

INFLUENCE OF ELECTRICAL STIMULATION AND ELECTRICAL MASSAGING ON CALPAIN ACTIVITY AND INTRACELLULAR CONCENTRATION OF Ca^{2+} OF MUSCULAR TISSUE OF BEEF WITH DIFFERENT PATTERN OF AUTOLYSIS

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Introduction and background

The current tasks of meat processing industry to increase the production volumes, decrease the expenses and improve quality can be solved only using intensive technologies in maximum degree considering the peculiarities of the raw materials being processed. One of the ways of improving technologies is the use of electric pulses, accelerating fermentative processes in the autolizing muscular tissue. As a result of periodical contraction and relaxation of muscular fibers the activation of lysosomal enzymes takes place, and especially of proteinases, and acceleration of redistribution of curing ingredients in previously cured raw materials [1, 2]. According to existing knowledge, the fermentative processes on which ageing and curing of meat are based, are responsible for the increase of its tenderness, flavour and aroma.

Softening of tissues and increasing of tenderness are largely connected with proteolytic changes of protein structures under the action of tissue enzymes: cathepsins and calpains. Studying of their properties will make it possible to purposefully influence the technological characteristics of raw materials and quality of final products.

Objectives

Due to the fact that properties of cathepsins and their activities with regards to muscle proteins are sufficiently studied (3) we have investigated the influence of electrical stimulation (ES) and electrical massaging (EM) on calpains activity (calcium dependent neutral proteinases) of beef muscle tissue.

Several scientists (4, 5) consider that maximum activity of calpain is observed at specific concentration of Ca^{2+} . In this connection it is interesting to trace the changes of the level of ionized calcium in muscular tissue with different pattern of autolysis (PSE, NOR - traditional pattern of autolysis and DFD).

Methods

Calpain having more accessible for calcium ions thio groups - microcalpain or calpain I_{2+} , is more sensitive to calcium ions. Taking this in mind we have studied calpain I, activated by Ca^{2+} concentration, corresponding to physiological values in the cells of animal tissues. In the experiments M.Longissimus dorsi taken from beef sides 45 min after slaughter was used. The unsalted muscle and injected with the brine muscle (10% of brine to the mass of raw materials) was subjected to treatment by electrical current (industrial frequency, voltage - 220 V, duration of pulses - 0.4 s with intervals 0.6s during 4 min). The activity of calpain was determined according to Anson M.L. with some modifications and additions. The concentration of ions of calcium in muscular tissue was fixed by digital pH-meter 5170 and calcium-selective electrode "Kritur" (Czech Republic), with the level of sensitivity $10^{-7} \text{ M Ca}^{2+}$.

Results and discussion

Analysis of data has shown (Figs 1 and 2) that independent of the pattern of autolysis the activity of calpain of muscular tissue of beef is reduced during storage. At the same time it was found that the activity of calpain in the first hours after slaughter of the animal is to some extent higher in DFD muscle as compared to NOR. However, by 24 hours of meat storage at $2 \pm 2^\circ\text{C}$ these differences become insignificant.

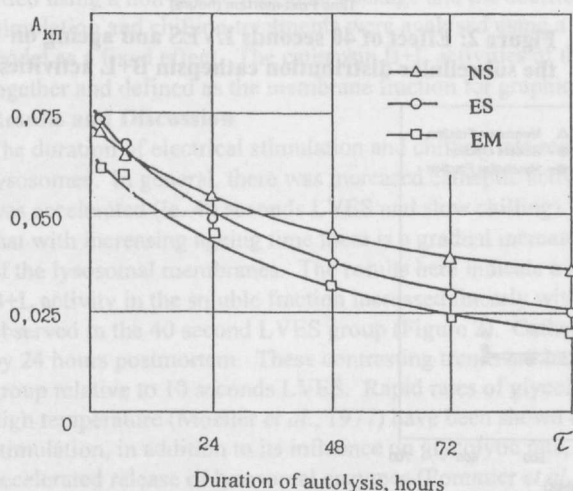


Fig. 1. Influence of electrical stimulation and electrical massaging on activity of calpain (A_c) of NOR beef (NS- non stimulated raw materials)

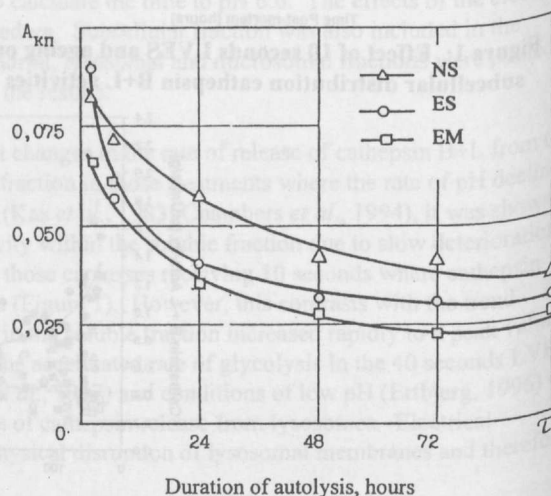


Fig. 2. Influence of electrical stimulation and electrical massaging on activity of calpain (A_c) of DFD beef

The presented materials show that the effect of electrical current on unsalted (ES) and salted (EM) muscular tissue leads to more intensive fall of calpain activity in DFD meat, and by 24 hours of autolysis it is by 16.7 % lower, than after ES, and by 11,2 % lower of the level in EM samples as compared to the raw materials of traditional pattern of autolysis.

Probably, a lower concentration of hydrogen ions in DFD muscular tissue pH_{24} 6.74 as compared to NOR pH_{24} 5.67 and the effect of electric pulses (ES and EM) lead to more intensive inhibition of the active center of the enzyme. By 72 hours of storage of meat at low negative temperatures the activity of calpain in DFD and NOR meat is actually equalized and is 0.3 - 3 $\mu\text{M}/\text{h.g}$ of protein, which indicates an increase of inhibiting effects on calpain.

As the results of the investigations have shown, in PSE, NOR and DFD meat during storage at $2 \pm 2^\circ \text{C}$ without treatment (Fig. 3), after ES (Fig. 4) and EM (Fig. 5) an increase in the concentration of ionized calcium was observed in all instances.

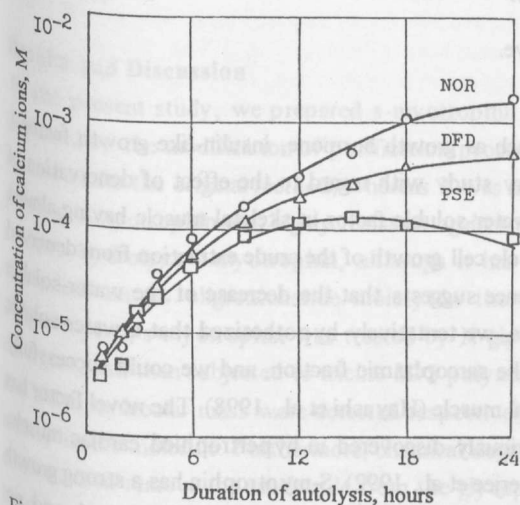


Fig. 3. Change of concentration of calcium ions during autolysis of beef muscular tissue

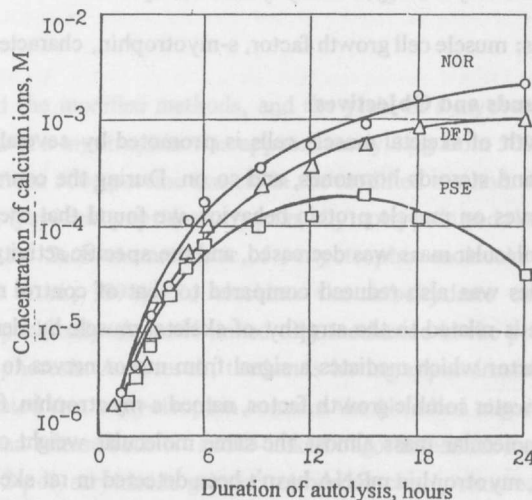


Fig. 4. Change of concentration of calcium ions during autolysis of ES muscular tissue of beef

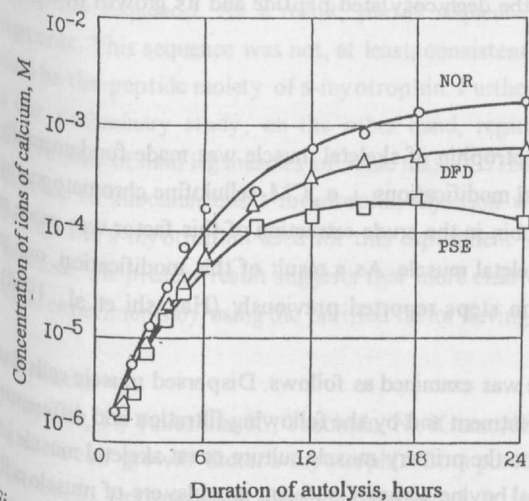


Fig. 5. Change of concentration of calcium ions during autolysis of EM beef muscle

One can state that the intensity of accumulation of calcium ions in the meat with different pattern of autolysis goes in the following sequence: NOR, DFD, PSE, independent of the investigated methods of action. Maximum concentration of calcium ions in NOR and DFD meat is achieved by 24 hours of storage at low positive temperatures, while in PSE meat — by 15 hours, after which, binding of Ca^{2+} by cellular structures is observed.

Conclusions

Based on the data obtained it can be stated that the rate of accumulation of Ca^{2+} in tissues during storage independent of the pattern of autolysis increases with ES and EM. In the autolizing, subjected to ES muscular tissue there is the most intensive accumulation of Ca^{2+} with their higher concentration.

The results of the investigations carried out indicate that the effects of electrical pulses both on unsalted and salted beef muscular tissue with different pattern of autolysis lead to a change of calpain inactivation rate and acceleration of calcium ions release from cellular organelles.

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