The influence of electrical stimulation and alternating temperature schedule of chilling upon changes in stored subfrozen meat

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Subfreezing is known to be one of the methods of meat cold treatment which allows to raise the output of freezers during seasonal deliveries of livestock to meat processing plants, to utilize more efficiently refrigerated cars for meat transportation from the main supplying areas to consuming regions. This method, however, involves a risk of muscle cold shortening in case of quick chilling of pre-rigor meat. The quality of subfrozen meat can be improved by means of electrical stimulation (ES), as well as by ES followed with alternating temperature schedule (ATS) chilling (1,2). The development of reliable and reasonable recommendations on the application of such technological procedures in commercial practice should be based on the results of comprehensive meat studies.

Purpose

The purpose of this work was to determine the effect of ES and ES combined with ATS on the changes in stored subfrozen meat by means of physical and biochemical methods.

Materials and methods

As experimental objects served semimembranosus and longissimus muscles dissected from beef sides. They were investigated rheologically (3), physico-chemically (4), citospectrophotometrically (5), histologically (6,7), morphometrically (7), electrophoretically (8); besides, protein solubility was studied (9). Meat quality was evaluated by its modulus of elasticity (E), pH, WHC (B), muscle esterase and lactate-dehydrogenase activity ($\mathcal{E}$) (by the optical density of the microphotographic negatives of specially stained meat samples), the number of disruptions and slot-like gaps (N_1), segmentations (N_2) and granular dissociation (N_3) in muscle fibers; by the protein in electrophoretic fractions and by myofibrillar protein solubility (R).

The experiments were carried out in 3 steps.

At Step 1 an electronic electrostimulating device was to be built. At Step 2 electrophoretic phenomena in meat were followed when electric current was passing through it. At Step 3 meat treated with the following methods was studied.

Method 1: meat chilling at -25°C down to 1°C in the deep tissue and storage at -2°C.
Method 2: meat stimulation (150V, 25 Hz) for 100 s; chilling and storage as in 1.
Method 3: stimulation as in 2, conditioning at 13°C for 12 hr; chilling and storage as in 1.

Results and discussion

An electronic ES device was designed and built; it is illustrated in Fig.1 together with a block diagram (11).

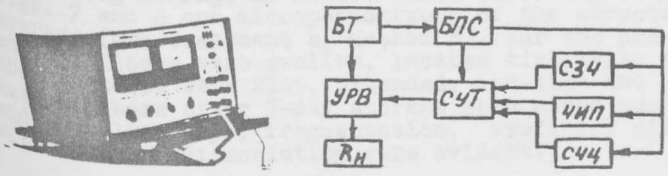

Fig. 1. The appearance and block diagram of the electrostimulator

The device consists of a block of power transformers (БТ), a power block (БПС), a system for specifying the frequency (СЗЧ), a system for specifying the number of impulses (ЧИП), a system for specifying the number of cycles (СЧЦ), a system monitoring thyristors (СУТ), a controllable reversible rectifier (УРВ); $R_н$ is a meat sample. The electrostimulator is a programmable impulse generator. The operating voltage can be regulated from 30 to 700 V, amperage - up to 10 A. Passing current trhough muscles demonstrated that impulse stimulation is accompanied by a number of light and acoustic phenomena caused with glow and spark discharges. To quantify electrophysical phenomena in meat, a coefficient K_τ was used, it representing the ratio of the time to reach the maximum amperage during ES to the moment of the completion of post-breakdown phenomena (10). It was established that K_τ /U (voltage) relation can be represented as equations for semimembranosus and loggissimus, resp.:

$$K_\tau = 18.23 \times e^{-0.0069 \times U} \quad (1) \quad \text{and} \quad K_\tau = 18.01 \times e^{-0.0074 \times U} \quad (2)$$

Thus, with increasing U, K_τ is decreasing, this being due to a faster break-down of tissue. Let us assume a 10% change in the amperage of the initial value as a permissible limit and think that above this value changes in the tissue will be significant. Then, the maximum possible time of impulse stimulation as related to the applied voltage can be approximated with the equations (for semimembranosus and longissimus, resp.):

$$\tau_B = 631 \times e^{-0.00118 \times U} \quad (3) \quad \text{and} \quad \tau_B = 450 \times e^{-0.0094 \times U} \quad (4)$$

With account for specific muscle changes during ES, the following schedule of impulse current effect was chosen: 150 V, 25 Hz, 100 s. Figs. 2-4 show changes in pH, WHC (amount,%, of water bound per 100 g of meat) and E in stored meat treated as is indicated in Methods 1, 2 or 3.

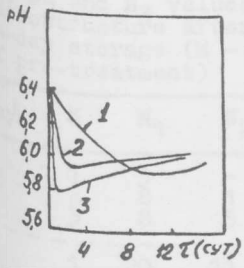

Fig.2.

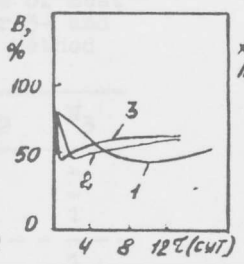

Fig. 3

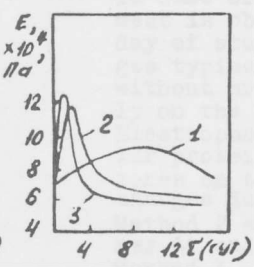

Fig. 4

Figs. 2, 3, 4. Relations of pH = f(τ), B = f(τ) and E = f(τ) for the meat treated by Methods 1,2 or 3. τ is storage time (days)

The data indicate that ES or ES+ATS improve meat juiciness and tenderness and that ES accelerates the mechanochemical processes in meat by 7-10 times and ES+ATS - by approximately 20 times as compared to the meat without pre-treatment.
Figs. 5 and 6 illustrate the pattern of changes in the esterase and lactate-dehydrogenase activity within the first 3 days of storage.

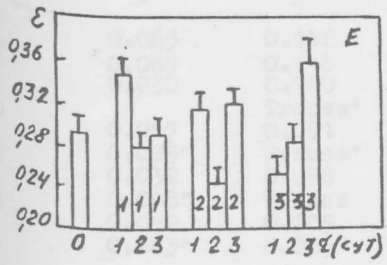

Fig. 5

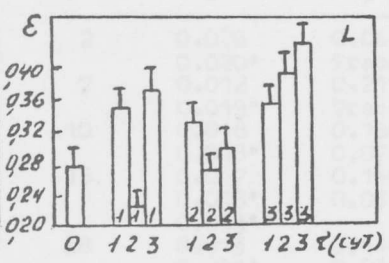

Fig. 6

Figs. 5 and 6. The pattern of changes in the esterase (E) and lactate-dehydrogenase (L) activity in meat, pre-treated by Methods 1, 2 or 3, during storage

Esterase is known to be a marker enzyme of lysosomes and lactate-dehydrogenase is an enzyme of anaerobic glycolysis (5,6). The pattern of changes in the activity of both esterase and lactate-dehydrogenase is nearly identical for all the technological treatments of meat; this, evidently, may indicate a conjugacy of the lysosomal mechanism functioning in the anaerobic chain of biochemical oxidation. A progressing rise in the activity of both enzymes during storage of meat treated according to Method 3 is noticeable.
Figs. 7 and 8 are microphotographs of the structure of meat before rigor and after 7-day storage (pre-treatment by Method 3). For the pre-rigor meat (Fig.7) it was found that muscle fibers were swollen, located tightly to each other, striation was clear and pronounced, nuclei were flat, extended, rod-like and oval. When meat was pre-treated by Method 3, muscle fibers after 7-day storage were moved apart, there were more fibers with disruptions and slot-like gaps; fragmentation, myofibril dissociation, local destruction of myofibrils with granular dissociation were evident.

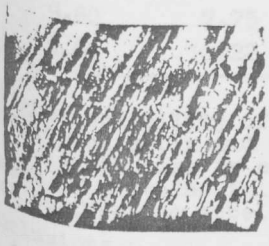

Fig. 7

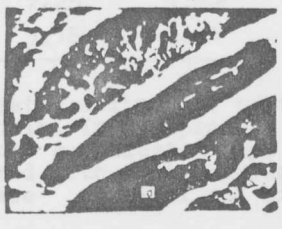

Fig. 8

Figs. 7 and 8. Meat microstructure before rigor and after 7-day storage, respectively; pre-treatment by Method 3

Table 1 presents morphological data reflecting microstructural changes in the meat after technological treatment.

Table 1
N_1, N_2 and N_3 values of meat microstructure after 3- and 14-day storage (M - method of pre-treatment)

τ, days	M	N_1	N_2	N_3
3	1	3	-	-
	2	8	1	-
	3	8	5	1
14	1	20	11	1
	2	30	20	4
	3	30	24	6

In case of Method 3, granular dissociation in meat is observed (N_3) starting from the 3rd day of storage; thus, there are structural changes typical of aged meat. In subfrozen meat without pre-ES treatment changes are noted only on the 14th day.
Electrophoretic separation of muscle myofibrillar proteins resulted in 14 fractions (protein zones on the electrophoregrams). Most important changes during storage of meat pre-treated by Method 2 are the appearance of new protein zones 6* and 9* and in case of pre-treatment by Method 3 - of zones 6*, 6** and 9* (Tables 2 and 3).

Table 2

τ, days	S	
	6	9
0	0.065	0.142
2	0.069	0.148
7	0.030	0.140
10		Traces*
	0.017	0.091
15	0.025*	Traces*
	0.032	0.098
28	0.048*	Traces
	0.052	0.205
	0.083*	0.055*

Table 3

τ, days	S	
	6	9
2	0.028	0.061
	0.020*	Traces*
7	0.012	0.215
	0.019*	Traces*
10	0.018	0.160
	0.018*	0.074*
15	0.017	0.140
	0.018*	0.081*
	0.013**	
28	0.013	0.114
	0.051*	0.035*
	0.011**	

Tables 2 and 3: Protein levels in fractions 6 and 9 of stored meat pre-treated by Method 2 (Table 2) or by Method 3 (Table 3). τ is storage time (days), S - protein level in grams per 100 g of muscle tissue

The experiments demonstrated that the molecular weight of proteins in zones 6*, 6** and 9* was 73000, 68000 and 30000 (respectively). It should be noted that for subfrozen meat without pre-ES the above zones appear at a later stage, this evidencing faster processes causing tenderisation of meat treated by Methods 2 and 3. Table 4 gives data on the solubility of meat myofibrillar proteins (R) after pre-treatment by Methods 1 and 2. The R-values are the total proteins in all the fractions obtained through muscle tissue extraction in solutions with the ionic strength $\mu = 0.6$ and $\mu = 1.2$. The data indicate that during the storage of meat subfrozen just after slaughter (Method 1) myofibrillar protein extractibility was reduced on the 2nd day, whereas in case of pre-treatment by Method 2 it increased.

M	τ, days			
	0	2	7	10
1	9.80	7.75	7.25	7,82
2	9.80	10.22	9.18	8.64

Table 4. R-values of the myofibrillar proteins of the meat treated by Method (M) 1 or 2 (g protein/100 g muscle); τ is storage time (days)

A higher solubility of meat myofibrillar proteins is obviously due to an increased extractibility of actin and myosin. In the process of further storage the extractibility of proteins is changing in a more complicated way. It may be caused with intramolecular rearrangement of proteins followed with their aggregation. Thus, ES and ATS of chilling before meat subfreezing accelerate physical and biochemical processes occurring in it.

Conclusions

1. An electrostimulater was built, and its suitability to treat meat electrically was shown.
2. Mathematical expressions were derived which characterize the relation of the maximum ES time to the voltage of impulse current.
3. Studies of meat with physico-chemical, rheological, citospectrophotometrical, histological, morphometrical and electrophoretical methods, as well as the analyses of protein solubility demonstrated that ES during subfreezing accelerated mechanochemical processes by 7-10 times, whereas ES combined with ATS provided a 20-fold increase.
4. The results obtained were used to substantiate meat subfreezing procedures which are covered by Author's Certificates (1,2).

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