The Analysis of Biocatalytic Properties of a Proteolytic Enzyme under the Influence of Physical Factors

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Abstract. Due to the relevance of biotechnological methods application in the meat processing industry, consisting in the reuse of collagen-containing raw materials, as well as the use of enzymes and enzyme preparations to improve quality and extend the shelf life, which is confirmed by the research results conducted in the field of food biotechnology, the purpose of the work was formulated. It consists in studying the changes in the biocatalytic activity of the collagenase enzyme after treatment with a blue light spectrum. In the course of the work, it was revealed that the activity of the collagenase enzyme, which was treated with a blue light spectrum, has a wider optimum pH in comparison with the control samples. Treatment of the solution with blue light collagenase with a luminous flux of 35 μW / cm2 for 1 hour increases the optimum biocatalytic activity of the enzyme, which is confirmed by studies conducted in two alternative methods (according to Anson and Telishevskaya). The experimental data showed that the activity of the collagenase enzyme in the experimental samples increases from 10 to 20 % under the influence of table salt in concentrations up to 3.5% compared to the control group.

1 Introduction

It is known that physical factors (mechanical, wave) can affect the activity of enzymes, for example, incubation of the studied enzyme preparations in 0.5% hydrochloric acid solution significantly reduces the activity of amylase and lipase. In the enzyme preparations produced in the form of tablets, the greatest decrease in the activity of amylase and lipase after incubation in hydrochloric acid is observed, which indicates a weak acid-resistant property of the shells of the studied drugs. This is due to the destruction of the protective shell and partial inactivation of the enzymes in the studied preparations [1].

Scientists have studied the effects of wave and optical effects on enzyme preparations in order to increase the activity of target enzymes. The results of light treatment effect with wavelengths from the range of 364-980 nm of the enzyme preparation Amilorizin P10x on its amylolytic ability showed that photo-treatment under experimental conditions allowed to

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increase the amount of hydrolyzed starch by 20-70 % compared to the control, depending on the wavelength of light used for processing [2].

Processing with visible-range monochromatic light significantly affects the technological properties of brewer's yeast and top fermentation, changing the nature of reproduction of the population of these microorganisms. The degree of such influence depends significantly on the wavelength of the light used for processing. By choosing a specific wavelength, you can change specific targets for yeast reproduction: the increase in the total number of cells and the consumption intensity of nutrient medium components. Processing with monochromatic light allows intensifying the process of top yeast development with low technological characteristics [3].

The researches of acoustic and light exposure effect on the enzyme preparation "APSubitilin P" showed that the starch content during acoustic treatment (2000 Hz) increased by 3 times, and during light treatment (346 nm) by 6 times. The amylolytic capacity of sound processing (1200-14000 Hz) was reduced by 50%, and when exposed to blue light (750 nm), the reduction in amylolytic capacity reached 70% [4].

The study revealed the inhibitory effect of red and blue light on the content of transcripts of the mitochondrial and cytoplasmic forms of aconitase on the level of transcription of aco1 genes in green corn leaves and showed a decrease in the concentration of their mRNA under the influence of a physical factor, which is associated with a change in the rate of aconitate hydratase functioning under these conditions. The results indicate the participation of the phytochromic and cryptochromic systems in the regulation of aconitate hydratase functioning [5].

In the studies conducted on the effect of light on linear growth, biomass accumulation and melanin synthesis in I. obliquus under the influence of coherent and incoherent light, the greatest stimulating effect was found when the mycelium was irradiated with blue light. Laser light exposure has a greater effect on the growth and accumulation of biomass in the mycelium of the fungus than incoherent light exposure. Light treatment significantly reduces the duration of fermentation [6]. The study of blue and red light influence on the physiological and biochemical characteristics of wheat plants showed that the influence of blue light stimulated the synthesis of proteins, and the synthesis of carbohydrates in wheat cells was affected by the radiation of the red light spectrum [7]. The influence of the blue light spectrum on dynamic growth is also confirmed in a study on the rate of photosynthesis under the influence of red and blue light, when irradiated with blue light, growth indicators increase, when exposed to red light, the effect slows down [8].

2 Materials and methods

In this regard, the aim of this work is to study the catalytic activity of the collagenase enzyme after treatment with blue light and under the influence of physical factors.

The object of the study is determined to be collagenase type I, which has the form of a fine sterile white powder with an activity of 125 u / mg, and as a collagen-containing raw material, the skin of broiler chickens. The objects were divided into control and experimental groups. The control group with the collagenase enzyme was not treated with light, the experimental group with the collagenase enzyme was treated with blue light for 1 hour with a luminous flux of 35 μW/cm2.

The activity of the enzyme was studied using the Anson method [9] and the Telishevskaya express method [10], which consist in the hydrolysis of gelatin with a collagenase solution. According to the method, the study should be carried out at a temperature of 37 °C. However, this negatively affects the gelatin, destroying its structure, which does not allow to determine the activity of the enzyme. In this regard, a temperature of 24 °C was selected to study the activity of collagenase. The collagenase solution must be prepared in advance in water

purified by distillation in the ratio 1:10, 1:20, 1:30 etc. The activity of the enzyme is determined by applying drops of the solution to the gelatin film with their delay on it for 10 minutes, after which they are washed off with water. Places with the applied solution are visually evaluated. According to the Anson method, the proteolytic activity of the enzyme is calculated by hydrolysis of gelatin.

3 Results

The pH environment in which the chemical reaction takes place affects the activity of the enzyme. Each enzyme is characterized by a special pH indicator, at which the peak of its activity is recorded. The deviation of the pH from the optimal one leads to a decrease in the reaction rate due to changes in the ionization of the functional amino acid residues of the protein molecule. A change in the reaction of the medium to the acid side causes a shift in the proton of free amino groups, causing a change in the rate of attachment of the substrate and its specificity. If the pH changes significantly, the enzyme undergoes denaturation and loses its biocatalytic activity. Table 1 contains indicators reflecting the relationship between pH and the activity of the collagenase enzyme at a temperature optimum of 42 °C.

Table 1. The effect of pH on the Activity of Control and Experimental Samples of the Collagenase Enzyme at Temperature of 42 °C, %.

Control		Experiment	
pH	Activity according to the Anson method, %	pH	Activity according to the Anson method, %
5	90	5	96
6	94	6	99
7	97	7	100
8	100	8	100
9	98	9	98
10	89	10	96

From the data presented in Table 1, it follows that when exposed to blue light on the studied objects, in the control group at pH = 8, the activity of the enzyme is 100%, in the experimental group this indicator is achieved at a pH range from 7 to 8.

When conducting studies according to the method [19] at a maintained temperature of 42 °C, the manifestation of activity in the control samples is 1:270, in the experimental samples – 1:320, which correlates with the data obtained from studies conducted according to the method [18] and indicates a difference in enzyme activity between the control and experimental groups of 29,000 units/mg.

The results of pH effect on the activity of the enzyme according to the method of Telishevskaya L. Ya. are presented in Table 2.

Table 2. The relationship Between pH and the Activity of Control and Experimental Samples of the Collagenase enzyme at a Temperature of 42°C, %.

Control		Experiment	
pH	Activity according to the Telishevskaya method, %	pH	Activity according to the Telishevskaya method, %
5	90	5	96
6	94	6	99
7	97	7	100
8	100	8	100
9	98	9	98
10	89	10	96

While studying in alkaline environment, the activity of the experimental samples was 96%, in the control samples it was 7% lower. In a neutral environment, the indicators in both groups were 100%.

An increase in temperature leads to a change in the rate of the chemical reaction, this is due to an increase in the speed of the molecules and their interaction, which leads to a change in the active center of the enzyme.

The temperature effect on the activity of the collagenase enzyme at an optimum pH of 8 is shown in Table 3.

Table 3. The Effect of Temperature on the Activity of Control and Experimental Samples of the Collagenase Enzyme at an Optimum pH of 8.

Control		Experiment	
t, °C	Activity according to the Telishevskaya method, %	t, °C	Activity according to the Telishevskaya method, %
0	0	0	0
10	28	10	34
20	43	20	56
30	95	30	98
40	100	40	100
50	82	50	96
60	24	60	28
70	0	70	0

The data presented in Table 3 indicate that the optimum of the catalytic activity in the control samples is in the range of 35-45 °C. For prototypes, this indicator is 28-54 °C.

For 1 hour, the solution with the collagenase enzyme was irradiated with blue light with a radiated flux power of 35 μW / cm2, which led to an increase in the range of biocatalytic activity of the enzyme. These data correlate with the indications obtained in studies using the methods of Teleshevskaya and Anson.

The inhibition of the catalytic activity of the enzyme was studied by the Anson method, which consists in applying a 0.5 – 4.0% solution of table salt to gelatin.

Table 4. The Effect on the Activity of Control and Experimental Samples of the Collagenase Enzyme NaCl at an Optimum pH of 8 and a Temperature of 41 °C., %.

Control		Experiment	
Table salt concentration, %	Collagenase activity, units / mg	Table salt concentration, %	Collagenase activity, units / mg
0	118	0	147
0,5	112	0,5	138
1	104	1	123
1,5	95	1,5	111
2	76	2	89
2,5	52	2,5	64
3	37	3	49
3,5	12	3,5	18
4	0	4	0

The data presented in the table indicate that table salt is not competitive with an inhibitor of the gelatin cleavage reaction. It changes the location of the enzyme molecule, affecting the degree of collagenase ionization, reducing its activity. It follows from this that the activity of the collagenase enzyme in the experimental samples under the influence of table salt in the concentration range of up to 4% is 6-29 u/mg higher than in the control samples.

Table 5. The Effect on the Activity of Control and Experimental Samples of the Collagenase Enzyme NaNO2 at an Optimum pH of 8 and a Temperature of 41 °C., %

Control		Experiment	
Table salt concentration, %	Collagenase activity, units / mg	Table salt concentration, %	Collagenase activity, units / mg
0	118	0	147
0,1	107	0,1	134
0,2	104	0,2	119
0,3	98	0,3	106
0,4	91	0,4	100
0,5	84	0,5	95
0,6	78	0,6	91
0,7	63	0,7	82

NaNO2 interacting with the substrate at a concentration of up to 0.7 mmol / dm3 reduces the activity of collagenase in control samples by 46.6%, in experimental samples by 44.2%. This shows that the treatment of the collagenase enzyme with blue light for 1 hour with a luminous flux of 35 μW/cm2 increases the optimal values of its activity and reduces the negative effect of the chemicals NaCl and NaNO2.

It was found that the activity of the collagenase enzyme, which was treated with blue light, has a wider optimum pH. Treatment of the solution with blue light collagenase with a luminous flux of 35 μW / cm2 for 1 hour increases the optimum biocatalytic activity of the enzyme, which is confirmed by studies conducted in two alternative methods.

4 Conclusions

Solution treatment with blue light collagenase with a luminous flux of 35 μW / cm2 for 1 hour increases the optimum biocatalytic activity of the enzyme, which is confirmed by studies conducted in two alternative methods (according to Anson and Telishevskaya). The experimental data showed that the activity of the collagenase enzyme in the experimental samples increased from 10 to 20 % under the influence of table salt in concentrations up to 3.5% compared to the control group.

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