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THE ACTIVITY OF PORK MUSCLE PROTEASES AS EFFECTED WITH SODIUM CHLORIDE

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SUMMARY

The results of a study into the effect of NaCl upon free activity of pork muscle proteolytic enzymes are presented. NaCl has been found to inhibit cathepsin D, the greatest reduction of its activity being observed when NaCl concentration reached 5%. The enzymic activity falls down by 50%. Increasing NaCl concentration from 5 up to 15% slows down the inactivation rate and decreases cathepsin D activity by 8-15%. The analysis of the data obtained demonstrates the non-competing inhibition of proteolysis in the presence of sodium chloride. The inhibition constant K_i has been estimated as being equal to 1.07 M.

INTRODUCTION

At present, I.A.Smorodintsev's opinion (1,2) is universally accepted, according to which meat ageing is a complex of physico-chemical and biochemical processes effected with intracellular enzymes (cathepsins) of the muscle tissue. The intensity of the proteolytic changes determines the properties of raw materials and influences the development of structuro-mechanical and organoleptical properties of the finished product (3,4). It is known that in meat production raw meat ageing occurs in the presence of NaCl. A great number of papers on meat curing deals basically with the effect of curing conditions and ingredients on the changes in the protein substances and physico-chemical properties of the raw meat. The literature on NaCl effect on the proteolytic processes in the muscle is scarce, and the viewpoints are very often contradictory. Pavlovsky (5) found that 3% of NaCl depress the activity of cathepsind both in ground and intact pork muscle. Bryanskaya et al. (6) believe that during horsemeat curing cathepsin activity rises by about 3-3.5 times. Deng and Lillard (7) state that the specific activity of cathepsins within NaCl concentration range from 0.01 to 1 M is stable. Pezacki and Pezacka (8) demonstrated that in dry sausage curing the proteolytic activity of the cathepsin complex increased with moisture lowering. A number of researchers (Reddi et al.(9), Levanidov et al. (10)) showed that sodium chloride inhibited the cathepsin activity of fish muscle, especially in the acid environment at pH 3.5. The present work has been carried out to study the effect of NaCl upon the activity of proteolytic enzymes in pork muscle.

MATERIALS AND METHODS

Experiments were carried out on pork, Finish Grade 2, from 9-10-month pigs of the Kemerovo breed. Muscle cathepsin activity was assessed by incubating l.dorsi m. homogenates with NaCl and without it by means of modified Anson's method (11). As a substrate, ox hemoglobin denaturated in 8 M urea was us
Muscle was comminuted in a Potter-Elveiem homogenizer, the bowl and the pestle be made of teflon; comminution was performed for 90 min in a buffer solution having pH 3.0. Together with the buffer solution, Na was added to the incubation mixture in su amounts that salt final concentration was 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5 7.0, 10.0 and 15.0%. Samples were incubat at 37°C for 1 hr, then 12% of a sol tion was added to stop the reaction. The s ples were chilled for 24 hr at 2-4°C and filtered; the optical density of the solu on was measured spectrophotometrically at 280 nm. The proteolytic activity of cathep sin D was ex pressed in mcM of tyrosine/hr g of the muscle.

RESULTS AND CONCLUSIONS

The test results on the activity of pork muscle cathepsins in the presence of NaCl in the system are illustrated in Fig.1.The indicate the inhibiting action of NaCl on the tissue proteases. The greatest reducti of cathepsin D activity occurs at the 5% Na concentration in the reaction mixture, in which case the activity is lowered by 50% compared to the initial sample without NaCl A further increase of NaCl concentration h a much lower influence on the activity of the tissue proteases, and at 15% NaCl the cathepsin D activity was decreased by 8-15 as compared to 5%NaCl. As is clear from Fig 1, irrespective of NaCl, of the highest ac tivity are the muscle cathepsins post 72 hr holding at 2-4°C. These results agree with Pavlovsky and Simbiryova's data (12), who established that the highest free activity of beef cathepsins was reached by 72-96 hr, Thus it was found that pork muscle cathepsi were inhibited with NaCl, the inactivation rate depending on the concentration of the latter. To substantiate meat curing parame res, it is necessary to find the kinetic characteristics both of the processes of cu ring ingredients accumulation and distribut on, and of the enzymic reactions. Of specia importance here becomes the knowledge of th inactivation rate constant of muscle cathep sins with sodium chloride, this allowing to develop and to practically use the mathema tical models aimed at solving the tasks of meat curing optimization. To calculate the constant of reaction rate inhibition as re lated to the concentration of the reactants it is useful to apply the method suggested by Webb (13). It is seen from Fig.2 that th experimental data are located on the straig ht line the continuation of which cuts off length on the abscissa, equal to the inhibi tion constant K_i. The analysis of the relat ons obtained evidences a non-competing type of the inhibition of proteolysis in the pre sence of NaCl. The enzymic reaction rate (V for non-competing systems is expressed as

$$V = \frac{V_m \cdot S}{(K_m + S)\left(1 + \frac{C}{K_i}\right)} \qquad (1)$$

where: V_m - the maximum reaction rate;
S - hemoglobin concentration;
C - NaCl concentration;
K_m - the Michaelis-Menten constant;
K_i - the inhibition constant.

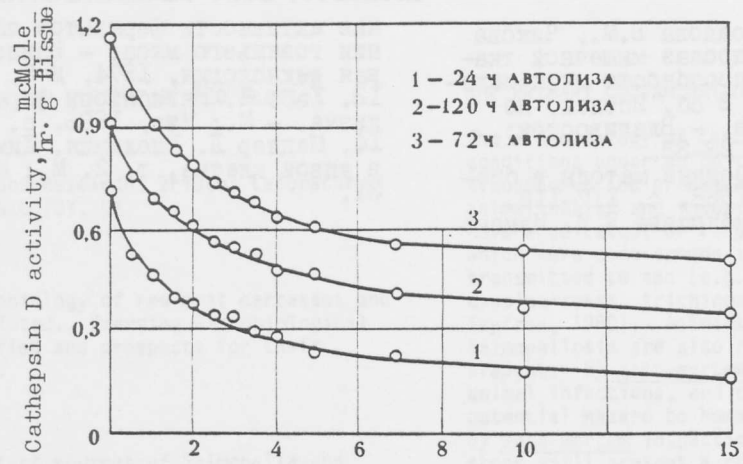

Fig.1.

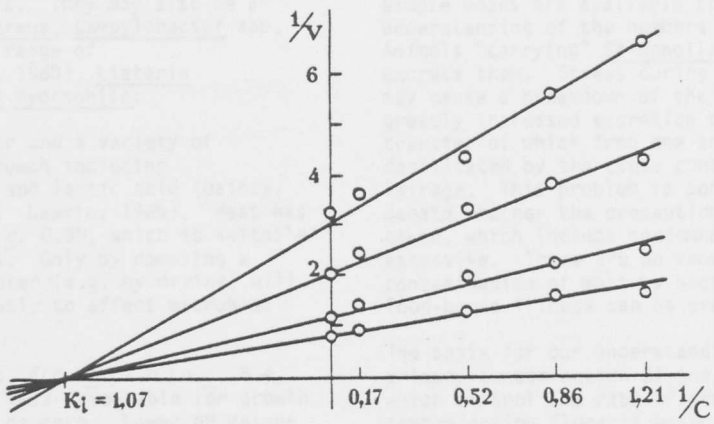

Fig.2.

Using this equation, it is possible to decide whether any given enzymic model corresponds to the non-competing inhibition, as well as to determine the K_m and V_m values. The non-competing inhibition of pork muscle cathepsin D with NaCl indicates that NaCl binds both with the free enzyme and with the enzyme-substrate complex. Due to the fact that in this case the inhibitor differs from the substrate in its structure, it may be suggested that NaCl interacts with the centre other than that of substrate binding. Here, as Metsler thinks (14), cathepsin activity inhibition is due to a distortion of its three-dimensional structure caused with inhibitor binding. In addition, the bound NaCl can effect the catalytic process, screening partially the active centre. As is seen from Fig.2 and Equation 1, non-competing inhibition is characterized with a lower maximal reaction rate as compared to the maximum rate in the presence of NaCl. The same Figure demonstrates that, however high is the concentration of the substrate, it is impossible to prevent inhibition. Thus, the results of the study serve the ground to believe that sodium chloride used to cure meat can inhibit the free activity of tissue proteases. The highest rate of cathepsin D inactivation was noticed with increasing NaCl concentration from 1 to 5%, the latter causing a 50% reduction of the initial activity. NaCl was found to be a non-competing inhibitor. The inhibition constant K_i was estimated, it equalling 1.07 M.

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