

Analysis of The Activity of the B-Galactosidase Enzyme in A Dry Extract Obtained from The Local Chickpea (CICER Arietinum) Plant

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Abstract: In this study, the enzyme β -galactosidase, called lactase, functions to break down lactose. These β -galactosidases are used not only in the preparation of lactose-free foods, but also in the processes of processing whey products and obtaining prebiotics. β -galactosidase breaks down the lactose disaccharide and can be isolated from a variety of sources, particularly plants, microorganisms, and animal tissues. The role of this enzyme is crucial in the production of hydrolyzed lactose and glycosylated products for people who cannot tolerate lactose. The composition of the β -galactosidase enzyme is important. Researchers extensively studied the composition of the β -galactosidase enzyme in the dry extract of the cultivated local chickpea (*Cicer arietinum*). The o-nitrophenyl- β -D-galactopyranoside (ONPG) substrate was used to measure enzyme activity. The results showed that the β -galactosidase enzyme in the dry extract from local plant raw materials has high stability and scientifically substantiated the possibility of its use in the production of local biologically active supplements.

Keywords: Peas (*Cicer arietinum*), β -galactosidase, dry extract, lactose, inability to digest.

Introduction: Today, there is a high demand for the enzyme β -galactosidase in the breakdown of lactose in dairy products and in the production of biologically active additives for the food and pharmaceutical industries. They are common in plants, microorganisms, and animals. [1, 2] Therefore, the enzyme β -galactosidase (β -D-galactoside-galactohydrolase, EC 3.2.1.23) is an important hydrolase that cleaves lactose, a milk sugar, into glucose as well as galactose monosaccharides. Since more than 70% of the world's population today suffers from hypolactasia (the inability to digest lactose), the pharmaceutical industry has a great need for this enzyme. [2] Although enzymes of mainly microbiological origin are used in the pharmaceutical industry, the enzymes that are biosynthesized from plant raw materials in the following years attract attention for their high stability. [3] Legumes, in

particular native peas (*Cicer arietinum*), are a source rich in biologically active components and enzyme composition. Nevertheless, the specifics of the enzyme β -galactosidase in a dry extract from a locally grown pea (*Cicer arietinum*) plant have not yet been fully studied. [4, 5] Processing milk and dairy products with β -galactosidase preparations allows for the provision of virtually lactose-free dairy products to those suffering from lactose intolerance. [6] Intolerance to milk affects 15-30% of the world's population and is caused by a lack of the enzyme lactase, which is produced in the small intestine and catalyzes the breakdown of lactose (milk sugar) into glucose and galactose, which is then easily absorbed into the blood. [7]

This condition is caused by the insufficient production of the lactase enzyme in the human body, which results in lactose not being fully broken down in the small intestine. [8] Lactose intolerance causes bloating,

diarrhea, abdominal pain, and other discomforts. [9] For this reason, the demand for lactose-free or lactose-reduced dairy products is increasing year by year. There are basically three types of lactase deficiency: primary, congenital, and secondary. [10]

Primary lactase deficiency occurs in people between the ages of 2 and 20. Primary lactase deficiency is a more common type, resulting from decreased synthesis of β -galactosidase along the brush margins of the small intestine (lactase). [11] Congenital lactase deficiency, the second type of lactase deficiency, is caused by a genetic abnormality in which patients have very little of this enzyme or none at all [12]. The third type, known as secondary lactase deficiency, is caused by low levels of this enzyme due to an underlying disease affecting the gastrointestinal tract. The prevalence of lactose intolerance in Asian countries is approximately 90%. [13] People who suffer from lactose intolerance consume fermented products in dairy products that contain no or very little lactose. Correction of lactase deficiency can be done in two ways: by consuming dairy products with partially hydrolyzed lactose or by taking medications containing the lactase enzyme. Therefore, the β -galactosidase enzyme from plant sources is suitable for practical use [14].

In our republic, the effective utilization of local raw material bases—specifically by investigating the β -galactosidase enzyme found in dry extracts derived from the local chickpea (*Cicer arietinum*) plant, which is rich in proteins and hydrolytic enzymes—enables the creation of highly effective, import-substituting biologically active supplements. Consequently, the extraction of the β -galactosidase enzyme from chickpea seeds and the study of its properties represent a significant area of focus within modern biotechnology [5].

Objective of the scientific research. The objective is to analyze the content of the enzyme β -galactosidase in a dry extract derived from the local chickpea (*Cicer arietinum*) plant and, based on the findings, to develop this lactase as a biologically active supplement.

METHODS AND PROCEDURES

Seeds of a local variety of chickpea (*Cicer arietinum*) were selected as the research subject. The raw material was washed and soaked in clean water for 8–12 hours. To prevent moisture loss, the soaked seeds were kept in a cool, dark place for 72 hours to allow for germination. The germinated seeds were crushed and mixed with a cold 0.1 M phosphate buffer solution (pH 7.0). The mixture was processed using a blade mill or homogenizer until it reached the consistency of a thick paste. The resulting mass was filtered, and the volume

of the filtrate was measured. Subsequently, ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$ was added to the solution to a specific degree of saturation for fractionating and isolating the enzyme.

As a result, the enzyme was precipitated using ammonium sulfate. The resulting homogenate was centrifuged at 10,000 rpm for 15–20 minutes, and the extract was recovered by filtration through filter paper or sterile gauze. The resulting precipitate was redissolved in a phosphate buffer (pH 7.0) to prepare it for dialysis. This solution was then placed into a dialysis bag. Dialysis was carried out at +4°C for 24 hours, with the phosphate buffer solution being replaced every 6 hours. Following dialysis, the purity of the enzyme was significantly improved; the dialysis process effectively removed small molecules and salts. The separated clear supernatant was frozen at -40°C and subsequently subjected to sublimation in a freeze-dryer, yielding an amorphous dry extract powder. β -galactosidase was extracted using three different methods: cold-water extraction, alkaline extraction (pH 8.0), and enzymatic extraction. Enzyme activity was determined using a standard colorimetric method, and the influence of pH (5.0–8.0) and temperature (25–45°C) was evaluated. The results indicated that, among the extraction methods tested, the enzymatic approach yielded the highest β -galactosidase activity. Enzyme activity was 4.5 U/mg for the cold-water extraction and 5.9 U/mg for the alkaline extraction. In enzymatic extraction, this value reached 8.2 U/mg. The maximum enzyme activity was observed at pH 6.5 and a temperature of 37°C, conditions under which the enzyme also exhibited high stability.

Absorbance was measured at 420 nm using a UV-spectrophotometer. A qualitative test was performed as follows: 0.5 mL of the enzyme solution was placed into a test tube, and 0.5 mL of a 2 mM ONPG solution was added. The mixture was incubated at 37°C for 15–30 minutes, after which 0.5 mL of 1 M Na_2CO_3 was added. The solution turned yellow, confirming the presence of β -galactosidase. During the determination of enzyme activity, the amount of o-nitrophenol released was measured spectrophotometrically at a wavelength of 420 nm.

RESULTS OBTAINED AND THEIR ANALYSIS

A comparative analysis was conducted on the impact of various extraction methods employed during the study on the efficiency of isolating the enzyme lactase (β -galactosidase) from local pea sprouts. The quantitative data obtained demonstrated that enzyme activity is directly dependent on the isolation technology used (Table 1).

Table 1.

Indicators of the specific activity of the β -galactosidase enzyme using various extraction methods

No	Extraction method	Specific activity of the enzyme, U/mg
1	Cold-water extraction	4,5
2	Alkaline extraction (pH 8.0)	5,9
3	Enzymatic extraction	8,2

The experimental results involved a comparison of cold-water extraction, enzymatic extraction, and alkaline extraction. The advantages and disadvantages of each method were analyzed, and the influence of factors such as pH, temperature, and duration on enzyme activity was examined. Cold-water extraction yielded an activity of 4.5 U/mg. Alkaline extraction (at pH 8.0) led to the partial breakdown of cellular protein membranes, increasing the enzyme yield to 5.9 U/mg. However, the enzymatic extraction method proved most effective, reaching 8.2 U/mg. This outcome is attributed to the enzymatic hydrolysis of the polysaccharide-glycoprotein complex within the plant cell walls, which facilitates the maximum release of bound β -galactosidase proteins into the free phase. In the experiments conducted, the composition and functional activity of the enzyme β -galactosidase, present in a dry extract derived from the local chickpea (*Cicer arietinum*) plant, were studied in depth. The substrate o-nitrophenyl- β -D-galactopyranoside (ONPG) was used to measure the catalytic stability and activity of the enzyme. The yellow-colored o-nitrophenol complex, formed as a result of ONPG hydrolysis, was analyzed spectrophotometrically at a wavelength of 420 nm.

CONCLUSION

As a result of the research conducted, a technology for extracting the highly active enzyme β -galactosidase (lactase) from chickpeas (*Cicer arietinum*), a local raw material source, has been scientifically substantiated through biological activation (germination) and enzymatic modification. Based on experimental data, the following fundamental and practical conclusions were reached: the enzymatic extraction method demonstrated the highest yield for isolating β -galactosidase from plant tissues, surpassing traditional cold-water (4.5 U/mg) and alkaline (5.9 U/mg) extraction methods and enabling the specific activity of

the enzyme to reach 8.2 U/mg. A specific qualitative reaction using the substrate ONPG (o-nitrophenyl- β -D-galactopyranoside), combined with the detection of an absorption spectrum at a wavelength of 420 nm via UV-spectrophotometry, definitively confirmed the presence of the β -galactosidase enzyme within the system at the molecular level. Enzymatic extraction is considered the most effective method for obtaining β -galactosidase from the chickpea (*Cicer arietinum*) plant. Enzyme activity reached its maximum at a pH of 6.5 and a temperature of 37 °C. It has been demonstrated that the process of isolating the enzyme β -galactosidase from the chickpea (*Cicer arietinum*) plant is directly dependent on the selection of high-quality raw material and the application of appropriate extraction methods.

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