DEVELOPMENT OR INTENSIVE TECHNOLOGIES FOR THE PRODUCTION OF MILK CLOTTING ENZYMES FROM MEAT BY-PRODUCTS

G.E. LIMONOV, R.V. ILUKHINA, N.V. NAZARENKO
The All-Russian Scientific Research Institute of Meat Industry
Talalikhina 26, Moscow 109316, Russia

W-6B.24

The increase in supply of high quality foods to population is associated with scientific and technical progress in food industry, rational usage of raw materials, development and using new advanced technologies in all the industries, including the meat and dairy industry.

The raw materials of meat industry contain not only indispensable ingredients of wholesome nutrition, but also are the sources of biologically active substance s, for instance, milk clotting enzymes, which are used in the production of cheese.

A classical enzyme, which is used in cheese-making is chymosin (a rennet enzyme) which is produced from rennets of calves and lambs at the age from 15 days to 3 months. The slaughter of the animals at such age is not economically advantageous, therefore at the present time there is a deficiency of raw materials for the production of rennet enzymes. In connection with this, investigations are carried out in search of substitutes of rennets. At the present time, beef, pork and sheep pepsin, as well as the enzymes of microbial and vegetable origin are used for milk clotting (Volik, Dolgikh, 1978; Stoeva et al., 1987). However, by their clotting effect and ability leading to the production of high quality cheeses, the rennets surpass all the existing substitutes. As a matter of fact, the need in milk clotting enzymes will increase by 1995 by 1.3-1.5-fold and will reach 270 tons per year.

The existing technology of milk clotting enzymes production is not perfect, as its time duration is about 200 hours, and enzyme extraction takes 72 hours. Extraction is a batch process and is carried out in a bulky equipment, taking large area. The existing extraction procedure involves a decrease in enzyme activity due to its low stability in aqueous medium.

To improve the process of extraction the investigation were carried out with the use of liquid nitrogen for pre-treatment of rennets, the exposure of raw materials to ultrasound or magnetic field, as well as to electromagnetic treatment. There exists a method of enzyme production using the electric pulses at the stage of extraction. The use of electroplasmolysis makes it possible to reduce the period of extraction and increase the enzyme yield; however, a temperature increase, observed during the passage of the current through the cell membranes results in local inactivation of the rennet and the loss of quality of the final product. The analysis of the existing method of production of milk-clotting enzymes in this country and abroad shows that all of them have large limitations and do not meet the demands of the present-day state of technique and technology of production of high-quality enzyme preparation.

Under the present-day conditions, the studies on the possibility of the application of low frequency oscillations for the intensification of milk-clotting enzymes extraction procedure, aimed at increasing their yield and enzymic activity, are of practical and scientific interest. Thus, the experiments have been carried out to study the influence of vibration on the extraction process of rennet enzyme from the calves rennet (Veretova et al., 1984). It has been found that the use of mechanical oscillations results in a higher intensity of the extraction process, as compared to the application of such widely used method, as mechanical mixing, applying high temperatures and pressure.

One of the aspects of the investigation of vibration oscillations is the study of behaviour of biological objects under the influence of vibration. The biological effect of vibration is manifested in the disturbance of stability of different components of the conformers of the molecule or in the disturbance of the spatial structure of the molecule on the whole.

At the same time, due to specifity of the influence of vibration oscillations on animal tissues and on the substances extracted therefrom, it is necessary for each particular investigated process to determine the best parameters of vibration treatment, intensifying the technological process without loss of quality of the final product.

The objective of our investigations was the development of processing technology of high quality enzyme preparation production, using low frequency mechanical oscillations at the stage of its extraction from raw materials. In this case, vibration parameters have a great influence on the extraction process. The intensity of vibration mixing is determined by two parameters: frequency and amplitude of oscillations along with their function of simultaneous effect:

$$K = A^2 \cdot f^3,$$

where K - intensity of mixing;

A - amplitude of oscillations, m;

f - frequency of oscillations, Hz.

The influence of frequency and duration of vibration treatment on the activity and yield of the obtained enzymes extracts was studied.

A method of orthogonal-symmetric plan with the investigation of 4 factors on 3 levels was used to obtain the necessary information about the studied object and to deduce the dependence of the value of the rennet enzyme extract activity and the yield of the ready preparation from a number of technological parameters. In accordance with the stated problems, to study the composition and properties of the rennet enzyme, a set of indices was determined using the adequate methods of investigations (Matrozova, 1977; Lowry O.H., 1951). The obtained results of the experiments were processed by methods of mathematical statistics, using analysis of variance and regression analysis. The data were processed on a computing system "Iskra 1256".

The results of the investigations are presented in the Table and as plotted on graphs. It was found (Fig. 1) that the activity of the enzyme preparations obtained using the rational conditions of vibration was by 10% higher, as compared to the preparations manufactured by conventional technology. The extraction procedure at 10 and 16 Hz does not result in high yield of ready produce (Fig. 2), because, apparently, at low frequencies the "destructive action" of oscillations on the structure of rennets is not sufficiently strong, and the enzyme is not extracted in full. As the frequency of treatment of the raw materials during extraction process increases up to 38 Hz, the yield of the enzyme slightly falls, due to extraction of a certain amount of ballast protein. The optimum duration of extraction procedure ensuring the greatest yield, was $27.6 \cdot 10^2$ s at a frequency of 25 Hz.

Based on a set of physico-chemical, biochemical and microstructural investigations, the influence of vibration on a spatial structure of rennets tissue as well as on disturbance of intracellular links, and contributing to a more complete extraction of the enzymes from the raw materials being processed, was determined. As a result of vibration processing, the content of indispendsable amino acids, as well as of serine, aspartic and glutamic acids, that are contained in the active centre of the enzyme, increased.

As a result of the investigations, the processing technologies of production of milk-clotting enzymes from animal raw materials were developed, where the vibrations were used at the stage of extraction, which make it possible to increase the yield by 18-20% and decrease the duration of extraction by 5-7-fold, as compared to conventional methods of production of enzyme preparations.

References

1. Lowry O.H., Rosebrough N.L., Farr A.L., Randall R.I. (1951). Protein measurement with the folin phenol reagent. J. Biolog. Chem., v. 193, p.p. 265-275.
2. Matrozova S.I. (1977). Technological control in meat and poultry processing industry. M. Pischevaya promyshlennost, Russia.
3. Stoeva S.P., Mesrov B.K. (1987). Purification of milk clotting enzyme from Bacilus mesentericus strain 76. Milchwissenschaft, v. 37, pp. 220-224.
4. Teply M., Mashek Ya, Gavlova Ya. (1980). Milk-clotting enzymes of animal and microbial origin. M. Pischevaya promyshlennost. Russia.
5. Veretova T.V., Limonov G.E., Rogov I.A. (1984). Improvement of extraction process of rennet enzyme using vibration. Myasnaya Industriya SSSR, N 11, pp. 15-19.
6. Volik V.G., Dolgikh T.V. (1978). Production of high efficient substitutes of rennet enzyme from by-products of poultry. Trudy of NPO "Complex".

Table. Results of determination of activity (A, thousand of convertional units of rennet enzyme as dependent on conditions of vibration treatment (τ, f) at the first stage of extraction at different concentracion (c) of the extraction solution.

Fig. 1 Dependence of activity (A) of ready enzyme preparation on conditions of vibration extraction (τ, f).

Fig. 2. Dependence of yield (M) of rennet enzyme on conditions of vibration extraction (extraction (τ, f).