils. The results obtained confirm that not only does the TG activity have a positive effect on WHC, but further that the microorganisms in BC possessing the ability to synthesize EPS, which are hydrocolloids, enhance the ability of the meat system to hydrate. Consequently, the highest values of WHC were detected in the samples with TG, *i.e.* 48.6% ($p < 0.05$) in sample S-TG at 12 h of maturation, 51.2 and 49.8% ($p < 0.05$) in samples S-K+TG and S-AD+TG, respectively at 24 h of maturation.

Accordingly, the results obtained confirm the synergistic effect of the activity of TG and BC on the development of the functional and technological properties of the meat system. Exposure for 12–18 h was found to be optimal for the development of the required technological qualities of minced meat with the addition of TG and BC.

Chemical composition

The results of determination of minced meat physico-chemical indicators are presented in Table 4. The results obtained demonstrate that applying various biotechnological additives resulted in a significant decrease in the fat content in the experimental groups compared with the control group ($p < 0.05$). Protein contents of the samples containing BCs (S-Pro, S-AD, S-K) were significantly reduced ($p < 0.05$) in comparison to those in the control sample and samples with TG, which was probably due to the lower water binding capacity and water holding capacity of these samples and caused loss of nutrients. The moisture content of the samples with BCs (S-Pro, S-AD, S-K) was 67.1, 67.4, and 67.7 g/100 g, respectively, while the moisture content of the samples with TG (S-TG, S-Pro+TG, S-AD+TG, S-K+TG) ranged from 66.0 to 66.6 g/100 g. According to Atilgan & Kilic [2017], the use of TG did not cause significant differences in protein contents among groups of cooked ground beef. The protein content in our studies ranged from 15.2 to 16.6 g/100 g ($p < 0.05$); no statistically significant differences were found in its values in the control and experimental groups with TG (0.1 and 0.2%). Similar results were obtained by Uran & Yilmaz [2017], who showed no statistically significant differences in the protein content in the control and other groups containing the enzyme in different concentrations (0.2, 0.6, 0.8, and 1%) during burger production.

Rheological properties

Minced meat has a plastic-elastic structure characterized by a complex of structural and mechanical properties. The deformation characteristics of the meat system depend on the moisture, fat content, and degree of grinding, and at the biochemical level – on the interaction forces between the functional groups of the molecules.

As a result of rheological studies, the effects of introducing TG and BC containing various types of microorganisms on the elastic-plastic properties of the meat system have been established. During the initial stages of ripening, relatively high rates of total and plastic deformation were established in minced meat containing both 5% and 10% BC (Table 5), which indicates the softening of the raw meat.

The most pronounced elastic properties were determined in samples S-TG, S-Pro+TG, S-AD+TG, and S-K+TG. Moreover, there was a tendency towards a progressive increase in these indicators with increasing exposure time of the minced meat. The gradient increase in elastic deformation over 24 h of maturation in the S-TG (from 2.25 to 3.26 mm) and S-Pro+TG (from 1.99 to 3.32 mm) samples was very clearly observed. The lowest total and plastic deformations in the 12–24 h period were established in the samples with 0.2% TG at 10.6–12.2 mm and 8.0–9.8 mm, respectively. In the samples that contained bacterial starters (S-Pro, S-AD, S-K), a “hopping” change in the elastic properties was observed, with the maximum elastic deformation detected in the 12–18 h period.

It should be noted that after 12 h of maturation, the maximum strengthening of the meat system structure occurred under the influence of both TG and microorganism enzymes. The total deformation decreased to 10.57–18.06 mm, plas-

TABLE 5. Deformation characteristics of minced meat samples.

Samples	0 h	6 h	12 h	18 h	24 h
<i>Total deformation (mm)</i>					
S-C	14.56±0.12 ^{abF}	15.00±0.355 ^{aF}	14.07±0.049 ^{bd}	13.96±0.038 ^{bF}	13.77±0.152 ^{bF}
S-Pro	23.10±0.215 ^{aBC}	22.57±0.474 ^{aBC}	16.05±0.025 ^{dB}	19.98±0.091 ^{cB}	21.44±0.215 ^{ba}
S-AD	22.48±0.167 ^{aCD}	21.73±0.271 ^{aCD}	15.33±0.380 ^{cBC}	19.12±0.265 ^{bC}	18.65±0.535 ^{bB}
S-K	26.28±0.145 ^{aA}	25.63±0.166 ^{aA}	18.06±0.354 ^{cA}	22.01±0.080 ^{ba}	21.06±0.393 ^{ba}
S-TG	16.79±0.040 ^{aE}	16.49±0.173 ^{aE}	10.57±0.240 ^{cF}	11.51±0.205 ^{bG}	12.15±0.101 ^{bE}
S-Pro+TG	21.90±0.237 ^{aD}	20.98±0.225 ^{bd}	14.00±0.063 ^{cD}	16.69±0.090 ^{dE}	18.75±0.118 ^{cB}
S-AD+TG	23.47±0.037 ^{aB}	23.77±0.051 ^{aB}	12.93±0.099 ^{dE}	17.16±0.113 ^{cE}	18.07±0.126 ^{bBC}
S-K+TG	22.37±0.251 ^{aCD}	21.74±0.333 ^{aCD}	14.83±0.075 ^{cCD}	17.87±0.079 ^{bd}	17.14±0.396 ^{bC}
<i>Plastic deformation (mm)</i>					
S-C	13.53±0.232 ^{aC}	12.82±0.353 ^{abA}	11.88±0.349 ^{bcC}	12.03±0.084 ^{bcD}	11.21±0.199 ^{cD}
S-Pro	21.86±0.223 ^{aA}	20.43±0.368 ^{bBC}	12.71±0.162 ^{cB}	16.95±0.105 ^{dB}	18.51±0.190 ^{cA}
S-AD	20.82±0.132 ^{aB}	19.58±0.308 ^{aB}	12.28±0.320 ^{cB}	14.22±0.147 ^{bC}	15.58±0.657 ^{bB}
S-K	24.75±0.107 ^{aD}	23.92±0.191 ^{aE}	15.66±0.398 ^{cA}	19.42±0.247 ^{ba}	18.60±0.472 ^{ba}
S-TG	14.38±0.215 ^{aC}	13.62±0.192 ^{bd}	8.00±0.127 ^{cE}	9.81±0.073 ^{cE}	8.88±0.041 ^{dE}
S-Pro+TG	19.64±0.229 ^{aB}	18.56±0.216 ^{bb}	10.96±0.064 ^{cCD}	12.52±0.065 ^{dD}	15.58±0.087 ^{cB}
S-AD+TG	21.91±0.030 ^{aA}	21.38±0.024 ^{bc}	9.79±0.084 ^{cD}	12.25±0.195 ^{dD}	15.03±0.142 ^{cB}
S-K+TG	19.49±0.183 ^{aB}	19.56±0.398 ^{aB}	12.02±0.066 ^{cB}	14.97±0.060 ^{bC}	13.92±0.317 ^{bC}
<i>Elastic deformation (mm)</i>					
S-C	1.986±0.040 ^{cB}	2.188±0.002 ^{bcB}	2.683±0.146 ^{aBC}	2.369±0.052 ^{abB}	2.652±0.054 ^{aDE}
S-Pro	2.024±0.048 ^{cAB}	2.142±0.107 ^{cB}	3.339±0.188 ^{aA}	3.118±0.044 ^{abA}	2.796±0.085 ^{bCD}
S-AD	1.930±0.037 ^{bb}	2.156±0.072 ^{bb}	3.050±0.061 ^{aAB}	3.028±0.062 ^{aA}	3.074±0.123 ^{aAC}
S-K	1.583±0.068 ^{bc}	1.708±0.025 ^{bc}	2.400±0.114 ^{aC}	2.194±0.029 ^{aB}	2.459±0.083 ^{aE}
S-TG	2.254±0.043 ^{dA}	2.828±0.020 ^{aA}	3.074±0.047 ^{baB}	3.089±0.027 ^{ba}	3.260±0.032 ^{aA}
S-Pro+TG	1.998±0.068 ^{dB}	2.414±0.008 ^{cB}	3.046±0.061 ^{baB}	3.007±0.055 ^{ba}	3.318±0.071 ^{aA}
S-AD+TG	2.115±0.069 ^{cAB}	2.390±0.075 ^{bb}	3.241±0.052 ^{aA}	3.180±0.047 ^{aC}	3.050±0.016 ^{aAC}
S-K+TG	1.956±0.029 ^{bb}	2.180±0.065 ^{bb}	2.915±0.159 ^{aAC}	3.008±0.055 ^{aA}	2.928±0.052 ^{aC}

Note: data are the mean values of five replicates ± standard deviation (n=5). Means in a row without a common superscript letter a, b, c – are statistically significantly different ($p < 0.05$). Means in a column without a common superscript letter A, B, C – are statistically significantly different ($p < 0.05$) as analyzed by one-way ANOVA and the TUKEY test.

S-C – control sample; S-Pro, S-AD, S-K – samples with the addition of 10% concentrate Bifilact–Pro, Bifilact–AD, Bifilact–Kefir; S-TG – samples with the addition of 0.2% transglutaminase; S-Pro+TG, S-AD+TG, S-K+TG – samples with the addition of 0.1% transglutaminase and 5% Bifilact–Pro, Bifilact–AD, Bifilact–Kefir.

tic deformation was up to 8.00–15.66 mm, and the extent of elastic deformation was 2.40–3.34 mm. Canto *et al.* [2014] reported that the increase in springiness and cohesiveness of meat systems containing TG might have been due to the enhanced protein cross-linking between particles of meat. A gradual decrease in total and plastic deformation and an increase in elastic deformation during the ripening period were observed in the control sample.

During the entire ripening period (24 h), it was noted that the S-K and S-Pro samples were characterized by the least

elasticity and most pronounced plasticity. The observed changes were caused by the proteolytic activity of microorganisms in the composition of BC capable of polypeptides release, which leads to the relaxation of raw meat structure. Nevertheless, with the combined use of starter microorganisms and TG involved in the binding of food systems biopolymers, all samples of minced meat showed an improvement in both the plastic and elastic properties. As a result, the meat system with developed elastic-plastic properties can be easily moulded into a dense monolithic structure, subsequently

forming the elastic consistency of the meat product. Ionescu *et al.* [2008] showed that the increase in viscosity of myofibrillar protein with TG treatment is due to protein chain cross-linking catalyzed by TG. The cross-linkage between TG and protein can affect the viscosity of food system, making it stiffer and more rigid than the control. Lesiow *et al.* [2017] demonstrated that the presence of TG increased the hardness of control pork meat batters. The interactions of proteins and water have important effects on water holding and gelling in meat products, and consequently on their technological properties.

CONCLUSIONS

The results of the studies showed a unidirectional positive effect of TG and BC on the functional and technological properties of the meat system. It has been determined that a ripening time of 12–18 h is sufficient for the development of optimal WBC, WHC and rheological properties of minced meat with the addition of TG and BC. The results of this research indicate that the meat industry may achieve the benefits of improved technological properties and reduced cooking loss by using a combination of BC with TG in their formulations.

RESEARCH FUNDING

The article was prepared with the support of the Government of the Russian Federation (Act No 211 dd. 16.03.2013), Contract No 02.A03.21.0011, and the financial support of state assignments No 40.8095.2017/БЧ (2017123-ГЗ), No 19.8259.2017/БЧ and RFBR grant No 18–53–45015.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Submitted: 13 March 2019. Revised: 22 May and 13 July 2019.
Accepted: 21 August 2019. Published on-line: 1 October 2019.