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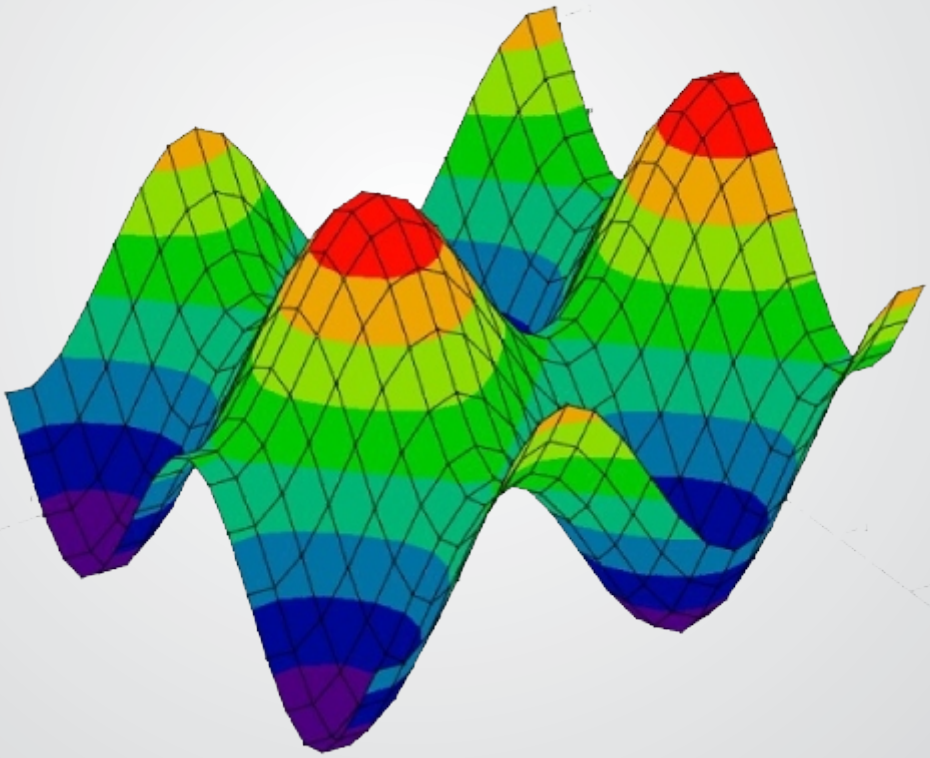

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USE OF COLLAGENASE IN TECHNOLOGY GERODIETETIC PRODUCTS

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Abstract: *The topicality of the work is to justify the choice of low-grade meat raw material as a matrix for tying together calcium ions. A safe, effective and affordable in Ukraine enzyme preparation is chosen from literature sources in order to increase the number of functional groups in the raw material.*

Keywords: meat, gerodietetic, tripe, proteolysis enzyme, collagenase.

I. Introduction

XX century was the age of population growth, XXI can become the age of aging population. According to the UN, it is expected that by 2050 the world population will increase by 2,5 billion people, while the number of people at the age of 60 and older will increase by 1 billion people. The current demographic situation in Ukraine is characterized by persistent tendency - a dynamic increase in the share of people older than working age, which contributes to global aging process of population. Today, 20,7% of the population in Ukraine, that is every fifth citizen - retired due to age, and every fourth, crossed the line of 50 year old.

Today we are talking about reaching potential immortality. The goal of scientists is to look for new tools and methods that will significantly improve the quality of life in order to increase one's performance during their life expectancy, advance the onset time of diseases that accompany old age (osteoporosis, type II diabetes, atherosclerosis, cancer, etc.). It is no accidently, that in the developed by UN Programme project on aging "Scientific research programmes on aging problems in the XXI century", the concept of healthy aging classified as the most priority areas.

Today gerontology has a number of methods and tools that improve health, psychological and physical possibilities of older and elderly age people. The most studied and effective of them is the rational mode of muscle activity and a balanced diet, with the obligatory inclusion in the diet ingredients that have geteroprotective and protective effect. According to domestic and foreign research, through properly organized diet the number of illnesses (diabetes, arthritis - 50%, heart disease - 25%, diseases of eyes - 20%, etc.) can be reduced and significantly reduce the risk of premature aging. Today, there are very few of these substances in our diets, which reduces the protective properties of the organism. For this reason, it is necessary to create specialized food products with specifically declared properties. Therefore, a new look at the potential of biotech food raw materials, grounding of new biotechnological solutions in technology of gerodietic products are particularly important.

The product range of gerodietic products is limited, moreover, the bulk accounts dairy products and baked goods. Therefore, the purpose of this study was to improve the theoretical foundations and design principles of gerodietic products, which are based on the creation of balanced by their micronutritient structure of recipes, adequate needs of seniors, elderly and centenarians.

One of the priority tendency of the concept of the National program "Health 2020: Ukrainian Dimension" (for the period 2012-2020 years) in the sphere of healthy nutrition of Ukraine is to eliminate the deficit of nutrients, including important micronutrients - vitamins and minerals. The problem of solving calcium deficiency both in food and in the human body is of paramount importance. Physiologists have shown that one of the reasons of calcium metabolism violation in the background of its deficit, is a low percentage of absorption of macronutrients, since calcium is one of those nutrients that the body cannot synthesize, and its content in the natural food sources - is limited. Absorption of calcium depends on its relation with other nutrients (phosphorus, vitamin D, fatty acids, etc.). Amount of protein in the diet affects on the assimilability of calcium: with the high-protein diet about 15% of calcium is absorbed, and at low-protein - only 5%.

II. Materials and methods

Therefore, it is necessary to create such food systems, where the maximum amount of calcium will be associated with protein compounds for better assimilation in body. Therefore, as a readily

available source of protein we have chosen by-products of second category, in particular cattle rumen (collagen content 62%). However, to increase the number of functional groups, it was necessary to conduct cattle rumen fermentation.

From the analytical review of the literature due to indexes of collagenases activity (Table 1) collagenase nutritive produced by close joined-stock company "Bioprohres" according to industrial standards 9158-002-11734126-94 (Schelkovo, Moscow region, Russia) was selected as the enzyme.

In the complex research aimed at the solution of the question about possibility and feasibility of using collagenase nutrition in meat production technology, in the first place it is necessary to study the proteolytic activity of the enzyme preparation and the influence on it such process parameters as pH and temperature.

It was founded that the activity of collagenase nutrition in proteolysis of caseinate sodium during fermentation, given the requirements of GOST 20264.2 for neutral proteinase (pH 7,0 temperature 30 ° C and duration of 10 × 60 s) was 288 un / g.

Accepted in the experiment pH range represented interest, as meat pH is 5,6 ... 5,8 and , therefore, the use of collagenase nutrition for enzymatic proteolysis of meat pH environment is slightly shifted to the acid side in comparison with an optimum action indicated in the standard. The results are represented in Figure 1.

Table 1. *Biochemical characterization of enzyme complexes of preparations*

Enzyme preparation (Source)	Activity	
	Proteolityc un/g	Collagenase un/mg
Protosubtylin G10h (Bacillus subtilis)	400	0,07
Protomegateryn G20H (Bacillus megaterium)	119	0,09
Pepsin (Mucous membrane of stomach)	30	0,01
Trypsin (pancreas)	240	0,01
Pancreatin (pancreas)	120	0,13
Collagenase nutritive (Kamchatka crab)	125	0,3
Papain(Papaya)	150	0,15

III. Result and discussing

As can be seen from the data in Figure 1 , collagenase nutrition activity accounts for most per zone pH 5,0 ... 7,5, while shifting the pH to slightly acidic zone the preparation stores 72 ... 90% of the maximum value of its proteolytic activity. It follows that the use of the preparation for cattle rumen fermentation, slightly acidic environmental conditions should not significantly affect its activity.

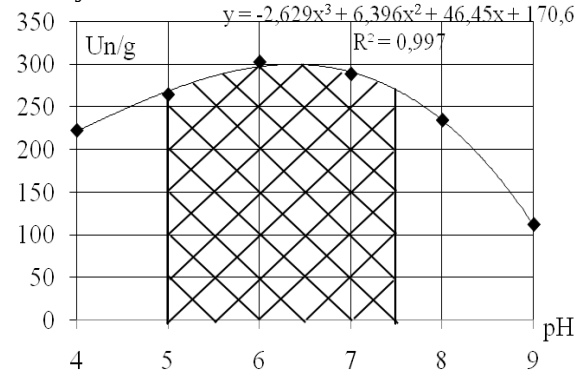

Figure 1. *Dependence of proteolytic activity of collagenase nutrition on the values of pH (t = 30 ° C, τ = 10h60s).*

The dependence of the proteolytic activity of collagenase nutrition on the duration of the process at different values of the ambient temperature measured in the range 0 ... 90 ° C at pH 7,0 during 10 x 60 s. The data presented in Figure 2.

Accepted in the experiment temperature range was suitable for technological production process and, thus, allowed to predict the intensity of enzymatic proteolysis of the protein in a particular technology. Increase of the ambient temperature to 35 ° C leads to an increase in enzyme preparation activity. Further increase in temperature above 45 °C causes partial inactivation of the enzyme preparation; the higher the temperature and longer duration of heat exposure, the more intensively enzyme preparation inactivates.

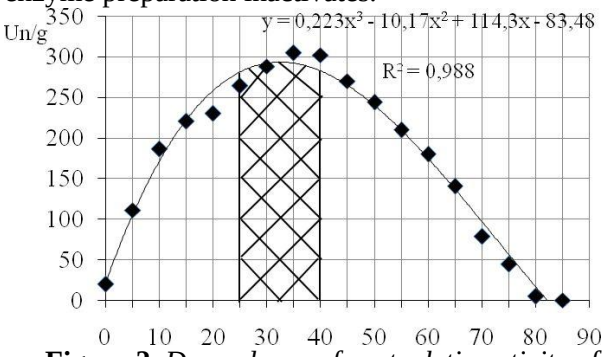

Figure 2. *Dependence of proteolytic activity of collagenase nutrition on temperature values (pH 7,0 τ = 10h60s).*

To determine the rational mode of enzymatic proteolysis a full factorial design method was used, followed by mathematical modeling in problem-oriented package MathCad. As a parameter of optimization amino nitrogen content in the hydrolyzate was selected. Within the two-

factor model experiment, the content of amino nitrogen in the rumen of cattle hydrolyzate according to the temperature and duration of enzymatic proteolysis was calculated by equation:

$$S=-1690,3+10,6\cdot\tau+55,6\cdot t-4,4\cdot10^{2}\cdot\tau-9,19\cdot10^{2}\cdot t-4,9\cdot10^{3}\cdot t$$

where S - content of water-soluble hydrolysis products, mg/g protein; τ - duration of enzymatic proteolysis, s; t - enzymatic proteolysis temperature, ° C.

In the ensuing mathematical modeling the region rational values of the investigated parameters was defined.

It is known that proteolytic enzymes preparations catalyze the cleavage reaction of protein molecules with water. However, the introduction of large amount of water to the rumen of cattle will lead to higher costs in its drying. Justification of the minimum duty water curve that ensures the efficient conduction of proteolysis was carried out by the intensity of accumulation of amino nitrogen in the water-soluble fraction of hydrolysates of cattle rumen at different values of duty water curve. The results are represented in Figure 3.

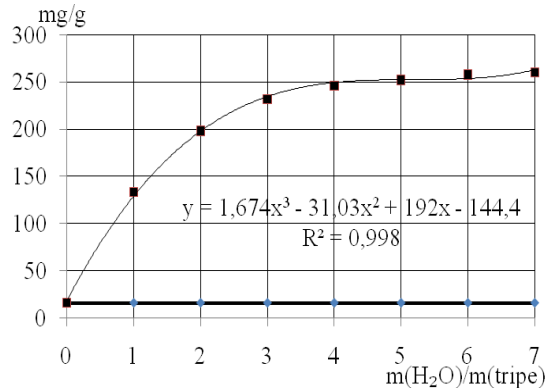

Figure 3. *Content of water-soluble hydrolysis products of cow rumen based on duty water curve: ■ - enzymatic proteolysis; ▲ - endurance in water without fermentation (control).*

According to received experimental data for the effective proteolysis of rumen of cattle sufficient duty water curve is "water: rumen of cattle" - 1:3. Further increase of water content in the environment does not lead to a significant increase in the degree of proteolysis.

To ensure the microbiological safety the mixture is heated to (85 ± 1) ° C and maintained for (10 ± 0,2) s 60. Taking into account the data on thermal inactivation of enzyme composition (Fig. 2), such parameters of heating will provide its full inactivation.

Enzymatic treatment leads to destructive changes of raw materials, increase of number of hydrophilic centers, increase of functional groups as a result of rupture of polypeptide chains, which further will be more accessible for reactions including calcium. However, our goal was not a complete hydrolyzate of protein molecules to amino acids, we tried to achieve only partial hydrolysis to increase the number of free functional groups, including those that are capable of binding calcium.

Processing of cattle rumen was held by 0,05% solution of the enzyme by weight of raw materials (recommendations of Tolstobokov Oleg Mykolayovych) at temperature regimes: 2 ° C (cold chamber), 12 ° C (in meat processing plants in the shops), 20 ° C (room temperature) and 37 ° and 50 ° C (thermostat) for 5 hours.

Proteolysis of protein of collagen containing tissue is observed in all modes, as evidenced by the accumulation of amino nitrogen. The highest rate of proteolysis of proteins is observed during the first time, as shown by angle curves from the second processing time it is reduced. The largest number of amino nitrogen was observed at 37 ° C in each period, minimum - at 2 ° C.

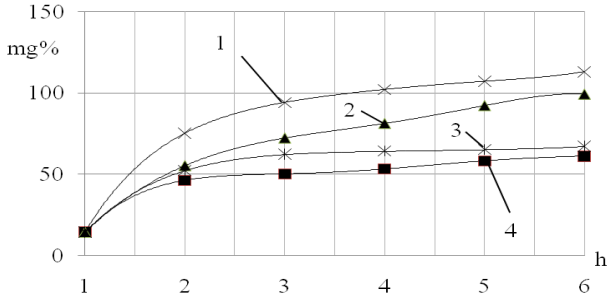

Figure 4. *Diagram of accumulation of aminobitrogen in the processing of the rumen of cattle depending on the ambient temparture. 1. treatment at 2 °C; 2 treatment at 12°C; 3. treatment at 37 °C; 4. treatment at 50 °C.*

So, after 2 hours of fermentation amount of amino nitrogen in samples that were treated at 37 ° C increased by 5,8 times at 12 ° C – 4,5 times, at 2 ° C - 3 times, further the rate of decay of proteins to peptides and amino acids gradually decreased. Thus, the most effective fermentation temperature is 37° C.

In conditions of production the support of 37 ° C entails additional costs for equipment and energy, which is undesirable in the development of new technologies. Also such temperature creates optimal conditions for microbial growth. Therefore, temperature 12 ° C is more suitable,

which is chosen for further studies because it is constantly maintained at a meat processing enterprises in manufacturing plants, but also increased the concentration of enzyme to 0,1%.

It is evidently from the graphs,that the treatment with the double number of enzyme allowed to get after 2 hours such amount of amine oxide, which was achieved at 37 ° C for 4 hours. Therefore, further treatment of collagen containing raw material was conducted by collagenase solution in an amount of 0,1% by weight of raw materials at 12 ° C for 3 hours.

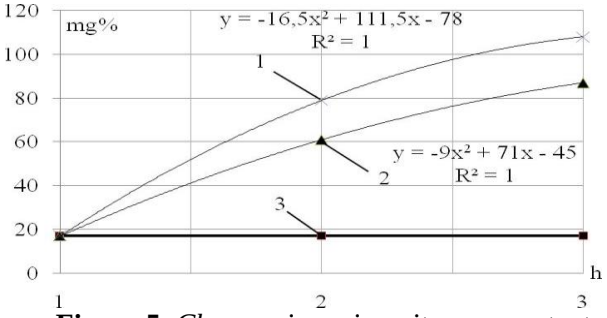

Figure 5. *Changes in amino nitrogen content at different concentrations of enzyme preparation*

Collaterally, the dynamic changes of content in soluble protein in processing by collagenase nutrition solution in an amount of 0,05 and 0.1% by weight of raw material.

Table 2. *Changes in the content of soluble protein by the action of collagenase nutritive*

Duration of fermentation, h	Water-soluble protein,%	
	0.05% by weight of raw	0.1% by weight of raw
1	1,14±0,03	1,72±0,07
2	1,3±0,01	2,04±0,02
3	1,38±0,01	2,17±0,05
4	1,4±0,07	2,26±0,04
5	1,405±0,09	2,3±0,02
6	1,41±0,05	2,32±0,03

Water-soluble protein of native form scar cattle equal 0,6%. The results are represented in Table 2.

The analysis of the received data showed that there is a direct correlation with the amino nitrogen, that is processes of accumulation of soluble fractions and accumulation of amino nitrogen are going in parallels. As expected, during the processing by 0,1% solution of enzyme the amount of soluble protein is higher, moreover, the greatest increase of soluble protein is observed within the first hour. Since the second hour of fermentation, the rate of formation of soluble protein decreases and slows down at fourth hour of fermentation.

IV. Conclusion.

1. Analysis of experimental data and also their mathematical treatment, allowed to justify the use of enzyme preparation - nutritive collagenase to increase functional groups in the by-products of II category (rumen of cattle).

2. It is shown that the effective concentration of nutritive collagenase during proteolysis of the cattle rumen is – 0,1% by weight of raw material.

3. It is founded that the maximum proteolytic activity of enzyme preparation - nutritive collagenase at pH - 5,0-7,5; duty water curve - 1:2; temperature - 25-40 ° C, proteolysis duration - 3 hours.

4. It is determined that for cattle rumen fermentation ,aiming at technology efficiency , temperature 12 ° C can be used, which is kept in production facilities meat processing enterprises, but the duration of proteolysis increases by 1:00.

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