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Investigation on Autolytic Processes and Microstructural Changes in Red Deer Meat

Leila A. Kaimbayeva[1,*], Elena S. Malysheva[2], Rashit Kazikhanov[3] and Saule R. Kazikhanova[4]

[1]*Department of Chemistry and Chemical Technologies, North Kazakhstan State University, Petropavlovsk, Republic of Kazakhstan*
[2]*Barnaul Veterinary Department of the Altai Region State Veterinary Service, Barnaul, Russian Federation*
[3]*Department of Production Technology and Processing of Livestock, Saken Seifullin Kazakh Agrotechnical University, Astana, Republic of Kazakhstan*
[4]*Department of Hunting and Fisheries, Saken Seifullin Kazakh Agrotechnical University, Astana, Republic of Kazakhstan*

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Key words
Autolysis of meat, Red deer meat, Changes after death, Microstructure, Autolytic processes.

ABSTRACT

This study was designed to evaluate markers of autolysis and to effectively assess the optimal maturity of red deer meat before trading, and utilization in the production of meat products. For these purposes, meat samples were collected from quadriceps of 2.5-4 years old female red deer, immediately processed for isolation of muscular fibers and were evaluated for the pH, water-binding power, activities of tissue enzymes and microstructure changes. The results of chemical analysis revealed the nature of protein substances, altered pH value of the meat and the intensity of glycolysis. All these parameters were indicative of autolysis process in the meat. The levels of water-binding capacity and structural-mechanical properties of red deer meat that occurred during the course of autolysis were further confirmed by the histological changes in the meat. Specifically, it was observed that post-mortem changes were characterized by multiple destruction and disintegration of fibers in the muscular tissue, and lysing of cores and their structures. Collectively, these results clearly articulate the irreversible destructive changes in the lean tissue. The data presented in this study highlight the mechanisms of the autolytic process and its influence both on the morphological composition of the lean tissue and on the shelf life of the meat.

INTRODUCTION

Production of quality red deer meat though selective breeding and high-technology processing has positive knock on effects on the economies of developing countries including Republic of Kazakhstan. Specifically, the mountains and the piedmont parts of the Altai Territory and the East Kazakhstan Region are unique natural and climatic zones that favour the breeding of central anthlers red deer. While meat and meat-products are important source of food especially for poor communities, the meat productivity and quality of Altai red deer has not been studied comprehensively.

Recent studies have indicated that functional and technological properties of raw materials as well as the implementation of advanced technologies for the production and processing of red deer meat are inevitable and invaluable for food security (Satija *et al.*, 2017). Owing to high-grade and high-quality raw material, red deer meat offers an enormous sources of food with acceptable biological value (Abbas *et al.*, 2017).

According to its biochemical properties, the red deer meat is characterized by high correlation of native proteins to deficient proteins, large content of nitric extractive matters, vitamins, macro- and microelements (Bureš *et al.*, 2015). Thus, according to the content of such non-replaceable amino acids as lysin and leucine, the red deer meat exceeds beef, pork and lamb (Kaimbaeva and Gurinovich, 2016). Due to the fact that there is a steady demand for this commodity, the quality of the meat is considered an important selection criterion (Kaimbaeva, 2008). One of the conditions of quality formation and product yield is the level and development nature of the autolytic processes (Kaimbaeva and Uzakov, 2015). Study of tissue composition will allow identifying functional and technological indicators of red deer meat quality (Malysheva and Zhukov, 2013).

In order to define benchmark of quality, the onset of autolysis was studied in red deer meat. Several indicators including pH, water-binding power, activity of tissue

* Corresponding author: dr.kaim2017@yandex.ua
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enzymes and microstructural changes were assessed to propose the optimal periods of red deer meat maturation. These findings are fundamental to not only improve the quality of by-products but also to offer the most suitability time for processing of red deer meat.

MATERIALS AND METHODS

Terms of the study

A female red deer (2.5-4 years) was euthanized by electric current (50 Hz) and bleeding.The muscular tissues were collected from the quadriceps and were immediately processed for muscle fibre collection. These samples were then individually packed and stored at 2-4ºC temperature until further analysis.

The first sampling was performed within 2-3 hours post-euthanasia, and were regarded as zero point in the analysis, representing a change of the studied indicators. The meat was considered fresh when it was maintained at the temperature of 35-36ºC. Afterward the sample analysis was performed at 30 min, and 24, 48, 72, 96 and 120 hours post-euthanasia. For the potentiometric measurement of the active acidity in the meat, a pH tester millivoltmeter was used (pH-150).

Permission on Animal Handling and Euthanasia

The experiments were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and were approved by the Ethics Committee of the Saken Seifullin Kazakh Agrotechnical University (Protocol No. 1289-RM (November 18, 2017)).

Methodologies

In order to define the activity of proteinase, the Anson method was used (with modification of E. Kaverzneva), where the catalytic properties are judged by the degree of splitting of protein standard with the formation of low molecular weight products: peptides and amino acids, in particular, with regards to accumulation of tyrosine. Casein was used as a substrate when defining the activity of cathepsin D and calpain (Antipova *et al*., 2000).

The yield point (YP, Pa) was defined with the help of the device PM-3 (Antipova *et al*., 2000). The water-binding power (WBP %) was defined by the compaction method (Antipova *et al*., 2000). Histological examinations were conducted according to Standards of Organisation Federal State-Funded Educational Institution of Higher Professional Education 00493184-001-2013 'Red deer meat (Gnat *et al*., 2015). For histological examination, the traditional methodology was used for cross-sectioning of tissues. Following staining approach was applied; formalin-fixation of the samples within 7 days; carry-through by alcohol solutions, starting with 50% of concentration and finishing with 100%, with the interval of 4-6% and the length of each stage of experiment of 24 h; filling with paraffin within 6-10 days and weight fraction of 8-12% within 3-5 days. The cross-section of the samples was performed at the sledge microtome for further histological examinations after staining with heamatoxilin-eosin.

Measuring instruments

The received preparations were studied with the help of the miscroscope, Biolam P1У4, under the object lens of 3.2-40 with the increase of the ocular to 13x. Further on, the analysis of the microscopical changes in the norm and in the post-mortem condition was performed with the help of micronets and a microruler (Russian Federation, 2013).

RESULTS AND DISCUSSION

Changes in the physico-chemical (pH, units), functional-technological (WBP, %) and structural-mechanical (YP, Pa) indicators of red deer meat were studied in studying during the process of autolysis. The data represented in Table I showed that during the process of autolysis, the water-binding capacity of red deer meat in the fresh state, under the temperature of 37°C, is equal to 66.80%. However, within an hour it was decreased insignificantly dropped as low as 65.20%.

After 24 h, the red deer meat revealed the WBP indicators of 52.32%, and after 72 h – the value dropped to 54.6%. With the following resolution of the post-mortem carcass stiffening, an increase of the hydration of the muscular tissue occured. The minimum of the hydration of proteins in the muscular tissue as marked approximately post 20–24 h of slaughtering.

The water-binding capacity of the meat after the carcass stiffening was continuously and slowly observed. The water-binding capacities increased during the whole period of its storage under positive temperatures. However, it failed to reach the initial level of the fresh meat and approached its maximum level of 85–87 %. The reduction of water-binding capacity in the muscular tissue within the first 24 h post-euthanasia was conditioned due to the reduction of pH value and the formation of actomyosin. It leads to the reduction of yield during the thermal treatment of meat and meat products. This is one of the most important practical consequences of stiffening.

The active-acidity of red deer meat at the beginning of autolysis approached to 5.6 units and after 24 h it reached its maximum value of 5.2 units. After 96 and 120 h (5 days) it was stabilized to the value of 5.5 units (Table I). In connection with this, the process of autolysis was not studied after 5 days.

Table I.- Changes of structural-mechanical and functional-technological indicators of red deer meat during the process of autolysis.

Length of autolysis (h)	pH	WBP (%)	YP (Pa)
30 min	5.6	66.80±0.26	308±2.52
24	5.2	52.32±0.56	312±2.52
48	5.3	53.35±0.62	315±2.54
72	5.4	54.6±0.72	330±2.64
96	5.5	56.6±0.68	350±2.66
120	5.5	57.24±0.82	360±2.89

During the maturing of meat, changes of structural-mechanical indicators occurred mainly as a result of biochemical transformations in the albuminous system (Rogov *et al*., 2013). Changes in the structural-mechanical indicators characterise tenderness of the meat (Nikiforova *et al*., 2011). The maturing process of processed meat is closely connected with the influence of proteolytic enzymes on the structural elements of tissues (Kudryashov, 2007). This fact proves the correlation between changes of protein substances, carbohydrates and mineral composition of the meat occurring during the maturing process (Cocolin, 2011).

The structural-mechanical indicators of red deer meat were assessed every hour within 5 days (120 h) after the killing, according to the results of yield strength. The received data showed that the structural-mechanical indicators of the muscular tissue of red deer meat depend on the structure of tissues and depth of development with regards to the autolytic processes. The results of performed experiments showed that the strength properties of the lean red deer meat is defined by the nature and depth of development of the said autolytic processes and depend considerably upon the grain of the tissues (Wiklund *et al*., 2014). Upon the death-mediated stiffening, the strength characteristics of the meat increased. As the obtained data confirmed, the changes of strength properties of red deer meat at the end of maturing has a tendency towards reduction. This process is explained by the conformational changes of the actomyosin complex and polyunsaturated fatty acids during the post-mortem period (Damez, 2008).

During the process of post-mortem changes, fermentative autolytic changes occur in the muscular tissue in the livestock, leading to the improvement of structural-mechanical indicators of the meat (Dutson, 1980). The tissue softening and the improvement of structural-mechanical indicators of the meat during the maturing stage are connected with the proteolytic degradation of protein structures under the influence of tissue proteolytic enzymes (Dwinger-Ron, 2008). The autolysis of meat occurs with the active participation of lysosome enzymes–cathepsins and calpains (Hope-Jones, 2010).

One of the conditions of quality and yield formation for the product would be the level and characteristics of development with regards to the autolytic processes (Krause, 2011). Study of the properties of the tissue proteinases will systematically change functional-structural indicators of red deer meat (Cheret, 2007). With regards to this, the changes in the activity of tissue proteinases of red deer meat are studied according to the stages of the autolysis process.

The research into the activity of tissue enzymes were also assessed – with regards to cathepsins and calpains – in the muscular fiber of red deer meat. The proteolytic activity of cathepsin D and calpains were expressed in micrograms (μm) of tyrosine during one hour for one gram (g) of tissue.

Results of the conducted research showed that during the process of autolysis, the release of cathepsins in the muscular tissues of red deer occurs as well as the manifestation of its activity (Fig. 1). The growth of free activity of cathepsin D is defined by the speed and depth of glycolytic changes. The data represented in Figure 1 confirmed the low activity of cathepsin D within 2-3 h after the slaughter of the animal. The low activity of cathepsin D after the slaughter is explained by the presence of native membrane in the meat retaining the cathepsines in the latent state.

During further maturing, the lysosome membrane permeability was increased and an active release of cathepsin D was observed as well as increase in the concentration of hydrogen ions in the sarcoplasm of the muscular fiber.

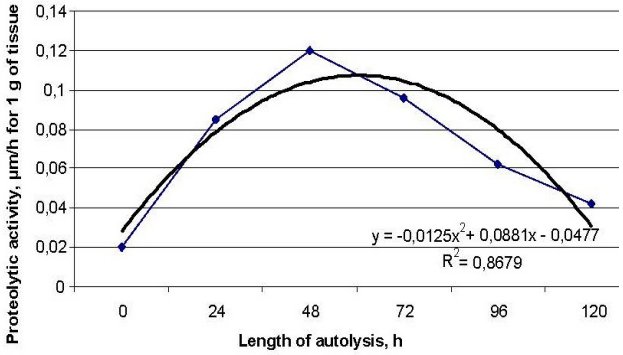

Fig. 1. Correlation of proteolytic activity of cathepsin D in the muscular fibers of red deer meat with regards to the length of autolysis.

Table II.- Quantitative indicators of striated musle tissue of red deer females in the norm and in the post-mortem state.

Time after the slaughter	Diameter (µm)		Area (µm²)		Relative volume (%)	
	Muscular fibers	Nucleus	Muscular fibers	Nucleus	Muscular fibers	Nucleus
Carcass meat	93.2-95.7	15.6-19.8	396.2-511.2	2.8-3.6	100	100
24 h	89.4-90.2	13.2-17.6	359.9-450.4	1.8-2.5	95.1	20.5
48 h	72.3-85.8	12.1-16.5	305.5-420.7	1.2-2.3	83.7	14.0
72 h	61.6-79.8	9.7-13.6	260.1-396.2	1.2-1.8	78.0	11.5
96 h	57.2-72.2	7.1-12.1	235.9-305.5	1.4-1.7	72.2	10.5
120 h	38.3-59.4	4.4-8.6	223.8-275.2	0.6-0.9	78.0	5.0

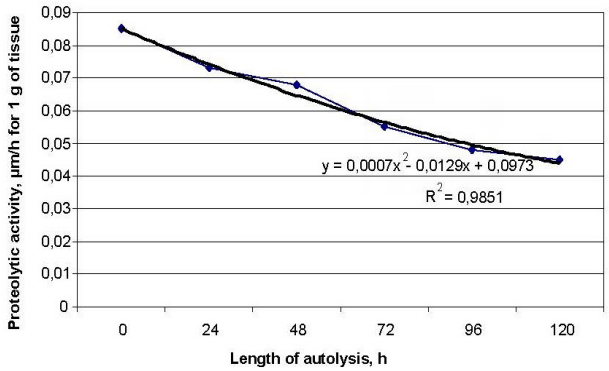

Fig. 2. Correlation of proteolytic activity of calpain in the muscular fibers of red deer meat with regards to the length of autolysis.

The maximum growth of proteolytic activity of cathepsin D for red deer meat was observed within 48 h after the slaughter – 0.12 µm/h for one gram of the protein. In the following hours of maturing, the enzyme inactivation occurred due to hydroxylation of hydrogen ions in the sarcoplasm of the muscular fiber.

The optimum proteolytic activity of calpains was observed in the fresh meat of red deer – 0.085 µm/h for one gram of protein (Fig. 2). The proteolytic activity of calpains is decreased sharply on the 2nd and 3rd days of the maturing process, whereas on the 4th and 5th days it is being stabilised (Skibniewski and Skibniewska, 2015). This process might be explained by the autoproteolysis and presence of an inhibitor of calpain enzyme – calpastatin.

It was revealed that the correlation of the activity of calpains with regards to Ca2+ might support the functionality of a regulatory mechanism, due to a change of the cation level in the cells and participation of Ca2+ - activated neutral proteases in the limited proteolysis. These changes in turn lead to altered activity of a number of enzymes (Krause, 2011). The quantitative indicators of the striate muscle tissue of female red deer in the normal and the post-mortem states are shown in Table II.

The outcome of performed experiments showed that the strength properties of the muscular fibers of red deer are defined by the nature and depth of development of autolytic processes and depend considerably on the structure of the tissues. Upon the start of death stiffening, the strength properties of the meat enhanced whereas changes in the strength properties of red deer meat at the end of maturing process has a tendency to decline (Cordeiro *et al*., 2017; Laghi *et al*., 2017). This circumstance is explained by the conformational changes of the actomyosin complex and polyunsaturated fatty acids during the post-mortem period. It was determined by the conducted research that the nature of changes of protein substances in the process of autolysis is determined by the pH value of the meat and the intensity of glycolysis (Dervilly-Pinel *et al*., 2017; Fowler *et al*., 2018; Prieto *et al*., 2017). The analysis of water binding capacity and structural-mechanical properties of red deer meat in the process of autolysis agreed with the results of the histologic examinations. The results of the quantitative investigations of histological sections of the muscular fiber of red deer females testify to the fact that the maximum diameter of the muscular fiber (95.7 µm), as well as the diameter of cell nuclei (19.8 µm) is peculiar for the samples received 30 min after the slaughter.

The maximum area occupied by the muscular fibers during this period was equal to 511.2 µm2, whereas the area occupied by the nuclei makes up 3.6 µm2. We can observe the least diameter of the muscular fibers (59.4 µm) and the diameter of cell nuclei (8.6 µm) 120 h after the slaughter. Under the post-mortem conditions, after 120 h, the area occupied by the muscular fibers made up 275.2 µm2, and the area taken by the nuclei was equal to 0.9 µm 2. A total of 24 h after the slaughter, the diameter of muscular fibers decreased by 5.5%, whereas the diameter of nuclei decreased by 2.2%.

During the process of changes post-death, under the influence of own enzymes, the disintegration of muscular

fibers and karyolysis was observed. The quantitative indicators were also changed during the process of autolysis (Myshalova *et al*., 2016). Therefore, 48 h post-euthanasia, the diameter of muscular fibers was decreased by 5.6%, whereas the diameter of nuclei was decreased by 1.1%. Later on, throughout the duration of 72 h, 96 h, the diameter of the muscular fiber and the nuclei decreased by 6-2.9%; 7.6-1.5% correspondingly. Upon completion of our experiment, during the process of autolysis, the diameter of muscular fibers decreased by 36.3%, diameter of nuclei decreased by 11.6%; the area occupied by the muscular fibers was reduced by 43.5%, whereas the area occupied by nuclei was reduced by 2.7%.

CONCLUSION

In conclusion, the obtained results indicated that changes after death are characterized by the multiple destruction and disintegration of the muscular fibers, lysing of nuclei and their structures. Afterwards, all of these characteristics lead to irreversible destructive changes of the lean tissue. Thus, the mechanisms of the autolytic processes influence both the morphological composition of the lean tissue and the shelf life of the meat for processing.

Statement of conflict of interest

Authors have declared no conflict of interest.

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