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# Effect of starter culture addition and processing method on proteolysis and texture profile of traditional dry-fermented sausage *Petrovská klobása*

Running title: Proteolysis and texture profile of *Petrovská klobása*

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## ABSTRACT

Proteolysis and texture profile of *Petrovská klobása,* made with addition of starter culture, were investigated during the ripening in different conditions (traditional vs. industrial). Due to intensive degradation of myofibrillar proteins, significant increases of non-protein nitrogen and free amino acids nitrogen contents were registered in all samples. Similar proteolytic changes were found in both starter and non-starter sausages, but were more pronounced in samples ripened in industrial room. In relation to ripening conditions hardness and chewiness differed significantly, while starter culture had significant effect only on hardness of sausages processed in industrial room.

**Key words:** dry-fermented sausage; ripening conditions; starter culture; proteolysis; texture.





# INTRODUCTION

One could say that there are almost as many types of traditional sausages as geographical areas and producers. The clear regional differences are recognizable although each production is based on a combination of fermentation and drying. In Mediterranean countries, Hungary and Balkan countries, air-dried, spicy sausages predominate, while in Northern and Central Europe most of the fermented sausages are smoked, and drying is less intensive [1, 2].

*Petrovská klobása* is dry-fermented sausage traditionally manufactured in north-western Serbia (Municipality of Bački Petrovac, Province of Vojvodina). Due to its production characteristics (smoking, long drying and ripening phase) and sensory attributes (aromatic and spicy-hot taste, dark red colour and hard consistency) it could be classified as a transitional type between the two basic kinds of the European fermented sausages mentioned above. *Petrovská klobása* is a part of Slovaks' heritage, who inhabited Vojvodina in 18$^{th}$ century. Presently, they are producing it in traditional manner, according to original recipe, without the use of chemical additives and microbial starters. In small/micro processing plants this sausage is made during winter, when temperatures are around 0 °C or lower. Consequently, it undergoes slow drying and ripening processes (90 days at least) [3-5].

As in the case of other traditional meat products, the increase in the economic value of *Petrovská klobása* production is limited by time consuming process. Thus, shortening of drying and ripening period would result in a reduction of the drying facilities, storage time, capital and labor, and would increase the profit margin and competitiveness of the product. The drying rate can be sped up by increasing the temperature and reducing the air relative humidity. Also, bacterial starter cultures, composed of selected lactic acid bacteria (LAB) and Gram-positive catalase positive cocci (GCC+), are widely used to accelerate the fermentation and ripening process, as well as to improve the quality and safety of the final product. The most promising bacteria for starter culture are those that are isolated from the indigenous microflora of traditional products. The most common LAB species identified in naturally fermented sausages are *Lactobacillus sakei, Lactobacillus curvatus* and *Lactobacillus plantarum*, while *Staphylococcus xylosus, Staphylococcus carnosus* and *Staphylococcus saprophyticus* are the most commonly identified among (GCC+) [1, 2, 6-9].

Texture is a multi-parameter attribute, and one of the most important components of meat products quality. Numerous factors affect final texture of dry-fermented sausages, including ingredients used, processing parameters, acidification method, ripening conditions, as well as interactions among these factors. When textural characteristics are evaluated throughout the manufacturing process instrumental measurements, most commonly texture profile analysis (TPA), are especially interesting since sensory analysis is only suitable for evaluation of texture on the final product [7, 10, 11]. Proteolysis occurring during the ripening of dry-fermented sausages is extremely important biochemical phenomena which determine the texture characteristics of the product. It is influenced by both muscle and microbial enzymes, and it results in formation of several low molecular weight components [6, 10, 12-14]. Generally, beside fermentation and proteolysis, drying is a major factor affecting binding and textural properties of fermented sausages [7, 8, 10, 15, 16]. Since dry-fermented sausage *Petrovská klobása* in traditional practice can be processed only in winter during long ripening period, the goal of this work was to investigate the possibility of shortening its processing period and prolonging the production season. Aiming to achieve that, the proteolytic changes and textural profile of sausages processed both with and without starter culture addition in different ripening conditions (traditional vs. industrial) were analysed.

# MATERIAL AND METHODS

## Sausage preparation

The sausages were manufactured from lean pork (80% w/w) and back fat (20% w/w), obtained from Landrace pigs. The animals were farmed in standard production system, with prolonged fattening period (9-12 months; live weight above 130 kg), and slaughtered in commercial slaughterhouse according to the routine procedure [17]. Meat and back fat were minced to 10 mm particle size and mixed with red hot paprika powder (2.5 g/100 g), salt (1.8 g/100 g), raw garlic paste (0.2 g/100 g), caraway (0.2 g/100 g) and crystal sugar (0.1 g/100 g), until a homogeneous composition was achieved. Half of the obtained mixture was inoculated with 15 mg/100 g of starter culture (about $10^7$ cfu/g; Quick-starter, Lay, Gewirze OHG, Germany) containing *Lactobacillus sakei* (25%), *Pediococcus pentosaceus* (25%), *Staphylococcus carnosus* (25%) and *Staphylococcus xylosus* (25%). The batters, control and starter culture inoculated (sc), were stuffed in collagen casings (500 mm long and 55 mm in diameter), forming two groups of raw sausages. One half of each group was ripened in a traditional smoking/drying room (control - T and starter inoculated - Tsc) and the other half was ripened in an industrial ripening room (control - I and starter inoculated - Isc). The environmental conditions in traditional room were characterized by low air temperature (average 7.11 ± 3.20 °C) and high relative humidity (RH) (average 82.6 ± 5.98%) (Fig. 1(a)). On the other hand, thermo-hygrometric conditions in industrial ripening room were set to imitate conditions present in traditional practice, i.e. low air temperature (average 11.1 ± 4.58 °C), but also to enable faster processes of fermentation (higher air

temperature in first 10 days, ≈15 °C) and drying (low air relative humidity, average 76.1 ± 8.48%) (Fig. 1(b)). The drying process of sausages lasted until the required moisture content (<35%) was achieved [18].

## Samples

For sampling, the seasoned butter prior to stuffing (0) and three randomly selected sausages from each batch were taken after 2, 6, 9, 15, 30, 60 and 90 days of processing. At each sampling time sausages were taken from different places in drying and ripening room in order to prevent the influence of possible air temperature and relative humidity fluctuation. Physicochemical and texture analysis were carried out at the day of sampling, and the rest of the sausages were homogenized, vacuum packed and stored at -20 °C for further analysis. Analyses for all samples were carried out in duplicate.

## Physicochemical analysis

The pH of samples was measured using the portable pH meter Testo 205 (Testo AG, USA) equipped with a combined penetration tip with temperature probe. Moisture content of sausages was determined according to recommended ISO standard [19].

## Microbiological analysis

For microbial analysis, 10 g of each sample were mixed with 90 ml of saline/peptone water (8 g/L NaCl, 1 g/L peptone) and homogenised [20]. After preparing serial dilutions, higher dilutions were plated onto MRS agar (Torlak, Belgrade, Serbia) for enumeration of LAB and onto Manitol Salt Agar (MSA, HiMedia Laboratories, Mumbai, India) for enumeration of *Micrococcaceae*. After solidification, MRS agar plates were covered with a thin layer of the same medium to establish microaerophilic conditions. Incubation was performed at 30 °C for 3 days and plates containing 30 to 300 colonies were enumerated. All data are presented as the average values of three enumerations.

## Nitrogen fractions

The non-protein nitrogen (NPN) and free amino acid nitrogen (FAAN) were determined according to the methods described by Ikonić et al. [4]. The nitrogen fractions contents were expressed as g/100 g dry matter (dm) of sample.

## Electrophoretic separation of myofibrillar proteins - LoaC method

Myofibrillar proteins were extracted according to the method described by Toldrá et al. [21]. The concentrations of the obtained protein extracts were determined by method of Lowry et

al. [22], using bovine serum albumin as standard protein, and adjusted with deionised water to give a final concentration of 9 mg/mL. The chip-based separations were carried out using Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA) in combination with the Protein 230 Plus LabChip Kit and the dedicated 2100 expert software. The chips were prepared according to Agilent Protein 230 Kit Guide (assay protocol) and successively placed into the bioanalyzer. The complete analysis of 10 protein samples, including sizing and quantification, took 25 minutes (including the start-up phase of the instrument).





## Instrumental Texture Analysis

Texture profile analysis (TPA) was performed as described by Bourne [23], at room temperature, using TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable MicroSystems, Godalming, UK) equipped with a standard cylindrical plate of 75 mm in diameter. The samples (cylinders) 2 cm thick and 2.54 cm in diameter, after discarding the external layer of the sausage, were compressed twice to 50% of their original thickness at a constant test speed of 1 mm/s. The following parameters were determined: hardness (g), springiness, cohesiveness and chewiness (g). Hardness was defined by peak force during the first compression cycle. Springiness was defined as the rate at which a deformed sample goes back to its un-deformed condition after the deforming force is removed. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Finally, chewiness was obtained by multiplying hardness, cohesiveness and springiness.

## Sensory analysis

The sensory analyses were performed by an eight-member panel with previous experience in testing dry fermented sausages, at the end of drying process, when the sausages were considered as ready for consumption ($60^{th}$ and $90^{th}$ day). The casing was removed and the sausages were cut into slices of approximately 3 mm thickness and served at room temperature on white plastic plates. Panel tasters were asked to score samples by using a 0 (worst) to 5 (best) scale for each attribute to be evaluated: chewiness, juiciness and overall acceptability.

## Statistical analysis

One way (ANOVA), Post-hoc (Duncan test) was performed using the software package Statistica (Version 12.0 for Windows, 2013; Stat Soft, Tulsa, OK, USA). Differences were considered significant at $P<0.05$.

# RESULTS AND DISCUSSION

## Moisture and pH

The changes in moisture content and pH along the ripening period showed the usual trends observed in this type of products (Fig. 2). However, different thermo-hygrometric conditions in ripening rooms (traditional vs. industrial) resulted in different drying intensity of sausages. Consequently, moisture content in industrially dried samples was lower throughout the entire processing period. Moreover, due to higher initial temperatures (≈15˚C) in industrial ripening room the fermentation process in I and Isc sausages was considerably faster, causing intensive pH decline toward the isoelectric point (pI) of actomyosin (≈5.0). After only 6 days the pH value in industrially ripened samples had fallen below 5.0, both in control (4.99) and in starter-inoculated sausages (4.80). Therefore, when the pI of myofibrillar proteins was reached the drying process of these sausages was facilitated, owing to low water holding capacity of meat [24-26]. Additionally, the pH drop in I and Isc sausages was higher (0.7-0.8 units), compared to T and Tsc (0.5-0.6 units). After reaching the minimal value pH started gradual increase in all samples, due to degradation of proteins and liberation of peptides, amino acids, ammonia and amines, as it was proposed by other authors [10, 27]. According to Serbian Regulation [18], the required moisture content in dry-fermented sausages ready for consumption has to be lower than 35%. The adequate value of this parameter in T and Tsc samples was achieved after 90 days of processing, while the same level of moisture content in I and Isc sausages was achieved 30 days earlier (60$^{th}$ day).

## Microbial growth

Fig. 3 shows the evolution of LAB and *Micrococcaceae* cell counts during the processing of sausages. The initial LAB counts were 4.60 and 6.09 log CFU/g for control and starter-inoculated samples, respectively. After 60 days of processing, the MRS agar counts reached maximum levels in traditionally ripened sausages (T - 8.28 log CFU/g, Tsc - 8.44 log CFU/g) and then considerable decrease was registered in both control and starter-inoculated products. Different pattern was observed in industrially processed sausages. Due to higher initial temperature in industrial room the LAB growth in these batches was fast, reaching the highest levels after 6 (I - 8.25 log CFU/g) and 30 days of ripening (Isc – 8.45 log CFU/g). The high growth rate of LAB in I and Isc samples was consistent with steep pH drop, confirming the well-known correlation between the fast LAB growth and high acidification in fermented sausages [10, 28, 29, 33].

The initial *Micrococcaceae* counts of the control and starter-inoculated samples were 4.50 and 5.81 log CFU/g, respectively. During processing of T, Tsc, I and Isc sausages, approximately 0.5, 1.5, 1.0 and 2.0 log units reduction of MSA agar counts was observed, respectively. In both ripening conditions, *Micrococcaceae* counts appeared to be considerably affected by rapid acidification or growth of LAB. Nevertheless, decrease in MSA agar counts was more pronounced in starter inoculated sausages (Tsc and Isc). This result is in concordance with previously published work of several authors who found that *Micrococcaceae* are poor competitors in the presence of active acidogenic bacteria (LAB) which have a good adaptation ability and fastest growth rate during fermentation and ripening of sausages. [12, 13, 29-31].

## NPN and FAAN

The content of NPN increased progressively ($P<0.05$) during processing in all analyzed samples (Table 1), being approximately 80% higher in finished products compared to raw sausage batter. In general, the obtained results are in agreement with other studies, indicating the increase in NPN content during the ripening of fermented sausages [12, 13, 27, 29, 32, 33]. Based on these investigations, the increase in NPN level is particularly pronounced in starter-inoculated sausages, indicating the importance of microbial peptidases. According to them, low molecular weight peptides and free amino acids, i.e. the compounds which predominantly contribute the NPN increase, are formed in a last phase of proteolysis due to combined activity of muscle and microbial enzymes. Comparing the results of this study with findings mentioned above certain conflict was noted, which is quite understandable after analysis of the results related to the changes in *Micrococcaceae* counts. Namely, until 60$^{th}$ day of processing in traditional conditions the level of NPN was generally higher in starter-inoculated sausages (Tsc), while in next 30 days the higher content of this nitrogen fraction was registered in naturally fermented sausages (T). Likewise, the content of NPN in Isc sausages was higher in first 9 days of ripening in industrial conditions. Thus, the positive impact of the starter culture addition on the intensity of the proteolysis process was evident in the first half of processing period, when *Micrococcaceae* counts in these sausages were higher. Moreover, added LAB induced rapid pH decline and consequently faster activation of cathepsin D [34-37]. Subsequently, strong acidification in starter-inoculated sausages most likely affected the inhibition of added and naturally present *Micrococcaceae (Staphylococci)*,

which have a proteolytic activity [12, 28-31, 38]. Hence, more intensive hydrolysis of proteins in sausages Tsc and Isc was omitted.

Regarding the FAAN, similar considerations could be applied. During first 30 days its content slightly increased in traditionally ripened sausages, when it reached the value of 0.36 g/100 g dm in both groups (T and Tsc) (Table 1). Unlike, similar level of this nitrogen fraction was reached after only 6 days of ripening in industrial (controlled) conditions, whether the samples were produced with or without starter culture. As mentioned before, this phenomenon was probably caused by higher temperature in the industrial room during the smoking process, which affected positively the growth of LAB, i.e. rapid pH decline, and consecutively the activation of the muscle proteinases (cathepsin D-like enzymes) and degradation of myofibrillar proteins [34-37]. The differences in speed and intensity of proteolysis process between T and I batches were evident until day 30. During that period, the content of FAAN fraction in I and Isc sausages gradually increased, being significantly higher ($P$<0.05) compared to T and Tsc. After 30 days of manufacturing the air temperature in traditional room was few degrees higher and the pH had fallen, resulting in more intensive proteolysis in T and Tsc sausages. Hence, after 60 days of ripening similar ($P$>0.05) content of FAAN fraction was observed in each sample sausage (Table 1). Throughout last month of processing the level of this nitrogen fraction additionally increased, being slightly higher in Tsc sausages (0.53 g/100 g dm) compared to other batches.

## Proteolysis of myofibrillar proteins

LoaC gel-like electrophoretograms of myofibrillar proteins are given in Fig. 4, for traditional (A) and industrial (B) ripening processes, respectively. As it can be seen, during the processing of all examined groups of sausages notable changes in qualitative and quantitative composition of myofibrillar proteins extracts occurred. Nevertheless, some differences in the intensity of certain protein fractions between variously processed sausages were evident.

Contents of basic myofibrillar proteins with molecular mass ≈220 kDa (myosin heavy chain – MHC) and ≈45 kDa (actin) were significantly reduced at the end of the manufacturing of each batch of sausages, compared to the initial ones. Thus, after three months, severe degradation of MHC resulted in almost complete disappearance of this protein fraction in all samples. In this case the influence of starter culture was not evident, while drying process in industrial conditions, contributed faster degradation of myosin in I and Isc sausages. This finding is in accordance with the results of several authors [4, 10, 14, 29, 31], who previously reported an intensive degradation of MHC during the ripening of naturally fermented sausages. On the other hand, actin had shown greater stability even it was notably degraded as well. This finding was opposite to result of previous study where actin fraction was found to be stable during the ripening of traditionally produced *Petrovská klobása,* made of hot-boned meat and characterized with a slight pH drop [4]. Others [10, 12-14, 28], also reported actin hydrolysis during the ripening of both starter-inoculated and non-inoculated fermented sausages. As a result, at the end of processing (90$^{th}$ day) the intensity of protein band at ≈45 kDa was decreased, particularly in starter-inoculated sausages ripened in industrial room (Isc). Thus, the activity of muscle proteinases (cathepsin D-like enzymes) and certain microbial enzymes was probably positively affected by higher initial temperatures in this

room and lower pH in I and Isc sausages, as it was proposed in previously published work of a number of authors [28, 31, 37-39].

Due to co-migration of myosin (≈220 kDa) degradation products the intensity of protein bands at ≈140 and 160 kDa markedly increased during the ripening. This phenomenon was particularly pronounced for starter-inoculated sausages. MHC degradation into the polypeptides with molecular weights in the range of 120-150 kDa was previously reported [10, 39]. Furthermore, the appearance and accumulation of several polypeptides with molecular weights in the range from ≈15 to ≈42 kDa, as well as the increase in intensity of protein bands of ≈70 and 75 kD, was registered during the ripening of each sample sausage. Several studies reported the formation and increase of protein fragments in the ranges 50-100 kDa and 14-45 kDa [10, 12, 13, 37, 39]. Nevertheless, more newly formed fragments appeared in sausages ripened in industrial conditions at higher initial temperatures (I and Isc). Also, total degradation and disappearance of numerous polypeptides (≈50, 90, 100, 105, 195 kDa) were registered in these samples, especially in starter-inoculated, confirming the positive effect of higher temperature and presence of microbial enzymes on proteolysis intensity [28, 31, 37, 38].





## Texture profile

Results of texture profile analysis are shown in Table 2. The hardness and chewiness of all examined groups of sausages increased progressively during processing. The impact of the starter culture addition on hardness and chewiness of T and Tsc sausages was not noticeable, still at the end of ripening period (90 days) Tsc was higher in hardness (81.8 N) and

chewiness (10.2 N) comparing with T sausage (66.9 N and 8.71 N, respectively). On the other hand, Isc sausage was higher ($P$<0.05) in both of these parameters comparing to I, during almost the entire ripening period.

Furthermore, industrially processed sausages were significantly higher ($P$<0.05) in hardness and chewiness than their counterparts from traditional production. As mentioned before, these differences could be explained by considerably faster fermentation process and steep pH drop in I and Isc sausages. When pH declines the solubilized miofybrillar proteins aggregate to form a gel leading to the formation of an ordered protein network. This gel binds the meat and fat particles closely together, contributing to firmness. If pH is reduced below the pI, solubilization of protein is higher, producing firmer sausages. After fermentation, drying is a major factor affecting binding and rheological properties of fermented sausages. During the drying process water is constantly removed from the product, and as it continues the more tightly bound water is released as well, creating a more dense and chewy structure due to further protein denaturation and degradation by muscle and microbial proteolytic enzymes. Usually, the net result is an increase in firmness, but for desired texture profile of sausage a fine balance between forces contributing to hardening (drying) and softening (protein breakdown) is required [7, 8, 10, 15, 16, 40]. Significant negative correlations ($P$<0.05) between pH, moisture content and proteolysis on the one hand and hardness and chewiness on the other were registered previously [7, 16, 32]. Hence, at the end of drying period (90 days in traditional and 60 days in industrial conditions), when the similar levels of moisture content (Fig. 2(a)) and nitrogen fractions (Table 1) were found in T and I sausages and in Tsc and Isc sausages, the similar levels of hardness and chewiness in these samples were observed as well (Table 2). Generally, the hardness values of *Petrovská klobása*

samples, obtained in present study, were in agreement with results reported for sucuk [16], slightly higher than those reported for *Chorizo de Pamplona* [41] and higher than those registered in Italian low-acid sausage [10]. During ripening period there was no significant effect of processing conditions or starter culture addition on springiness and cohesiveness.

## Sensory analysis

The sensory analysis revealed significant ($P$<0.05) differences in chewiness, juiciness and overall acceptability of samples at the end of drying process (Fig. 5). Traditional production, that is, lower temperatures and longer ripening period resulted in better sensory characteristics. Industrially processed sausages, especially I, were rated with lower scores for chewiness and juiciness, what affected their overall acceptability. However, the addition of starter culture gave better results for both traditionally and industrially processed sausages. Hence, to obtain sausages of the higher sensory quality in industrial conditions, application of different starter culture, probably autochthonous microbes isolated from traditional sausage, might be better solution.

# CONCLUSIONS

Different ripening conditions had more noticeable effect on evaluated parameters, compared to starter culture addition. Hence, higher initial temperatures in industrial ripening room

(≈15˚C) affected faster fermentation process, i.e. more intensive pH decline in I and Isc sausages. This phenomenon promoted the drying and proteolysis processes, as well as increase of hardness and chewiness in these samples. Consequently, moisture content in sausages dried in industrial room was lower throughout the entire processing period, and the required one (<35%) was achieved 30 days earlier (60 days), when compared to those counterparts from traditional conditions (90 days). Likewise, the proteolytic changes were more significant in I and Isc samples, resulting in higher contents of nitrogen fractions (NPN and FAAN) and more intensive degradation of myofibrillar proteins. At each sampling period hardness and chewiness were significantly higher for sausages ripened in industrial room, being almost at the same level when the required moisture content was achieved in different ripening conditions. The starter culture addition had positive effect on the sensory characteristics of *Petrovská klobása*, but for industrial production the use of autochthonous starter culture might be considered.





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**Figure captions**

Fig. 1 Environmental temperature and relative humidity recorded throughout the ripening of *Petrovská klobása* in traditional (a) and industrial (b) conditions

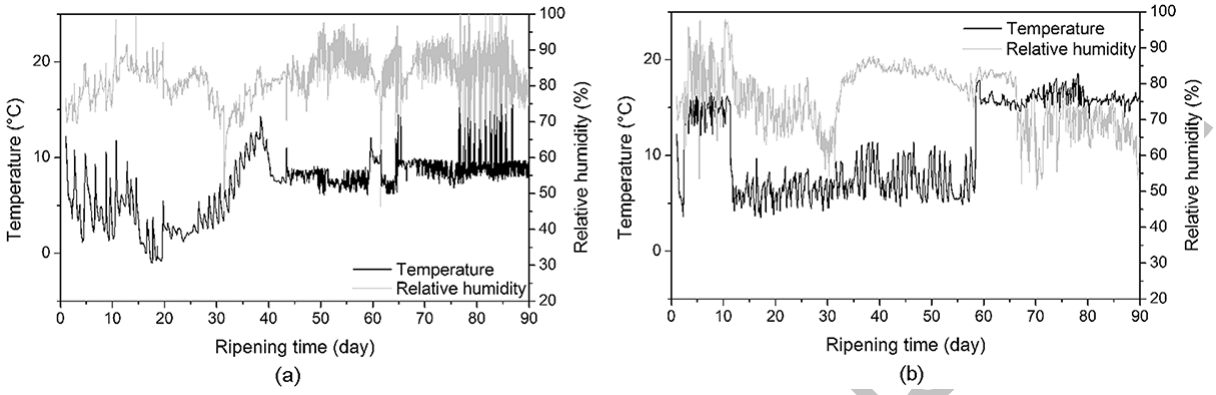

Fig. 2 Evolution of moisture content (a) and pH (b) in *Petrovská klobása* sausages throughout the ripening process in traditional (control - T, starter inoculated - Tsc) and industrial (control - I, starter inoculated - Isc) conditions

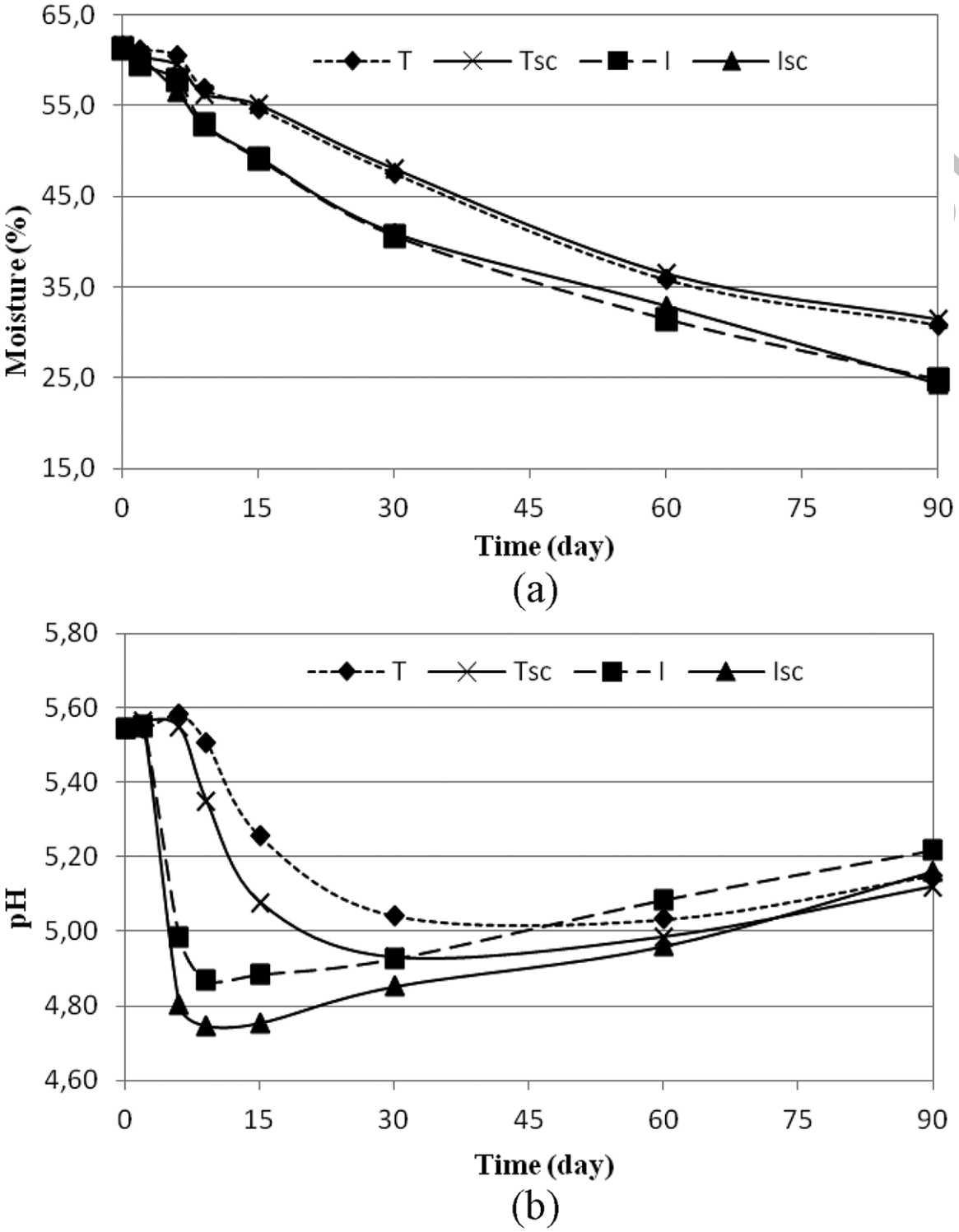

Fig. 3 Evolution of LAB (a) and *Micrococcaceae* (b) counts in *Petrovská klobása* sausages throughout the ripening process in traditional (control - T, starter inoculated - Tsc) and industrial (control - I, starter inoculated - Isc) conditions

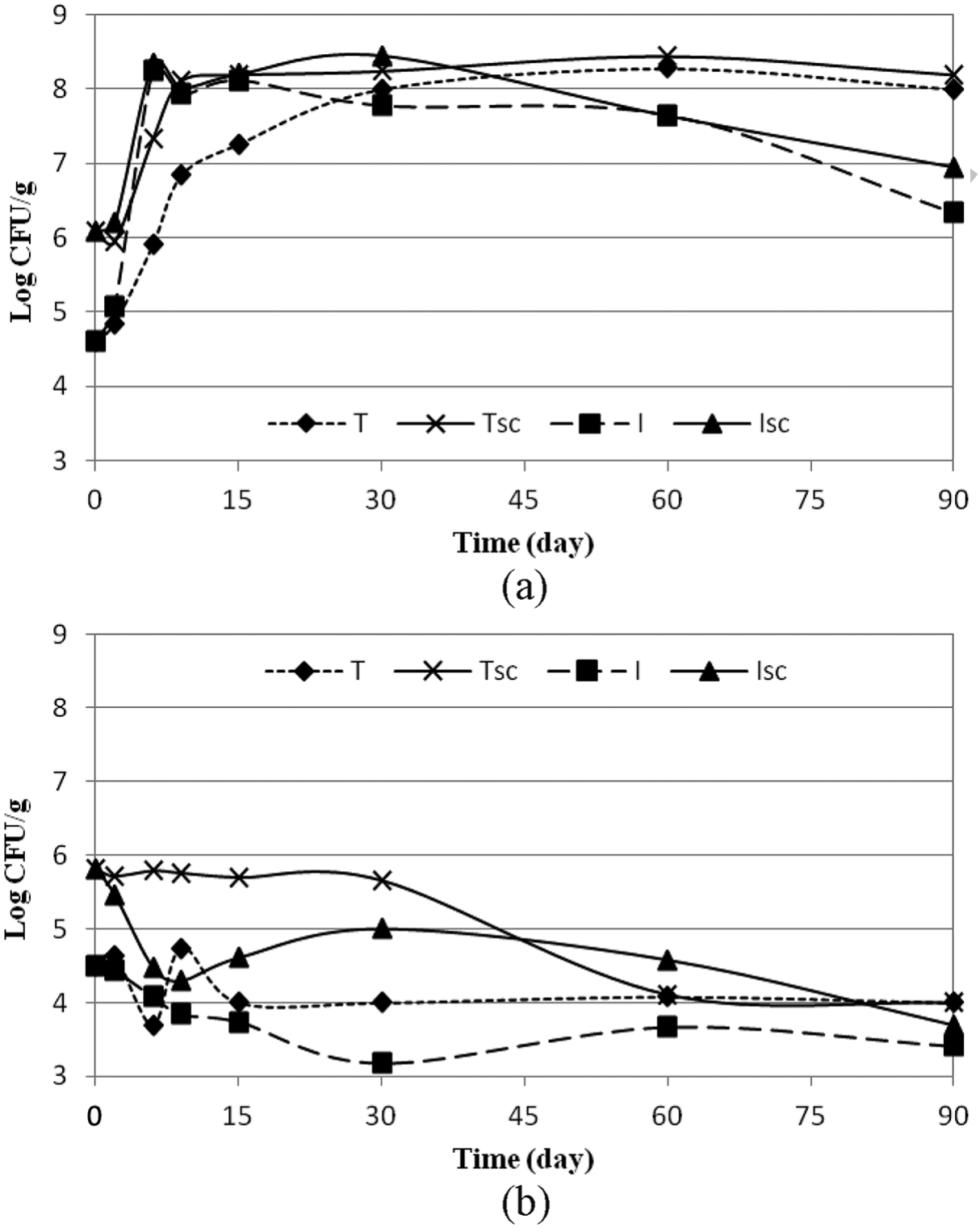

Fig. 4 LoaC gel-like image of myofibrillar proteins throughout the ripening of *Petrovská klobása* in traditional (A) and industrial (B) conditions (lanes 1-8): (a) control (non-inoculated), (b) starter inoculated. Lane L (Ladder), molecular weight standards ranging from 4.5 to 240 kDa

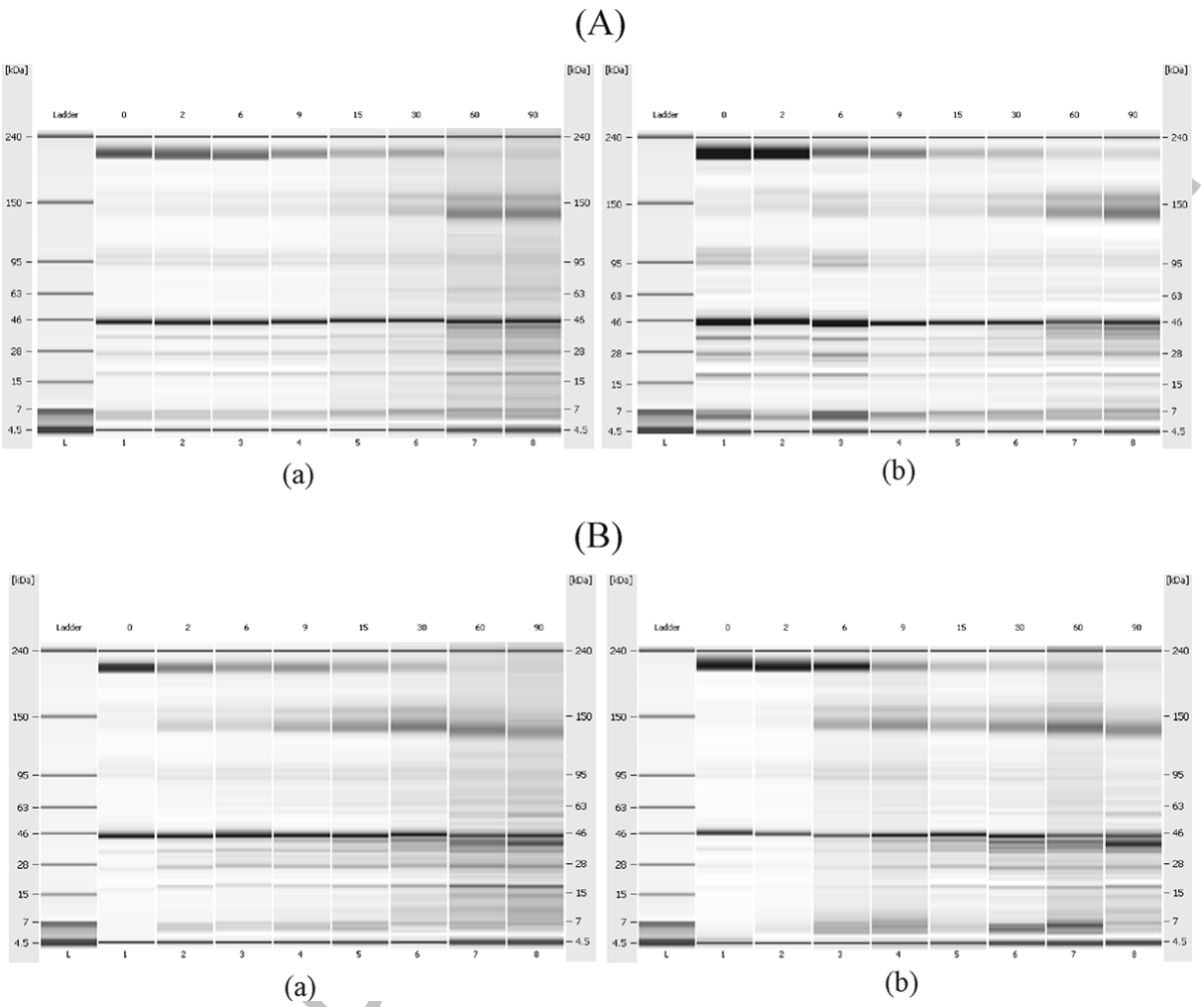

Fig. 5 Sensory scores of *Petrovská klobása* sausages at the end of drying process in traditional (control - T, starter inoculated - Tsc) and industrial (control - I, starter inoculated - Isc) conditions

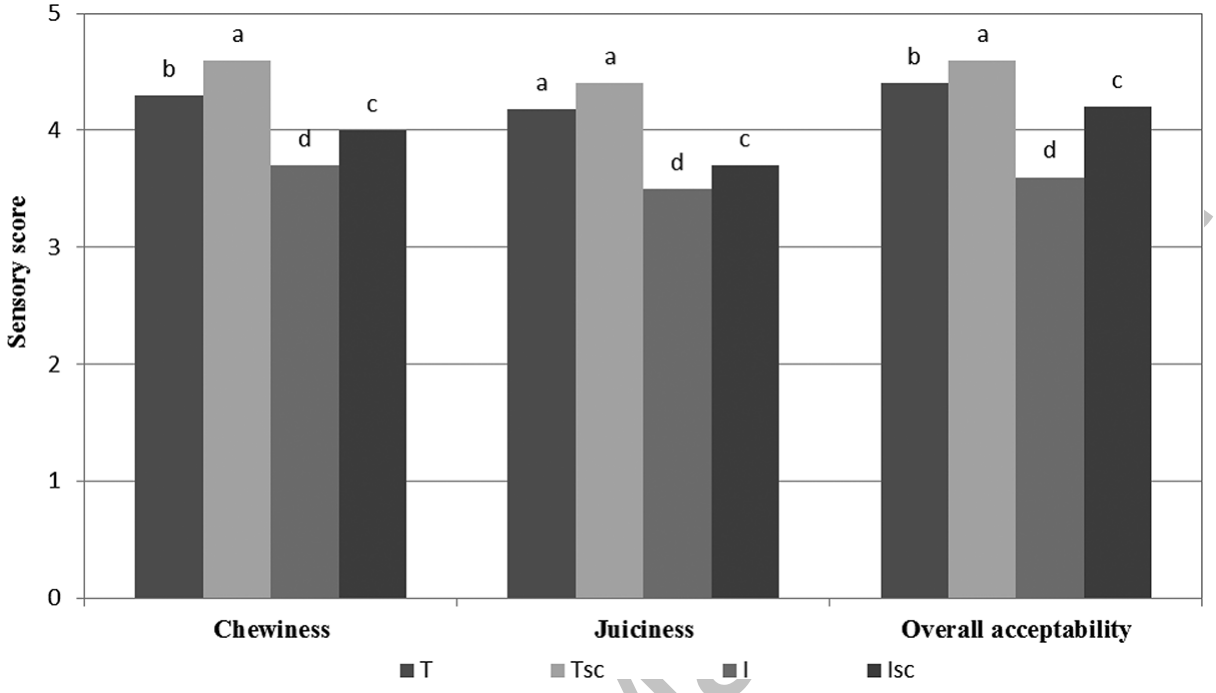

Table 1. Evolution of NPN and FAAN content in *Petrovská klobása* sausages (expressed as g/100g dm) throughout ripening process

| Nitrogen fraction | Batch | Time (day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 6 | 9 | 15 | 30 | 60 | 90 |
| NPN | T | $0.63^{a,B}$ | $0.64^{b,B}$ | $0.80^{a,A}$ | $0.76^{a,A}$ | $0.76^{a,A}$ | $0.86^{b,C}$ | $1.06^{b,D}$ | $1.17^{d,E}$ |
| | | ± 0.01 | ± 0.02 | ± 0.02 | ± 0.02 | ± 0.05 | ± 0.02 | ± 0.01 | ± 0.01 |
| | Tsc | $0.63^{a,C}$ | $0.74^{c,A}$ | $0.72^{b,A}$ | $0.74^{a,A}$ | $0.79^{a,D}$ | $0.97^{a,E}$ | $1.14^{a,B}$ | $1.15^{c,B}$ |
| | | ± 0.01 | ± 0.02 | ± 0.02 | ± 0.02 | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.01 |
| | I | $0.63^{a,B}$ | $0.69^{a,C}$ | $0.81^{a,A}$ | $0.81^{b,A}$ | $0.92^{b,D}$ | $0.98^{a,E}$ | $1.13^{a,G}$ | $1.10^{b,F}$ |
| | | ± 0.01 | ± 0.01 | ± 0.02 | ± 0.01 | ± 0.02 | ± 0.01 | ± 0.00 | ± 0.01 |
| | Isc | $0.63^{a,B}$ | $0.70^{a,C}$ | $0.85^{c,A}$ | $0.87^{c,A}$ | $0.89^{b,D}$ | $0.93^{c,E}$ | $1.09^{c,G}$ | $1.06^{a,F}$ |
| | | ± 0.01 | ± 0.01 | ± 0.02 | ± 0.02 | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.01 |
| FAAN | T | $0.25^{a,B}$ | $0.26^{a,C}$ | $0.28^{a,D}$ | $0.31^{a,A}$ | $0.31^{a,A}$ | $0.36^{a,E}$ | $0.47^{b,F}$ | $0.48^{a,G}$ |
| | | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.00 | ± 0.01 | ± 0.00 | ± 0.01 | ± 0.00 |
| | Tsc | $0.25^{a,B}$ | $0.27^{a,C}$ | $0.29^{a,D}$ | $0.31^{a,A}$ | $0.31^{a,A}$ | $0.36^{a,E}$ | $0.46^{a,F}$ | $0.53^{b,G}$ |
| | | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.00 | ± 0.00 | ± 0.01 | ± 0.00 | ± 0.00 |
| | I | $0.25^{a,B}$ | $0.27^{a,C}$ | $0.36^{b,A}$ | $0.36^{b,A}$ | $0.38^{b,D}$ | $0.39^{b,E}$ | $0.46^{a,F}$ | $0.49^{a,G}$ |
| | | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.00 | ± 0.01 | ± 0.01 | ± 0.00 | ± 0.00 |
| | Isc | $0.25^{a,B}$ | $0.29^{b,C}$ | $0.34^{b,D}$ | $0.37^{b,A}$ | $0.38^{b,A}$ | $0.40^{b,E}$ | $0.45^{a,F}$ | $0.48^{a,G}$ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ± 0.01 | ± 0.00 | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.01 | ± 0.01 |

[a - d] Means within the same column with different superscript letters are different ($P<0.05$).

[A - G] Means within the same row with different superscript letters are different ($P<0.05$).

NPN - non-protein nitrogen, FAAN - free amino acids nitrogen.

Table 2. Evolution of texture profile of *Petrovská klobása* sausages throughout drying/ripening process

| Parameter | Batch | Time (day) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 2 | 6 | 9 | 15 | 30 | 60 | 90 |
| Hardness (N) | T | $4.69^{a,D}$ | $6.82^{bc,D}$ | $6.54^{c,D}$ | $7.65^{d,D}$ | $15.9^{c,C}$ | $31.9^{c,B}$ | $66.9^{b,A}$ |
| | | ± 1.26 | ± 0.82 | ± 1.11 | ± 1.01 | ± 3.74 | ± 16.2 | ± 6.02 |
| | Tsc | $4.58^{a,D}$ | $4.94^{c,D}$ | $6.16^{c,D}$ | $9.65^{c,CD}$ | $15.2^{c,C}$ | $45.5^{c,B}$ | $81.8^{b,A}$ |
| | | ± 0.81 | ± 1.21 | ± 0.39 | ± 1.01 | ± 2.86 | ± 11.1 | ± 9.70 |
| | I | $5.08^{a,D}$ | $8.67^{b,D}$ | $11.2^{b,D}$ | $16.1^{a,CD}$ | $25.6^{b,C}$ | $65.2^{b,B}$ | $107^{a,A}$ |
| | | ± 0.80 | ± 1.21 | ± 2.32 | ± 0.96 | ± 6.89 | ± 11.4 | ± 22.6 |
| | Isc | $4.26^{a,E}$ | $12.7^{a,DE}$ | $14.9^{a,D}$ | $14.2^{b,D}$ | $32.0^{a,C}$ | $81.0^{a,B}$ | $117^{a,A}$ |
| | | ± 0.70 | ± 3.26 | ± 4.73 | ± 1.20 | ± 5.65 | ± 6.31 | ± 16.5 |
| Chewiness (N) | T | $0.40^{a,D}$ | $0.72^{c,D}$ | $0.59^{c,D}$ | $1.07^{c,D}$ | $2.42^{b,C}$ | $4.40^{b,B}$ | $8.71^{b,A}$ |
| | | ± 0.10 | ± 0.16 | ± 0.11 | ± 0.23 | ± 0.44 | ± 2.44 | ± 1.36 |
| | Tsc | $0.44^{a,D}$ | $0.50^{c,D}$ | $0.69^{c,D}$ | $1.30^{c,CD}$ | $2.59^{b,C}$ | $6.72^{b,B}$ | $10.2^{ab,A}$ |
| | | ± 0.09 | ± 0.13 | ± 0.07 | ± 0.20 | ± 0.73 | ± 2.34 | ± 1.87 |
| | I | $0.47^{a,D}$ | $1.23^{b,CD}$ | $1.46^{b,CD}$ | $2.26^{a,CD}$ | $3.46^{ab,C}$ | $10.1^{a,B}$ | $13.1^{a,A}$ |
| | | ± 0.07 | ± 0.21 | ± 0.35 | ± 0.28 | ± 1.37 | ± 3.07 | ± 4.40 |
| | Isc | $0.44^{a,D}$ | $1.83^{a,D}$ | $1.98^{a,D}$ | $1.75^{b,D}$ | $3.87^{a,C}$ | $10.7^{a,B}$ | $12.9^{a,A}$ |
| | | ± 0.11 | ± 3.26 | ± 0.65 | ± 0.24 | ± 0.75 | ± 1.55 | ± 3.27 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Springiness | T | $0.29^{a,C}$ | $0.33^{ab,C}$ | $0.30^{b,C}$ | $0.37^{a,B}$ | $0.41^{a,A}$ | $0.38^{a,AB}$ | $0.37^{a,B}$ |
| | | ± 0.02 | ± 0.06 | ± 0.02 | ± 0.03 | ± 0.02 | ± 0.05 | ± 0.02 |
| | Tsc | $0.30^{a,E}$ | $0.31^{b,DE}$ | $0.34^{da,C}$ | $0.36^{a,BC}$ | $0.41^{a,A}$ | $0.38^{a,B}$ | $0.36^{a,BC}$ |
| | | ± 0.02 | ± 0.03 | ± 0.03 | ± 0.02 | ± 0.03 | ± 0.03 | ± 0.01 |
| | I | $0.32^{a,C}$ | $0.33^{ab,BC}$ | $0.35^{a,ABC}$ | $0.36^{a,AB}$ | $0.36^{b,AB}$ | $0.39^{a,A}$ | $0.36^{a,AB}$ |
| | | ± 0.03 | ± 0.02 | ± 0.03 | ± 0.02 | ± 0.03 | ± 0.05 | ± 0.04 |
| | Isc | $0.32^{a,BC}$ | $0.36^{a,A}$ | $0.36^{a,A}$ | $0.32^{b,BC}$ | $0.34^{b,ABC}$ | $0.36^{a,A}$ | $0.38^{a,A}$ |
| | | ± 0.04 | ± 0.02 | ± 0.02 | ± 0.02 | ± 0.02 | ± 0.01 | ± 0.04 |
| Cohesiveness | T | $0.29^{a,C}$ | $0.32^{b,BC}$ | $0.30^{c,C}$ | $0.38^{a,A}$ | $0.37^{b,A}$ | $0.37^{a,A}$ | $0.35^{a,AB}$ |
| | | ± 0.03 | ± 0.02 | ± 0.03 | ± 0.04 | ± 0.03 | ± 0.02 | ± 0.03 |
| | Tsc | $0.32^{a,C}$ | $0.33^{b,C}$ | $0.33^{b,C}$ | $0.38^{a,AB}$ | $0.41^{a,A}$ | $0.38^{a,AB}$ | $0.34^{ab,C}$ |
| | | ± 0.02 | ± 0.01 | ± 0.02 | ± 0.03 | ± 0.02 | ± 0.03 | ± 0.03 |
| | I | $0.29^{a,D}$ | $0.43^{a,A}$ | $0.37^{a,BC}$ | $0.39^{a,ABC}$ | $0.37^{b,BC}$ | $0.40^{a,AB}$ | $0.34^{ab,CD}$ |
| | | ± 0.02 | ± 0.02 | ± 0.02 | ± 0.03 | ± 0.03 | ± 0.06 | ± 0.05 |
| | Isc | $0.32^{a,CD}$ | $0.40^{a,A}$ | $0.37^{a,AB}$ | $0.39^{a,AB}$ | $0.35^{b,BC}$ | $0.36^{a,AB}$ | $0.29^{b,D}$ |
| | | ± 0.04 | ± 0.04 | ± 0.02 | ± 0.02 | ± 0.03 | ± 0.02 | ± 0.06 |

$^{a-d}$ Means within the same column with different superscript letters are different ($P$<0.05).

$^{A-D}$ Means within the same row with different superscript letters are different ($P$<0.05).