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The study of stabilizing food enzymes using b-cyclodextrins

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ABSTRACT

Background: Today, biologically active additives, which contain hydrolytic enzymes (amylase, lipase, protease), are widely used in the digestive industry. These enzymes are often obtained from animal and vegetable raw materials, -for example, from the pancreas of a reindeer, the Fabricius bag of chickens, and Medusomyces Gisevii Lindau (Kombucha). However, in obtaining enzymes, the activity of amylase and lipase may decrease due to active forms --of proteases.

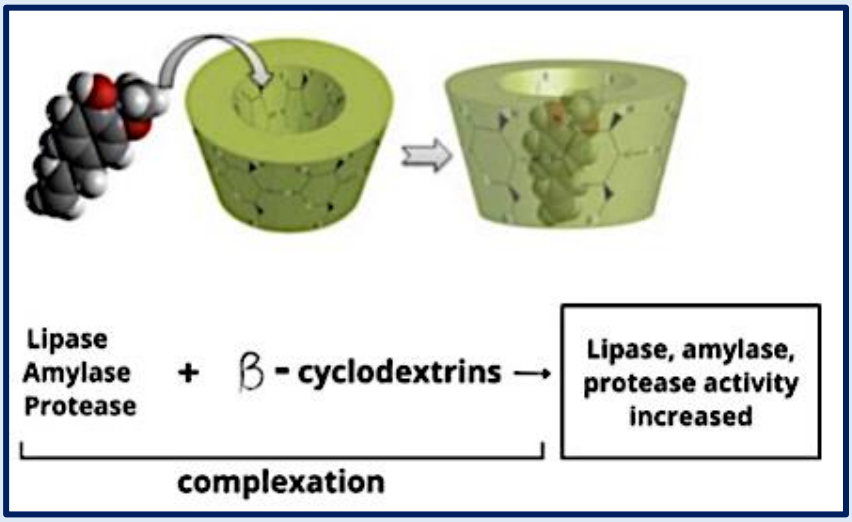
Objective: The main goal of this research work was to evaluate the stabilizing effect of nanostructures–cyclodextrins on the activity of hydrolytic enzymes (protease, amylase, lipase) obtained from animal raw material (chicken bursa of Fabricius) and microorganism cultures (Kombucha). Identify the relationship at which the negative influence of external environmental factors is leveled and, as a result, enzymatic activity is maintained for a certain period of time.

Methods: The protein concentration in the solution was determined using the Lowry method. A modified method by Rukhlyadiva was used to determine the amylolytic activity. The definition of lipolytic activity is based on modified Shibabi-Bishop method. To determine the amylolytic activity, a modified method by Rukhlyadiva was used.

Results: The relationship between b-cyclodextrin and enzymes was determined, at which enzymatic activity is maintained for a certain period.

Conclusion: Thus, cyclodextrins in solution stabilizes the activity of proteases, amylases, and lipases, which is of great practical importance in the food and pharmaceutical industries.

Keywords: kombucha, pancreas, enzymes, amylase, lipase, protease, cyclodextrins



INTRODUCTION

To date, biologically active additives containing hydrolytic enzymes such as protease, lipase, and amylase are widely used in the food industry. These enzymes help to speed technological processes, significantly increase the yield of finishing products, improve their quality, rationally use valuable raw materials that often end up in waste, and reduce the amount of garbage.

Enzyme preparations with amylolytic, proteolytic, and lipolytic activities are widely used in food technology. Hydrolytic enzymes are used in various areas of the food industry, such as brewing, winemaking, baking, the production of alcohol, fruit, and vegetable juices, yeast, cheese, meat and fish products, and protein hydrolysates.

The use of hydrolytic enzyme preparations in the baking process has several positive effects on bread's quality and freshness. These enzymes reduce flour and sugar consumption, the time required for dough maturation and increase bread's porosity and volume. Thanks to enzymes, bread retains freshness for a longer time and has a rich aroma.

Enzymes are essential in the brewing process, improving the beer's quality and shelf life. They help to minimize the loss of starch during malt splitting and allow you to reduce the consumption of barley by replacing it with regular grain. The use of enzyme preparations contributes to obtaining a higher-quality product with a rich taste, aroma, and improved storage stability. This enhances the consumer's overall experience and ensures the preservation of the beer for a long time.

It should be noted that enzymes such as protease, amylase, and lipase are used in these directions. Modern enzyme preparations are, to a greater extent, obtained from microbial and animal raw materials, as well as from raw materials of plant origin. An alternative source of

hydrolytic enzymes for industrial producers is Medusomyces Gisevii Lindau (Kombucha) and Fabricius bag of chickens.

It is known that during the fermentation process, kombucha produces and contains several components, including organic acids, sugars, water-soluble vitamins, amino acids, lipids, proteins, hydrolytic enzymes, ethanol, polyphenols, minerals, and others [1-2] According to the literature, kombucha has been shown to have physiological benefits such as improved digestive enzymatic activity and anti-inflammatory and antioxidant activity. Thus, kombucha should be classified as a functional food. [3-4]. In fact, studies show that many biologically active substances are prepared from kombucha [5-6].

According to the Functional Food Center (FFC), "functional foods are natural or processed foods containing known or unknown biologically active compounds that, when consumed in specific, effective non-toxic amounts, provide clinically established and recognized health benefits like prevention, management or treatment of chronic illness." [7] Functional foods can be of plant or animal origin. Bioactive compounds found in functional foods include flavonoids such as quercetin, polyphenols, vitamins, minerals, prebiotics, probiotics, essential fatty acids, polyphenols, vitamins, minerals, prebiotics, probiotics, essential fatty acids, antioxidants, carotenoids, phytosterols, polysaccharides, proteins, peptides, alkaloids, terpenoids, polyphenols, and other bioactive compounds. [7-8] Functional foods have high nutritional value and reduce the risks of serious diseases such as diabetes, cardiovascular diseases, and cancer. [8-12]

It is pertinent to remark that bioactive compounds are the main practical components of functional foods, which play a significant role in the prevention of many diseases, in maintaining the physical and mental health of our body, the resource state of the body. Functional foods treat metabolic disorders in the body: type 1 diabetes mellitus and type 2 diabetes mellitus. [9] Functional foods containing probiotics are effectively used in the prevention of obesity. [10,13] Plant enzymes, such as proteases and papain, have a solid thrombolytic and fibrinolytic effect and therefore can treat and prevent cardiovascular diseases. [14-15]

Medusomyces Gisevii Lindau (Kombucha) and Fabricius' bag of chickens are sources of hydrolytic enzymes. Hydrolytic enzymes, as noted earlier, are widely used in the food industry, as well as in preventive medicine and pharmacy. Hydrolytic enzymes are bioactive compounds that share pharmacological effects, which means hydrolytic enzymes positively affect the health of the body's digestive system. Hydrolytic enzymes are integral to systemic enzyme therapy and are important in preventing gastrointestinal diseases and systemic diseases.

In the process of isolating hydrolytic enzymes, it is known that proteolytic enzymes, which are not in the form of zymogens but in active forms, can inhibit the activity of amylase and lipase, namely, to reduce it, which prevents the production of active enzyme complexes. [21] Based on the literature data, some nanomaterials stabilize enzymes, maintain enzyme activity, and extend the shelf life of products and functional nutrition supplements. Thus, in this work we used nanostructures such as b-cyclodextrins, which were approved for use in the food, pharmaceutical, and medical industries. [22-26]. In this regard, cyclodextrins can help overcome the inactivating effect of active proteases on other enzymes in native solutions.

In industry, cyclodextrins are fermented from modified starch using cyclodextrin glucosyltransferase. Cyclodextrin is non-toxic, characterized by the fact that it is not absorbed in the upper gastrointestinal tract, and is completely broken down by the microflora of the colon. [26-27] Cyclodextrin is a cyclic molecule of glucose

subunits that forms an internal cavity capable of binding to hydrophobic molecules (hydrophobic inclusions). This property allows cyclodextrins to form stable complexes with hydrophobic molecules, including lipids, aromatic compounds, and some proteins.

α-CDs have small cavity sizes, so they cannot accept many molecules. γ-CDs have larger cavities than most other molecules with which γ-CDs can form complexes. [18,19] However, due to the capacity of many molecules in the CD cavity, there is stress on the hydrophobic bonds in the CD cavity, so the host-guest interaction is complex. The diameter of the β-CD cavity is believed to be most suitable for molecules such as hormones, vitamins, hydrolytic enzymes, and other compounds commonly used in tissue and cell culture. This feature makes β-CDs preferable for complexation [28-29].

Given the fact that cyclodextrins can form complexes with enzymes, and thereby nanostructures protect enzymes from proteolytic destruction, one of the urgent tasks was to study the effect of the cyclodextrins on hydrolytic enzymes obtained from Medusomyces Gisevii Lindau (kombucha), reindeer pancreas, and Fabricius bag of chickens.

MATERIALS AND METHODS

Model enzyme preparations were used to select the optimal enzyme: cyclodextrin ratio. To determine the optimal ratio of amylase and b-cyclodextrin, the model drug Amilosubtilin (G3H LLC Sibbiopharm, Russia) was used. . To determine the optimal ratio of lipase and b-cyclodextrin, a model preparation, Lipopan 100L (Novozymes, Denmark), was used.

The total protein concentration in the solution was determined using the Lowry method using the Folin-Ciocalteu reagent. This method is based on the reduction of the phenyl group of tyrosine of phosphomolybdic acid in the Folin–Ciocalteu reagent with the formation of a blu-colored compound, which is determined on a spectrophotometer at a wavelength of 750 nm and a cuvette thickness of 10 mm. [30]

To determine the optimal ratio of protease and b-cyclodextrin, the model drug Protosubtilin (G3X LLC Sibbiopharm, Russia) was used. There are three types of cyclodextrins: α-CD, β-CD, and γ-CD. Every kind of cyclodextrin has a different cavity shape to form a host-guest complex.

The research used β-cyclodextrins from the Leko style company (Russia). These nanostructures are odorless white crystalline powder, with a sweetish taste. They are soluble in water but practically insoluble in methanol, ethanol, propanol, and ethyl ether. Cyclodextrins have high heat resistance and can withstand temperatures up to 2000C. In addition, they are stable over a wide pH range, including values from 3.0 to 14.0.

To obtain the enzyme: cyclodextrin complex, several identical portions of the model enzyme preparation and various portions of β-cyclodextrin were taken to obtain the following ratios: 1:1,1:2,1:3. Enzyme: cyclodextrin complexes were obtained in multiple ratios. The complexes were obtained by grinding a mixture of β-cyclodextrin with an enzyme preparation with a pestle and mortar with the addition of minimal amounts of water. The resulting complexes were dissolved in purified water to maintain the calculated ratios. For 90 minutes, samples were taken for enzymatic activity (amylolytic, proteolytic, lipolytic).

Analysis Progress: To 1 ml of the test solution, 2 ml of 0.5 N. NaOH solution and 0.6 ml of Folin-Ciocalteu solution were added, and the solution was previously diluted three times with distilled water. The sample was kept for 20 minutes; then, the optical density was measured on a spectrophotometer at a wavelength of λ = 750 nm.

The measurement was carried out relative to the control: the same reagents were added to 1 ml of purified

water and in the exact amounts as in the sample. The concentration of the protein in the solution was determined by the calibration graph built on-albumin (Table 1). To 2 ml of 1% casein solution, pre-thermostated at 37 ^{0}C, 0.2 ml of the enzyme was added, and hydrolysis carried out for 10 minutes at 37 ^{0}C. 3 ml of 5% trichloroacetic acid (TCA) was added to interrupt the hydrolysis. The precipitate formed was allowed to stand for 20 minutes. Then, the optical density of the supernatant was measured at a wavelength of 280 nm in a 10 mm thick cuvette. A blank sample filtrate was used as a reference solution.

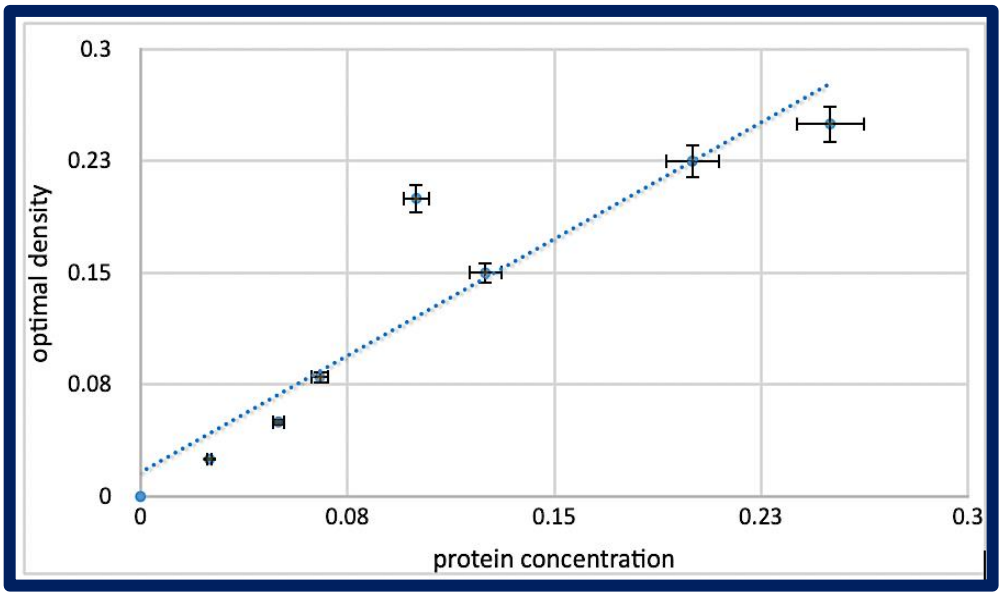


Figure 1. Albumin (serum) calibration curve for determining protein concentration using the Lowry method. Where the definition of proteolytic activity is based on the modified Kunitz method

The activity of pancreatic lipase was determined according to Shihabi and Bishop's modified method. This method is based on changing the rate of decrease in the optical density value of a stable olive oil emulsion under the action of the lipase enzyme.

Analysis Progress: To 2 ml of 1% casein solution, pre-thermostated at 37 ^{0}C, 0.2 ml of enzyme solution was added and, hydrolysis was carried out for 10 minutes at 37 ^{0}C. 3 ml of 5% trichloroacetic acid (TCA) was added to interrupt the hydrolysis. The precipitate formed was allowed to stand for 20 minutes. Then, the optical density of the supernatant was measured at a wavelength of 280 nm in a 10 mm thick cuvette. A blank sample filtrate was used as a reference solution.

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Analysis Progress: An olive oil emulsion (3 ml) was preliminarily incubated in a cuvette at 25^0 C for 3 minutes, 0.1 ml of enzyme material was added and shaken, and the initial reading of the device at 350 nm was immediately noted (this wavelength was selected empirically). Next, using a stopwatch, changes in the optical density of the emulsion were noted every minute for 3 minutes. The results obtained (E1, E2, E0, E3) can be expressed in turbidimetric units.

A laboratory using this method must calibrate and derive a coefficient to convert turbometric units to micromolytriglyceride bonds, since lipase activity is

expressed as the number of micromoleutriglyceride bonds hydrolyzed in 1 min of 1 liter of enzyme material.

The optical density of the emulsions is determined under selected conditions for photometry. An increase in olive oil concentration of 68 µmol/l should form a linear relationship.

Construction of a Calibration Graph: Samples of increasing concentrations of emulsified olive oil were prepared: 68, 136, 204 µmol/l, for 0.3, 0.6; 0.9 ml of a 1% solution of olive oil in ethanol is emulsified in 50 ml of a 0.2% solution of sodium deoxycholate in Tris buffer. The converting turbidimetric units into micromolytri-glyceride bonds: Preparation of an emulsion of olive oil

optical density of the emulsions is determined under selected conditions for photometry.

An increase in olive oil concentrations of 68 µmol/l should form a linear relationship. Conversion to molar concentration is made by the molecular weight of olive oil triolein - 880. From the graph, determine the amount of olive oil (in µmol/l), which corresponds to the division of the scale of the photometric device used.

Denoting this value as X µmol/l, we substitute iit into the formula for calculating the coefficient (K) for

$$K = \frac{X_{\mu mol/l} \bullet 0{,}003 \bullet 1000}{0{,}1}$$

0.015% in 0.2 sodium deoxycholate solution in Tris buffer pH=8.8, 1.5 mL of 1% olive oil solution (in absolute ethanol) was slowly added dropwise. Lipase activity was determined using a calibration graph. (fig 2)

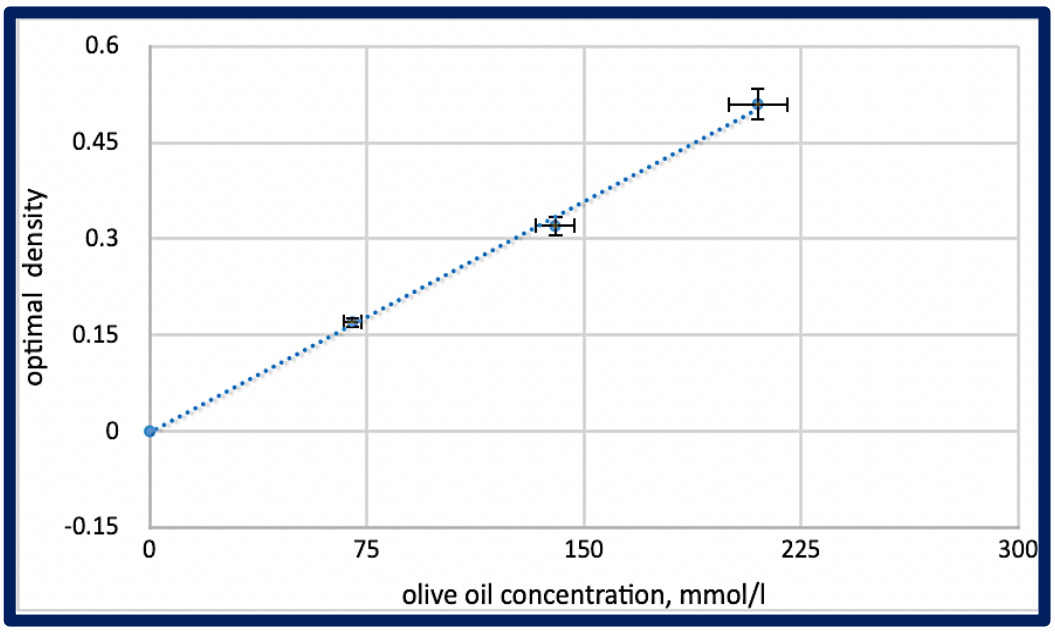

Figure 2. Standard curve for determination of lipase activity.

The determination of amylolytic activity is based on the modified method of Rukhdyadeva. According to this method, the unit of activity of α-amylase corresponds to the amount of enzyme that catalyzes the breakdown of 1

gram of soluble starch in 10 minutes at a temperature of 30^0C and pH=6 by 30.

Analysis Progress: 1 mL of the enzyme was added to pre-thermostated 2 mL of 1% starch solution and thermostated at 30 ^{0}C for 10 minutes. Then 0.2 ml was withdrawn and added to 2 ml of 0.1N HCl. After that, 5 ml of a working iodine solution was added to 0.5 ml of the resulting reaction mixture. A solution similar in preparation was used as a control, except that 1 ml of purified water was added instead of 1 ml of the enzyme solution.

SUBJECTS

The work used the pancreas of a reindeer, the Fabricius bag of chickens, and Medusomyces Gisevii Lindau (Kombucha) were used.

Statistical Analysis: The data from the study were statistically processed using the significance criterion.

The experiments were repeated 4-5 times to obtain more accurate results. The paper presented the arithmetic mean values and standard errors at a significance level of $p \leq 0.05$.

This approach allows for a qualitative analysis of the data obtained to determine their validity and reliability. The use of statistical methods is widespread in scientific research, pharmaceutical and medical industries when an objective evaluation of the results of experiments is necessary.

Presenting data as averages allows you to average the results of several experiments and get more reliable and accurate results. This helps to reduce the influence of random factors on the data obtained and gave a more objective picture of what happened during the experiment.

$$S = \sqrt{\frac{\sum_{i=1}^{n} (\overline{X} - X_i)^2}{n-1}}$$

Calculation of the arithmetic mean value of all measurements X was calculated by the formula:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where $\sum_{i=1}^{n} X_i$ - is the sum of all values of individual measurements (the sum of all options);

n - is the volume of the population (number of options);

As a measure of the spread of values from the average value, the standard deviation of the arithmetic mean was:

where $(\overline{X} - X_i)$ - is the deviation of each value from the arithmetic mean;

$\sum_{i=1}^{n}(\overline{X}-X_i)^2$ - is the sum of all squared deviations.

$(n-1)$ - is the number of degrees of freedom.

The error of the arithmetic mean value ***Sx*** was calculated by the formula:

$$S_x = \frac{S}{\sqrt{n}}$$

$$S_{\bar{x}} = S_x - t(n, p = 0.95)$$

An assessment of the significance of differences between the values obtained in the experiments and control conditions was calculated using the student's t-test according to the formula:

$$t = \frac{X_1 - X_2}{\sqrt{S_{\bar{x}_1}^2 - S_{\bar{x}_2}^2}}$$

The t-criterion calculated using experimental data was compared with tabular values, considering the number of degrees of freedom. This allows one to determine the statistical significance of the results and the reliability of the experiment.

The level of reliability of the obtained data was at least 95%, which is acceptable for studies of biological systems. This means that with a high probability, the experiment's results can be considered reliable and applicable in further work.

Assessing the validity of experimental results is an essential step in conducting scientific research, as it allows you to identify errors and deviations that may affect the results of the study.

RESULTS

Previously, data on the study of the enzymatic component composition of the native solution of Medusomyces Gisevii Lindau (Kombucha) [31] and the bursa of Fabricius in chickens [32] were published.

The study of the stability of kombucha enzymes in the presence of cyclodextrins was carried out using model solutions of protease enzymes (Protosubtilin, G3Kh OOO Sibbiopharm, Russia), amylase (Amilosubtilin, G3Kh OOO Sibbiopharm, Russia), and lipase (Lipopan, Novozymes, Denmark). The concentration of the model solutions was 2 mg/mL. From preliminary studies, the most optimal ratio [cyclodextrins: enzyme] was found to be 4:1.

Therefore, cyclodextrins were added to each model solution of the enzyme in a ratio of 8 mg/mL. No cyclodextrins were added to control samples. Enzyme

solutions were stored at a temperature of 5 ^{0}C during fermentation for 24 hours. During this time, a sampling was carried out, and the activity of enzymes: amylolytic, proteolytic, and lipolytic, was measured.

The results of the studies are shown in Figures 4-5, in the form of dependence Ai/Ain = f(τ), where Ai, Ain – enzyme activity in the sample and the initial solution, respectively; Ai/Aex is the relative activity of the enzyme;

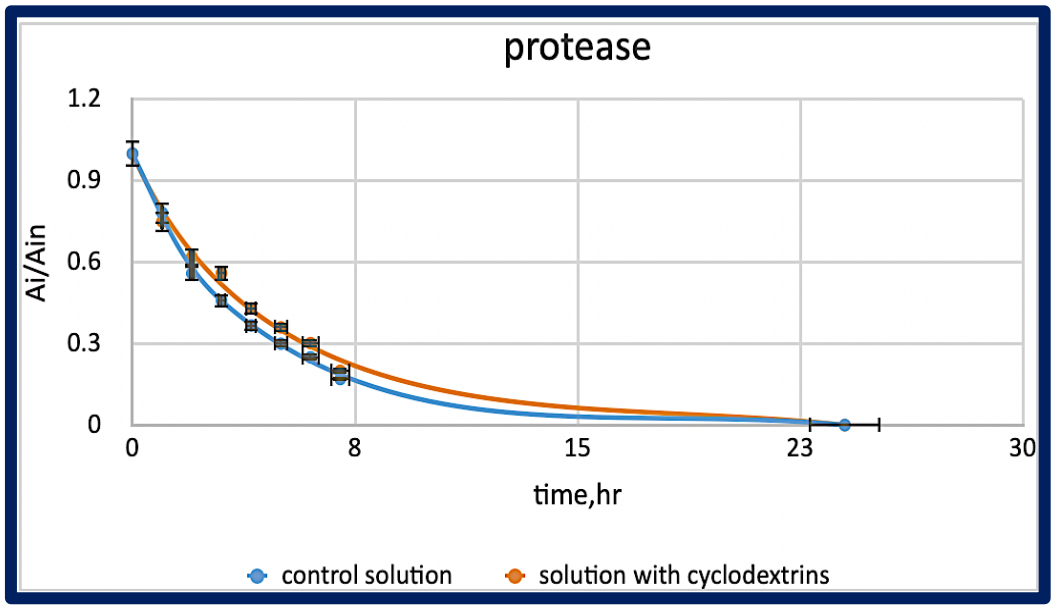

Figure 3. Dependence of the relative activity of the protease on the storage time in the presence of cyclodextrins

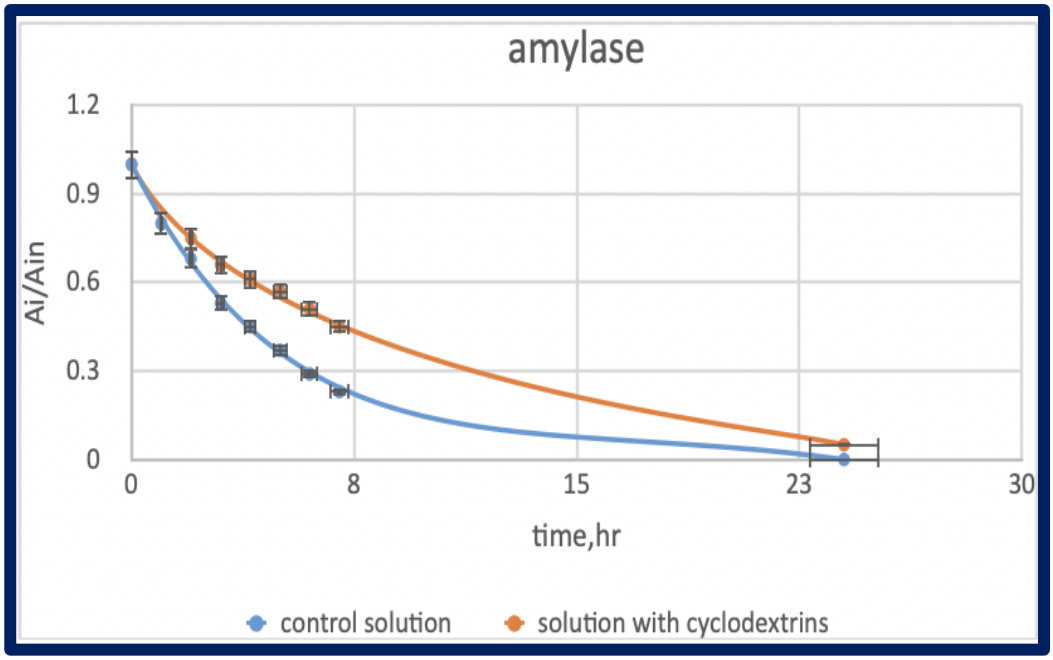

Figure 4. Dependence of relative amylase activity on storage time in the presence of cyclodextrins

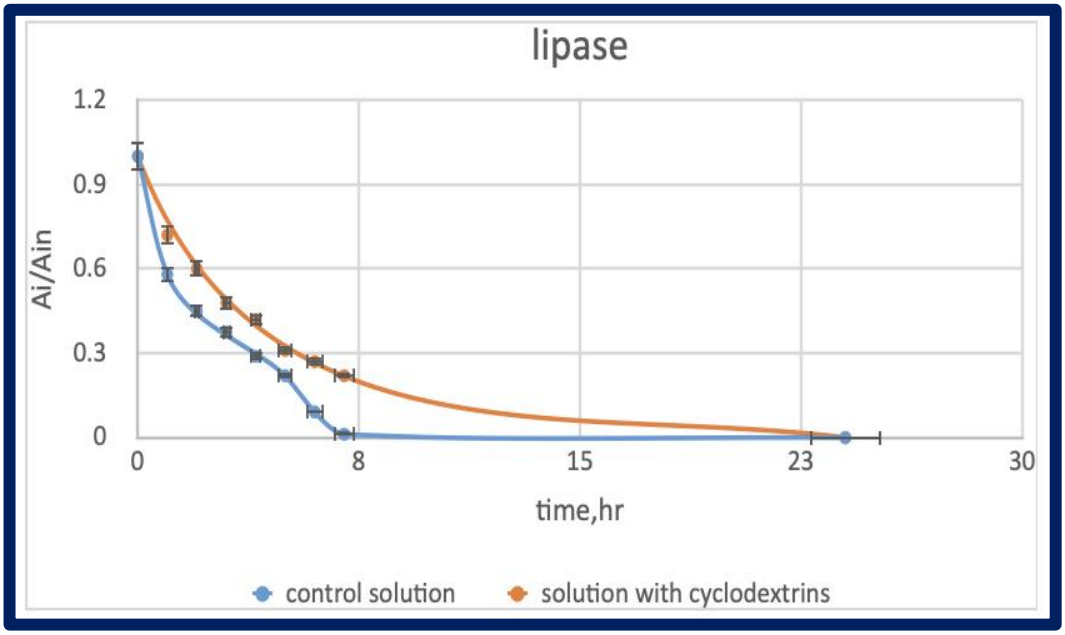

Figure 5. Dependence of the relative activity of lipase on the storage time in the presence of cyclodextrins

The study of stabilization of enzymes obtained from the bursa of Fabricium was carried out as follows: first, an extract from the bursa of Fabricium was obtained, desalted, and then the amount of protein in it was determined. Next, the required amount of β-CDs was calculated in various ratios to total protein to obtain the following ratios of 1:1, 1:2 and 1:3.

Since the proteolytic enzymes were in solution, before introducing β-cyclodextrins into the solution, the nanostructures had to be prepared accordingly. To achieve the desired result, β-cyclodextrins were dissolved in hot purified water with constant stirring until complete dissolution. Then the resulting solution was cooled, and a desalted supernatant solution was added dropwise.

Samples for the presence of enzymatic activity were taken within four days. In addition, a control sample was performed to evaluate the effect of different rations of β-cyclodextrins on target enzymes. In this case, samples were also taken from the desalted supernatant for four days without the addition of β-cyclodextrins. Experimental curves were built in the coordinates A = f(t) (where A is the enzymatic activity of the sample (PE / ml), and t is the time (days).) The results of studying the effect of various ratios of β-cyclodextrins to proteolytic enzymes are presented in Table 1 and in Figure 6.

Table 1. Effect of β-cyclodextrins on the activity of proteolytic enzymes

Day Relation	1	2	3	4
	Activity			
Stock Solution	35,5	17,5	11	7,5
1:1	34,25	19,25	15,5	10,25
1:2	36,5	30,5	28,75	25,1
1:3	35	15,5	10,5	9,5

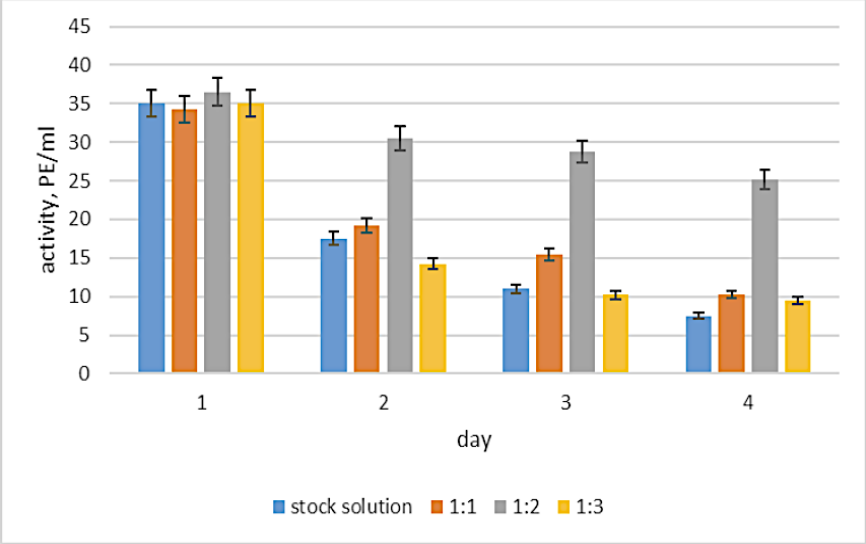

Figure 6. Effect of β-cyclodextrins on the activity of proteolytic enzymes.

RESULTS AND DISCUSSION

Villiers first described CDs in 1851 [33]. Cyclodextrins pay such close attention to the cylindrical shape of the molecule, which has specific properties and a wide range of applications. The residues of the glucose molecule have polar hydroxyl groups located on the cylinder's surface, so the cyclodextrin molecule's outer side is hydrophilic. In contrast, the inner side is non-polar and lipophilic (hydrophobic).

In nature, bacteria use the enzyme cyclodextrin glucosyltransferase (CGT) to convert starch into cyclodextrins. CGT cleaves the helical chain of starch in two places and then joins the ends of the cleaved chain to form cyclic structures. Typically, this process results in a mixture of two types of cyclodextrins because the split starch chain fragments vary in length. At present, a method has already been developed by which it is possible to make the CGT enzyme work more selectively, that is, to produce mainly one of the cyclodextrins of a given size [33-34].

Cyclodextrins or cyclodextrin ligands can "wrap" individual molecules of active ingredients. This process is called complex formation, which is called the "host-guest" complex. In this pair, cyclodextrin is the "host," and the molecule "wrapped" by it is the "guest." A guest particle can be any molecule that, on the one hand, is small enough to fit into the cyclodextrin ring and, on the other hand, is non-polar enough to interact with the internal lipophilic part of the cyclodextrin molecule. The complex formed by the cyclodextrin molecules, and the active substance (the so-called monomolecular container will be destroyed on the skin, releasing the active substance at the right time and place. [22,28,33-34]

β-cyclodextrins are common in the pharmaceutical industry, where they can stabilize active substances and mask unpleasant taste and odor. In fact, CDs use as excipients has increased significantly in various delivery systems in the pharmaceutical and food industries.

As part of the complex, cyclodextrin protects the "guest" molecule from damage by various reactive molecules, thereby reducing the rate of oxidation, steric rearrangements, hydrolysis, racemization, and enzymatic destruction. The ability of cyclodextrin molecules and molecules of the "guest" substance to form inclusion complexes depends on two factors:

1). Steric factor. The formation of inclusion complexes is possible if the structure of the cyclodextrin molecule is sterically consistent. This factor considers the

correspondence between the dimensions of the internal cavity of the cyclodextrin molecule and the size of the "guest" molecule or a particular functional group that must be placed in the cyclodextrin cavity. If the size does not match, the functional group or molecule cannot firmly attach to the cavity

2). Thermodynamic interaction between cyclodextrin, guest, and solvent. To form a complex, a state of the system must be formed in which the most energetically favorable is the placement of "guest" molecules in the internal cavity of the cyclodextrin.

In the hydrophobic cavity of cyclodextrin, a certain microenvironment is formed that is energetically favorable for the placement of non-polar regions of the "guest" molecule and the formation of a complex. The main driving factor in the formation of the complex is the insufficient complementarity of hydrogen bonds between water molecules with the functional groups of the cyclodextrin torus cavity. This leads to an increase in the enthalpy of water molecules and their release from the cyclodextrin cavity. Their place is taken by hydrophobic molecules that form non-polar bonds with cyclodextrins

Of great interest are studies where CDs are used as food additives. Experiments on animals when feeding CD preparations labeled with 14C showed that CDs are almost completely absorbed in the body, indicating their nutritional value. There is evidence that CDs as food additives regulate lipid metabolism and lower cholesterol levels in the body. In many countries, including France, the Netherlands, and Belgium, the use of CDs in the food industry as food additives is permitted. Substances that determine the taste and aroma of food often do not have high stability and decompose during storage, heat treatment, etc. It has been noted that complexation with CD leads to the stabilizing of flavor-forming and biologically active substances of aromatic plants. The production of complexes of such chemicals with CD solves many issues related to the technology to produce seasonings, their storage, the development of dietary supplements for dietary nutrition, etc. Dry complexes of CD with food components are quite stable, and in an aqueous environment, they dissociate with the release of active components.

In the food industry, the preparation of β-CD complexes with spicy-aromatic compounds and substances that influence the organoleptic properties

of food is a well-established process. For instance, in Hungary, a range of aromatic plant extracts (dill, onion, garlic, cumin) is produced in the form of inclusion complexes with CD, which are characterized by high stability and pronounced organoleptic properties.

This process is because cyclodextrin binds to these structures due to additional molecular interactions, which leads to increased stabilization and activation of amylase and lipase. Still, at the same time, the proteolytic effect of proteases on lipase and amylase is reduced.

Cyclodextrins has a wide range of applications in the pharmaceutical and food industries. They serve as stabilizers for the activity of vitamins and drugs, and importantly, they preserve the organoleptic properties of plant extracts, showcasing their potential in the food industry. However, these nanostructures—cyclodextrins—can also be used to stabilize enzymes to preserve their activity, further using these enzymes in the process of food production.

There is an example of research work carried out earlier at the Department of Biotechnology of the St. Petersburg Chemical-Pharmaceutical University, in which the stabilization of hydrolytic enzymes obtained from the pancreas of reindeer was studied. Proteases are inactive forms, which means they (proteases) tend to reduce amylase activity and lipases. The study observed significant lipase and amylase activity retention in the presence of protease with the stabilizing effect of nanostructures (cyclodextrins).

This paper presents the results of studying the effect of cyclodextrins on the activity of hydrolytic enzymes obtained from Medusomyces Gisevii Lindau (kombucha) and chicken bursa of Fabricius, a waste product from poultry farms.

In the presence of cyclodextrins, the activity of enzymes (amylase and lipase) obtained from kombucha increased markedly during storage compared to control samples. The stability of the protease in the presence of cyclodextrins remained virtually unchanged. They were observed at an optimal ratio of 4:1 (cyclodextrin: enzyme). This process is because cyclodextrin binds to these structures due to additional molecular interactions, which leads to increased stabilization and activation of amylase and lipase. Still, at the same time, the proteolytic effect of proteases on lipase and amylase is reduced.

The activity of the protease enzyme obtained from the bursa of Fabricius of chickens was maintained for four days at an enzyme: cyclodextrin ratio of 1:2. Thus, in this formed complex of enzymes with cyclodextrins, a stabilizing effect of nanostructures on proteases is observed. Moreover, the data obtained showed that cyclodextrins have a stabilizing effect on hydrolytic enzymes aimed at preserving the activity of enzymes, which is essential for food storage.

When using cyclodextrins in a ratio of 1:2 with enzymes (protease) obtained from the bursa of Fabricius and at an optimal ratio of 4:1 (cyclodextrin: enzyme) with enzymes (lipase and amylase) obtained from kombucha, significant preservation of the activity of these enzymes was observed. The use of cyclodextrins in the food industry to increase the shelf life of corresponding food products is promising.

CONCLUSION

Hydrolytic enzymes (amylase, lipase, protease) are widely used in the food and pharmaceutical industries for the prevention of diseases, namely in the production of dietary supplements, which is essential in the field of functional nutrition, and therefore for maintaining optimal health, reducing the risk of chronic diseases and managing their symptoms using functional nutrition.[35]

One of the primary challenges in hydrolytic enzyme production is preserving their activity.

experimental part. Kucherenko A.N., Karavaeva L.I., and Glazova N.V. participated in writing and editing the manuscript. Kiprushkina E.I. advised on manuscript writing and editing. All authors read and approved the final manuscript.

Fortunately, b-cyclodextrins have been found to enhance enzyme activity, making them a vital component in maintaining enzymatic functionality.y establishing an optimal cyclodextrin-to-enzyme ratio, it is possible to utilize a minimal amount of b-cyclodextrins while sustaining enzyme activity over an extended period.

At a 1:2 ratio of cyclodextrin/enzyme (protease) obtained from the bursa of Fabricius and at a 4:1 ratio of cyclodextrin/enzyme with enzymes (lipase and amylase) obtained from kombucha, significant preservation of the activity of these enzymes was observed for four days.

The use of cyclodextrins in the food industry holds great promise for extending the shelf life of functional foods. These foods, which are rich sources of bioactive compounds such as hydrolytic enzymes (lipase, amylase, protease), can benefit significantly from the stabilizing effect of cyclodextrins, thereby enhancing their role in functional nutrition.

List of Abbreviations: CD: cyclodextrin; CGT: cyclodextrin glucosyltransferase; TCA: trichloroacetic acid; FFC: Functional Food Center

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