Original Research Article

Purification of β-Glucosidase, one of the flavor-enhancing food enzymes, from Peppermint (Mentha piperita L.) and its Biochemical Characterization

Abstract

Aims: Determination of the biochemical properties of β-glucosidase in peppermint, which is rich in aromatic compounds.
Study Design: β-glucosidase was purified from mint, and biochemical characterization of the purified enzyme was performed.
Place and Duration of Study: This study was carried out in the Faculty of Arts and Sciences Biochemistry laboratory.
Methodology: Enzyme purification was performed by hydrophobic interaction chromatography using a Sepharose 4B-L-tyrosine-1-naphthylamine gel. Optimum pH, temperature, and substrate specificity of the purified enzyme were determined. The effects of glucose, δ-gluconolactone and some heavy metals on the enzyme activity were investigated.
Results: The enzyme was purified with 8-fold and 28% yield. The purified protein from mint was visualized at 65 kDa on SDS-PAGE. The substrate specificity of the purified β-glucosidase from mint was determined against *para*- and *ortho*-nitrophenyl β-D-glucopyranoside (*p/o*-NPG) substrates. The Km values were 0.4 and 0.9 mM, and the Vmax values were 102.2 EU and 96.6 EU, respectively. While the optimum pH for the purified enzyme was 6, the optimum temperature was 35 °C. Effects of heavy metals Ag^{+2}, Fe^{+3}, Zn^{+2}, Cu^{+2}, and Pb^{+2} on the purified enzyme activity were investigated. Relative activities of heavy metals were introduced into the reaction medium as 0.75 mM samples without any known inhibitors in the environment. Fe^{+3} increased the enzyme activity, and Ag^{+2}, Pb^{+2}, Cu^{+2}, and Zn^{+2} inhibited the enzyme, and their relative activities were 78, 76, 22, and 31%, respectively. Glucose and δ-gluconolactone competitively inhibited the enzyme activity when *p*-NPG was the substrate. *K*i values of glucose and δ-gluconolactone were determined as 0.034±0.001 and 0.038±0.002 mM, respectively.
Conclusion: Determination of the biochemical properties of β-glucosidase from mint, which has commercial and pharmacological importance due to the phenolic substances it contains, will contribute to studies on improving food quality.

Keywords: *Mentha piperita* L.; enzymes; hydrolyses; β-glucosidase; hydrophobic interaction chromatography; characterization

1. Introduction

Peppermint is a perennial herb cultivated in many places around the world. The most well-known properties of mint are its flavoring functions and scent. Fresh or dried mint leaves and the essential oil derived from the plant have been widely used in the food, cosmetics, and drug industries [1]. Peppermint is on the alternative medicine list due to its health benefits. It is also used therapeutically for gastrointestinal disorders (GI) such as biliary disorders, indigestion, enteritis, flatulence, intestinal colic, gastritis and spasms. Peppermint is also a popular herbal tea. Phenolic compounds found in plants consumed as tea are considered natural antioxidants [2]. The dry weight of peppermint leaves includes phenolic compounds, about 19-23%, while about 12% contain flavonoids such as hesperidin, eriocitrin, and rosmarinic acid [3]. Many studies suggest these compounds have antioxidant, antigenotoxic, antiallergic, and antitumor effects [4-7]. Mint contains various flavone and flavanone compounds such as luteolin, apigenin, and hesperidin. Peppermint is considered an antioxidant because flavones are involved in the linoleic acid oxidation system [8]. Plants contain phenolic compounds in the form of glycosides. The glycosidic bonds of glycosides are hydrolyzed by glycoside hydrolase and polysaccharide enzymes in plants [9]. As glycosyl hydrolases, β-glucosidases are the key enzymes of carbohydrate metabolism, taking place in almost every living thing, including plants, fungi, mammals, and bacteria [10].

There are many types of β-glucosidic flavor precursors in plants. Phenolic substances, released when their aglycones are separated from the glycon, increase the flavor of foods and beverages produced from these plants [11]. Compounds released from hydrolysis of glycosidic flavor precursors effectively improve the flavor of fruit juice and wine. Different types of grapes, fungus, and yeast glycosidase enzymes have been a broad subject of research as they can take part in the hydrolysis of glycosides [12-14]. The enzyme has been purified from several sources such as tea leaves [15, 16], vanilla [17], soybean [18], chayote [19], orange [20], olive [21], white button mushroom [22], and analyzed in terms of its biochemical properties. Generally, β-glucosidases are found in plant tissues and hydrolyze glucosidic flavor precursors to reveal the aromatic aglycone moiety. β-glucosidases obtained from source plants or other sources such as microorganisms are used as food enzymes. It can be added to food and beverages in food processing steps to improve food quality. For this purpose, β-glucosidases were investigated by many researchers in food technology and biotechnology [11].

Soluble or immobilized forms of β-glucosidases isolated from microorganisms are used as exogenous enzymes in the feed and food industries [12]. In the food and feed manufacturing industry, microbial strains are used increasingly to produce additives, flavorings, and food enzymes. Modified strains are also frequently found among these microbial strains. For feed additives, both live strains and their additives, including DNA, must not be present in the last stage of the feed additive product. In addition, the food enzyme manufacturer must prove that the strain used for production or its DNA does not exist [23].

The richness of mint in terms of its phenolic compound content and the antioxidant effects in teas and food have made it essential to determine the biochemical properties of β-glucosidase. This study aims to purify mint β-glucosidase and determine its biochemical properties. We consider that the obtained results will contribute to food improvement studies.

2. Materials and methods

2.1. Plant Material

The mint plant used in the study was obtained from Balıkesir/Turkey and collected in April and May. All parts of the plant were used in the experiments.

2.2. Chemicals

L-tyrosine, Sepharose-4B, 1-naphthylamine, *p*-NPG, *o*-NPG, *p*-NP, *o*-NP, glucose, δ-gluconolactone, EDTA, phenylmethanesulfonyl fluoride (PMSF), dithiothreitol (DTT), protein assay reagents and chemicals for electrophoresis were obtained from Sigma-Aldrich Company (product of USA). Buffers and other chemicals used for preparation were of the best available grade.

2.3. Enzyme Extraction

Firstly, acetone powder was prepared using Kara's method [21]. The obtained acetone powder was stored in the fridge at -20 °C until used. To prepare extract, 1 g of acetone powder was homogenized for 2 minutes with 25 mL of extraction buffer [(100 mM borate buffer containing 5 mM EDTA, 1 mM phenylmethane sulfonyl fluoride (PMSF), 0.25% (w/v) dithiothreitol (DTT), pH 9)] with Stuart SHM1 homogenizer. The homogenate was centrifuged at 15,000 rpm for 30 minutes, and the supernatant was used as the crude extract.

2.4. Enzyme Purification

Enzyme purification was performed in two stages by modifying the method described by Kara et al.: i) Ammonium sulfate precipitation and ii) Hydrophobic interaction chromatography [21]. First of all, the precipitation range of ammonium sulfate in the crude extract was determined by us. Ammonium sulfate precipitation in 40-80% intervals was applied to the raw extract, and the final precipitate was dissolved in minimum volume by 50 mM sodium phosphate buffer at pH 6.8. The resolving solution was implemented on a Sepharose 4B-L-tyrosine-1-naphthylamine hydrophobic gel column of 1x5 cm. Before the sample was applied, the column was balanced with 50 mM, pH 6.8, sodium phosphate buffer containing 1 M of (NH4)2SO4. After the sample was applied, it was eluted from the saline 50 mM, pH 6.8, sodium phosphate buffer containing 1 M $(NH_4)_2SO_4$ to saltless 50 mM, pH 6.8, sodium phosphate buffer by forming a gradient. The flow rate of the column during elution was 30 mL/h. In the stage of hydrophobic interaction chromatography, eluents were taken into 2 mL tubes; enzyme activities and qualitative protein amounts at 280 nm were determined for each tube. During the chromatography stage, the 1.0-0.0 M ammonium sulfate gradient started at the 24th tube and ended at the 35th tube. As a result of purification, tubes showing high β-glucosidase activity were pooled and used as pure enzyme sources.

2.5. Enzyme Activity Assay and Protein Determination

Mint β-glucosidase enzyme activity was assessed spectrophotometrically. 5 mM *p*/*o*-NPG solutions were used as substrates. They were dissolved in 50 mM, pH 5.5, sodium acetate buffer. 0.5 M Na2CO3 was used as the reaction-stopping solution. 70 µL substrate and 70 µL enzyme solutions were introduced into the reaction environment and incubated for 30 minutes at 37 °C. The reaction was stopped at the end of the 30 minutes with 70 µL of stopping solution. The resulting *p*/*o*-nitrophenols (*p*/*o*-NPs) were observed at 405 nm and 420 nm wavelengths, respectively, and absorbances were determined. The calibration curves of p/o-NPs were at respective wavelengths for calculating the enzyme activity. Enzyme activity measurements were made with the Thermo Scientific Multiscan GO device using a 96-well microtiter plate. An enzyme unit is defined as the enzyme that emits one µmol *p*/*o*-NP in one minute. Lowry method was used to determine quantitative proteins [24].

2.6. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The purity of mint β-glucosidase purified was checked by SDS polyacrylamide gel electrophoresis. SDS-PAGE was conducted based on the method described by Laemmli U.K. [25], and samples were subjected to 10% separation gel. Among the resulting fractions obtained from hydrophobic interaction chromatography, the sample showing the highest activity was subjected to the separation gel. Protein molecular weight marker containing the following standards was used; (β-galactosidase (116.0 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), REase Bsp98I (E. coli) (25.0 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.4 kDa). SDS-PAGE was achieved using Bio-Rad Mini-Protean Electrophoresis System.

2.7. Determination of Optimum pH and Temperature

The effects of pH on the purified enzyme were measured under pH values changed between 2.0 and 10.0. For this, 50 mM sodium acetate (2.0-6.5) and phosphate (6.5-10.0) were used as buffers, and *p*-NPG was used as substrate. Activity measurements were achieved as described in the enzyme assay section.

For optimum temperature determination, the reaction mixture was kept at temperatures ranging from 25 to 65 °C, and enzyme activities were measured. The reaction environment consisting of the enzyme and the substrate of 5 mM *p*-NPG was incubated in water bath at different temperatures (25 - 65 °C) for 30 minutes. The reaction was stopped using 0.5 M sodium carbonate. After the enzyme activities for each temperature value were measured, the temperature with the highest activity was taken as 100%, and percentage residue values were calculated for the remaining values.

2.8. Determination of Kinetic Parameters and Inhibition Studies

The Michaelis-Menten constant (Km) and Vmax values of purified mint β-glucosidase against (*p*-NPG) and (*o*-NPG) substrates were calculated from the

Lineweaver-Burk graph. The enzyme activities were found in different reaction environment concentrations of *p*/*o*- NPG substrate (0.35 - 2.3 mM), and 1/V – 1/[S] graphs were produced. Km and Vmax values were computed using graph equations.

The relative activities of heavy metals, Ag^{+2}, Fe^{+3}, Zn^{+2}, Cu^{+2}, and Pb^{+2}, were calculated to determine their effects on the purified enzyme activity. In order to achieve this, relative activities of heavy metals introduced to the reaction environment as 0.75 mM samples were calculated against the uninhibited enzyme activity in the reaction mix.

Inhibition types of glucose and δ-gluconolactone, known enzyme inhibitors, were determined by the Lineweaver-Burk plot. For this 1/[S] – 1/[V], graphs of glucose were drawn in $[I_1]$ = 0.1 mM and $[I_2]$ =0.2 mM concentrations, and the value was computed using graph equations. Similarly, 1/[S] – 1/[V] graphs of δ-gluconolactone were drawn in $[I_1]$= 0.05 mM and $[I_2]$= 0.25 mM concentrations and the value was computed using graph equations.

3. Results and Discussion

Extraction and Purification

Since the peppermint plant is rich in oil content, an acetone powder was prepared to remove the oil. Since the peppermint plant is rich in oil, an acetone powder was prepared to remove the oil. It is seen that acetone powder is also used in the purification of β-glucosidase from olive fruit [21] and tea leaves [15]. Additionally, in another study that compared the enzyme activities of enzyme extracts derived from the flesh fruit or the acetone powder, the usage of acetone powder as a starting material for β-glucosidase purification was recommended [26]. Peppermint is an oil-containing herb, and using acetone powder to prepare crude extract is suitable for getting rid of oil pollution.

The purification of the mint β-glucosidase was performed in two steps using ammonium sulfate precipitation with a 40-50% ratio and hydrophobic interaction chromatography using Sepharose-4B-L-tyrosine-1- naphthylamine. The specific activity of the purified enzyme was 1396.99 EU/mg protein with a yield of 31% and a 7.86 purification fold (Table 1). The enzyme activity with elution pattern on the hydrophobic interaction column is shown in Figure 1. The enzyme activity and the total protein concentrations (absorbance 280 nm) of all eluents collected were determined with the methods described in section 2.2.1. The tube with the highest enzyme activity was exposed to SDS-PAGE and was observed as a single band at 65 kDa, as seen in Figure 2. Our previous studies used the same method to purify β-glucosidase from olive fruit and *Galleria mellonella* [21, 27]. Asic et al. purified β-glucosidase from white button mushrooms by the same method [22]. Different researchers have preferred chromatography methods for β-glucosidase purification. Ion exchange and gel filtration chromatography were used to purify β-glucosidase from the tea leaf. In this study, the enzyme was purified 117.0-fold with a 1.26% yield [15]. In another study on tea, isoenzyme A was partially purified by ion-exchange chromatography with a fold of 5.95 with 13.7% efficiency. In comparison, isoenzyme B partially purified a factor of 14.16 with 23.83% efficiency in this study [16]. Ünal et al. purified the β-glucosidase from Muscat of Bornova grapes using ion-exchange chromatography with 7.2 purification fold and 6.69% efficiency [28]. The enzyme was

purified by a factor of 46.1 with 12.8% efficiency from apple seeds [29], by a factor of 6.8 with 26% efficiency from cherry fruit using ion-exchange chromatography and gel filtration chromatography [26], and by a factor of 7.2 with 8.4% efficiency from vanilla plants using ion-exchange chromatography first, and then by hydrophobic interaction chromatography and gel filtration chromatography [17]. In the purification made on soybeans, the enzyme was purified by a factor of 20 with 20% efficiency, using ion-exchange chromatography [18]. The purification from oranges has been reported that using ion-exchange chromatography followed by gel filtration chromatography can achieve a 489.5-fold yield of the enzyme [20]. Some studies use interaction chromatography for β-glucosidase purification [21, 22, 30].

Table 1. Purification steps of mint *(Mentha piperita* L.*)* β-glucosidase

Step	Volume (mL)	Activity (U/mL)	Total Activity (U/mL)	Content of Protein (mg/mL)	Total Protein (mg)	Specific Activity (U/mg)	% Yield	Purification Fold
Crude Extract	17.5	39.038	683.17	0.2196	3.843	177.77	100.0	1
Ammonium sulfate precipitation	4	53.750	215.00	0.2071	0.8284	259.54	31.5	1.46
Hydrophobic interaction chromatography	4	53.365	213.46	0.0382	0.1528	1396.99	31.2	7.86

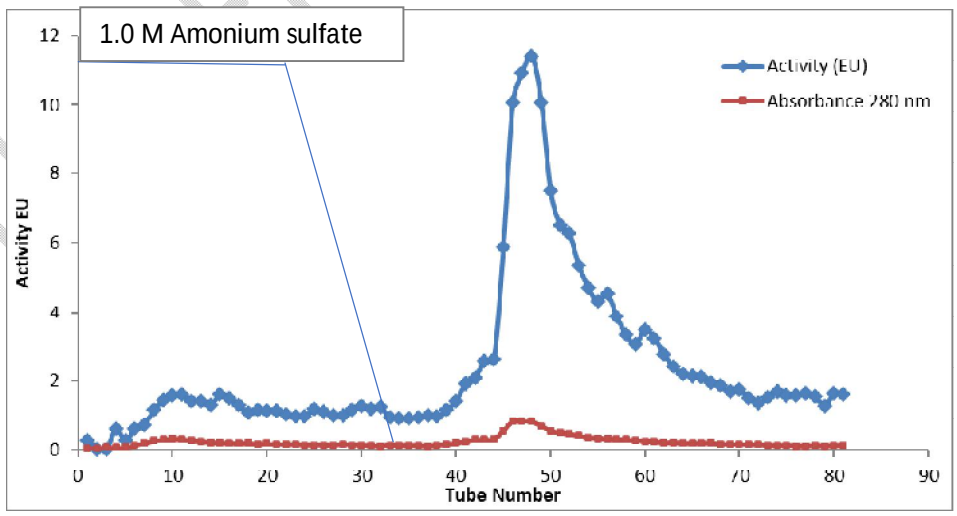

Fig. 1 Purification of mint *(Mentha piperita* L.*)* β-glucosidase by hydrophobic interaction chromatography. The enzyme activity and total protein amount collected in all eluents in the chromatography stage. Graphs were

created by processing the activity and protein amount data corresponding to the number of tubes collected. The decreasing ammonium sulfate gradient was applied as 1.0-0 M and shown as a straight line in the graph.

The purity of the mint β-glucosidase was visualized on SDS-PAGE. The molecular weight of the enzyme was approximately 65 kDa (Figure 2). It was reported that β-glucosidase purified from different plant sources was 68 kDa in cherry [29], 64 kDa in orange [20], 41 kDa in tea [15], 67 kDa in gentian plants [30]. The purified enzyme from vanilla was 50 kDa on SDS-PAGE; however, it was observed on the native PAGE at 201 kDa and reported tetramer [17]. Similarly, chayote β-glucosidase has been described as a 116 kDa protein composed of two 58 kDa subunits [19]. Romero-Segura et al. reported that olive β-glucosidase at 65.4 kDa [31], and similar Kara et al. reported it at 65 kDa [21]. The molecular weight of the mint β-glucosidase calculated at 65 kDa on the SDS-PAGE in our study is similar to the results of other β-glucosidase studies in the plants.

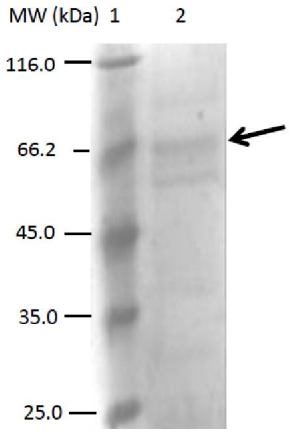

Fig. 2. SDS-PAGE image of the purified β-glucosidase from mint *(Mentha piperita* L.). Lane 1: Protein molecular weight marker Lane 1: molecular weight standards (β-galactosidase, 116 kDa; bovine serum albumin, 66.2 kDa; egg albumin, 45 kDa; lactate dehydrogenase, 35 kDa; Rease Bsp981 (E. coli), 25 kDa; β-lactoglobulin, 18.4 kDa; lysozyme, 14.4 kDa); lane 2: purified β-glucosidase.

Optimum temperature and pH

The reaction was carried out at temperatures changing from 25 to 70 °C to determine the optimum temperature for the enzyme. The effect of temperature on the peppermint β-glucosidase activity revealed the enzyme's maximal activity at 35 °C (Figure 3). The enzyme activity under pH values changing from 2.0 to 10 was determined using *p*-NPG as a substrate. The peppermint β-glucosidase activity was optimum at pH 6.0 (Figure 4). Different researchers have reported the optimum pH and temperature values for β-glucosidase 5.5 and 40 °C for tea [15], 5.5 and 42 °C for olive [21], and 5.0 and 40 °C for cherry [20], respectively. Bornova grape β-glucosidase had optimum pH of 5.0 and a temperature of 55 °C [34], while a different study on tea found isoenzyme A temperature of 40 °C and pH of 5.0 as optimum values [16]. The optimum temperature of mint β-glucosidase is lower than that of some plant β-glucosidases, while the optimum pH appears to be similar to β-glucosidases of various plants.

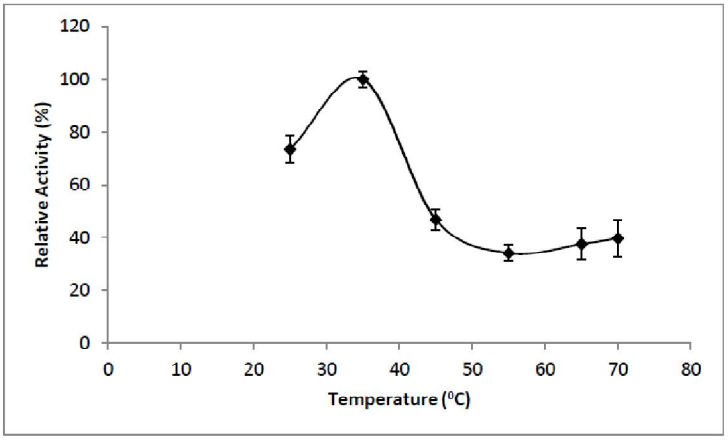

Fig. 3. Effect of temperature on purified β-glucosidase from mint (*Mentha piperita* L.)

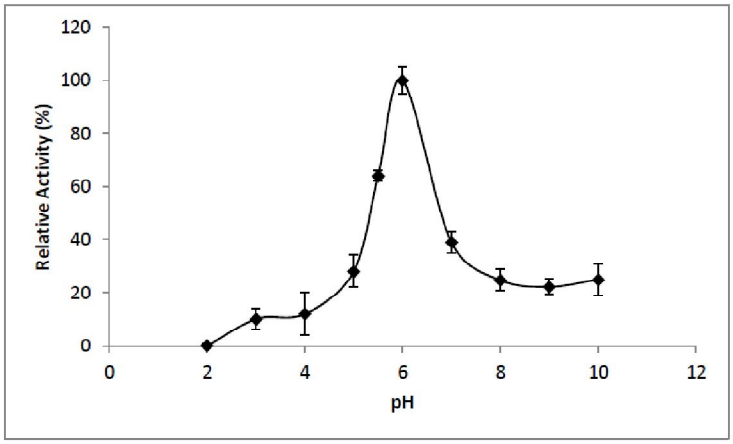

Fig. 4. Effect of pH on purified β-glucosidase from mint (*Mentha piperita* L.)

Kinetic Parameters of Substrates

The mint β-glucosidase kinetic parameters were determined over Lineweaver-Burk graphs with *p*/*o*-NPG substrates (Figure 5 A, B). The K_m and V_{max} values for *p*-NPG were 0.4 mM and 102.2 EU, respectively, while these values were 0.9 mM and 96.9 EU for the *o*-NPG substrate, respectively. These results show peppermint β-glucosidase has a higher affinity for the *p*-NPGlu substrate. The purified β-glucosidase from orange displayed the highest affinity for the *p*-NPG to other substrates [20]. Similarly, β-glucosidase purified from soybeans [18], vanilla [17], tea [15], olive [21], and white button mushroom [22] had a high affinity for the *p*-NPG substrate. However, contrary to these results, β-glucosidase purified from cherry fruits had a higher affinity for the *o*-NPG substrate [26]. In a study conducted on plum seeds, when *p*-nitrophenyl β-D-fucopyranoside (*p*-NPF) was used as a substrate, it

had an activity rate of 242.5% relative to the *p*-NPG substrate [35]. Kara et al. reported olive β-glucosidase enzyme's K_m and V_{max} values for the *p*-NPG substrate as 2.22 mM and 370.37 EU, respectively, and in the same study, the Km and V_{max} values for the *o*-NPG substrate were 14.11 mM and 48.54 EU, respectively [21]. In another study by Chen et al., β-glucosidase purified from plum seeds had K_m values of 3.09 mM and V_{max} value of 219.6 EU for the *p*-NPG substrate [32]. K_m and V_{max} values of the purified β-glucosidase white button mushroom were 1.751 mM and 833 U/mg using the *p*-NPG substrate, while they were 8.547 mM and 556 U/mg for the *o*-NPG substrate [22]. The mint β-glucosidase has a higher affinity for the *p*-NPG substrate than the *o*-NPG substrate.

Immobilization of β-glucosidase to magnetic nanoparticles improves the catalytic performance of the enzyme. [33]. Çelik et al. immobilized β-Glucosidase on the chitosan-MWCNTs carrier and investigated its flavor-enhancing effect in different tea samples. They detected aromatic precursors in tea samples, including black tea, yerba mate, green tea, balm tea, and sage, before and after treatment with immobilized β-glucosidase and showed the number of flavor compounds in all samples increased significantly [34]. In a similar study, β-Glucosidase was immobilized on the surface of nano-magnetic iron oxide, and its aroma and flavonoid aglycon enhancing effect in tea soup was investigated. Immobilized β-glucosidase treatment increased the flavor content of green tea and black tea preparations by approximately 16 and 48%, respectively. In green and black tea preparations, flavonoid aglycones, including myricetin, kaempferol, and quercetin, increased about 65 and 5 times, respectively [35]. In another study, β-Glucosidase and β-xylosidase were analyzed for their capability to improve the instant green tea flavor. Researchers found that β-glucosidase treatment significantly enhances floral and grassy features due to increases in geraniol, nonanal, and cis-3-hexene-1-ol [36].

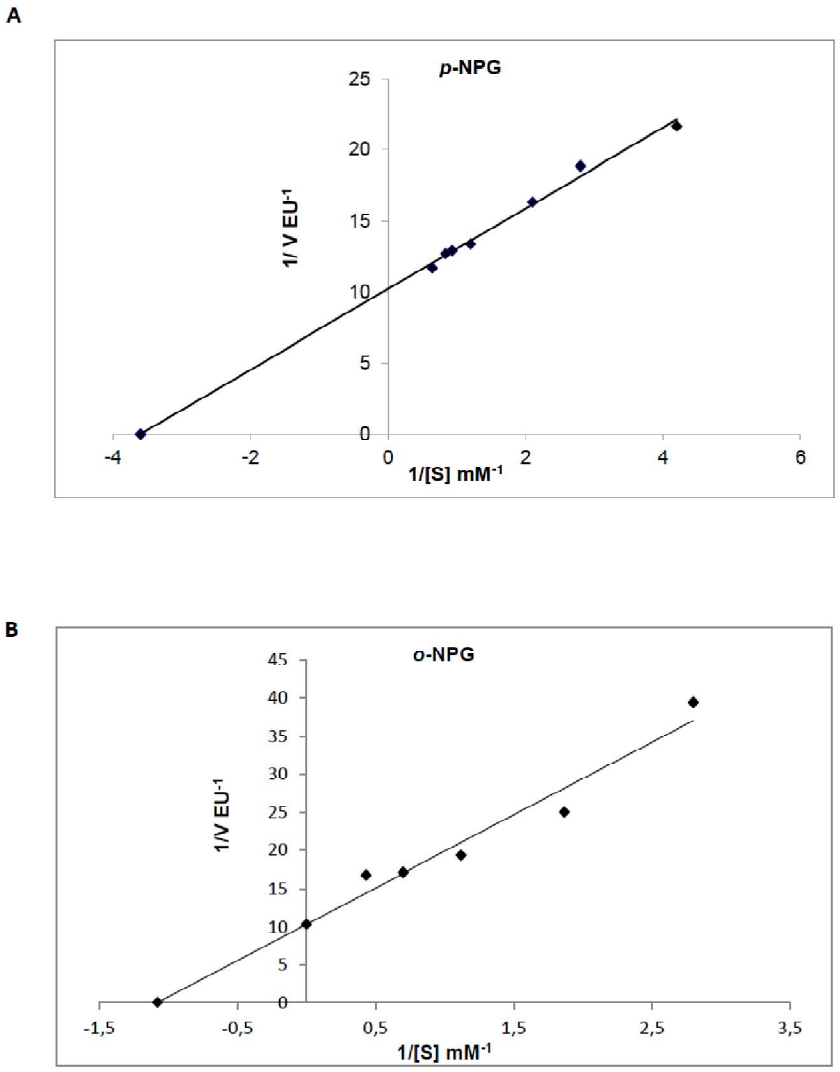

Fig. 5. Kinetic parameters of *p*-PNG and *o*-NPG substrate Lineweaver-Burk plots. **A.** Graph when using *p*-NPG as substrate. **B.** Graph when using *o*-NPG as substrate.

Effects of Heavy Metals

The effects of some heavy metals, including Ag^{+2}, Fe^{+3}, Zn^{+2}, Cu^{+2}, and Pb^{+2} on purified mint β-glucosidase activity are shown in Table 2. The relative activities of these heavy metals were calculated to determine their effects on the purified enzyme activity. Heavy metal ion concentration in the reaction medium was 0.75 mM, and relative activities were calculated against the non-inhibitor enzyme activity. When the effects of Fe^{+3}, Ag^{+2}, Pb^{+2}, Zn^{+2}, and Cu^{+2} on purified peppermint β-glucosidase were investigated, their relative activities were 117, 78, 76, 31, and 22%, respectively. While Fe^{+3} increased the enzyme activity, Cu^{+2} inhibited the enzyme most strongly among the investigated substances. Cameron et al. found that $FeCl_3$ did not affect the purified β-glucosidase activity from orange, and they reported that the relative activity of 1 mM $FeCl_3$ was 100% (20). Fe^{+3} increased β-glucosidase activity in olive

fruits [21]. Yu et al. reported the relative activities of Cu^{+2} and Ag^{+} with 5 mM concentration as 3 and 0%, respectively, on apple seed β-glucosidase [29]. Chen et al. reported that heavy metals with 1 mM concentration in the reaction medium of the β-glucosidase purified from plum seed had relative activities of 63.2, 64.2, and 0% for Cu^{+2}, Zn^{+2}, and Ag^{+} respectively [32]. We determined that Zn^{+2} inhibited the enzyme, but Zn^{+2} metal with 1 mM concentration had a relative activity rate of 112.0% and activated the citrus β-glucosidase [20]. Different results have been reported in the literature on the effects of Zn^{+2} on β-glucosidases. Cu^{+2} inhibited mint β-glucosidase strongly in our study, and the inhibitory effect of Cu^{+2} on plant β-glucosidases activity has also been reported in studies such as vanilla bean and apple seed, and olive [17, 29, 31]. In the study investigating the effects of Ag^{+} on soybean β-glucosidase activity, the relative activity of 1 mM of $AgNO_4$ was 35%, while 10 mM of $AgNO_4$ had a relative activity of 0% [18]. The relative activities of Ag^{+} were reported as 112 and 29%, at 0.1 mM and 1 mM, respectively, on vanilla β-glucosidase activity. We found that the relative activity of Ag^{+} on mint β-glucosidase activity was 78% at 0.75 mM, similar to the vanilla β-glucosidase [17].

Table 2. Effect of some heavy metal ions on purified β-glucosidase activity from mint *(Mentha piperita L.)*

Substance	[I] mM	% Relative Activity
Without inhibitor	0.00	100.0
Fe^{+3}	0.75	117
Cu^{+2}	0.75	22
Ag^{+2},	0.75	78
$Pb+^{2}$	0.75	76
Zn^{+2}	0.75	31

Effects of Inhibitors

The inhibition kinetic evaluation of the enzyme was performed by plotting the Lineweaver-Burk graph. The *p*-NPG was a substrate, while glucose and δ-gluconolactone were inhibitors in this experiment. Figure 6 A shows the Lineweaver-Burk graph plotted at 0.1 and 0.2 mM glucose concentrations, and Figure 6 B shows the Lineweaver-Burk graph drawn based on the δ-gluconolactone concentrations of 0.05 and 0.25 mM. The mint β-glucosidase activity was inhibited both by glucose and δ-gluconolactone with K_i values of 0.034±0.001 and 0.038±0.001 mM, respectively. Both glucose and δ-gluconolactone were found to be as competitive inhibitors.

We observed that glucose and δ-gluconolactone strongly inhibited the β-glucosidase from the mint. Glucose competitively inhibited the mint β-glucosidase activity with *a* K_i value of 0.034±0.001 mM (Figure 6A) towards *p*-NPG as substrate. Purified β-glucosidase from white button mushroom, sweet chery and Arabidopsis thaliana

were not inhibited by glucose [22, 26, 37], but the purified enzyme from orange was strongly inhibited by glucose [20].The inhibitor effect of glucose on β-glucosidases from various plants and microorganisms has been extensively studied since inhibition of β-glucosidases by glucose is not desirable when enzymatic hydrolysis of cellulose is carried out as an industrial process. Glucose did not inhibit the β-glucosidase from rice, vanilla bean, and Arabidopsis at concentrations up to 2 M [17, 37, 38]. In contrast, glucose at lower concentrations effectively inhibited the enzyme from orange [20]. The δ-gluconolactone inhibited the mint β-glucosidase competitively with a K_i value of 0.038±0.001 mM (Figure 6B). The potent inhibitory effect of δ-gluconolactone agrees with the other studies that investigated the inhibition of β-glucosidases from plant sources [17, 21, 22]. The survey conducted on vanilla reported that δ-gluconolactone inhibited β-glucosidase competitively with a K_i value of 670 µM. Our result agrees with the report by Odoux et al. [17].

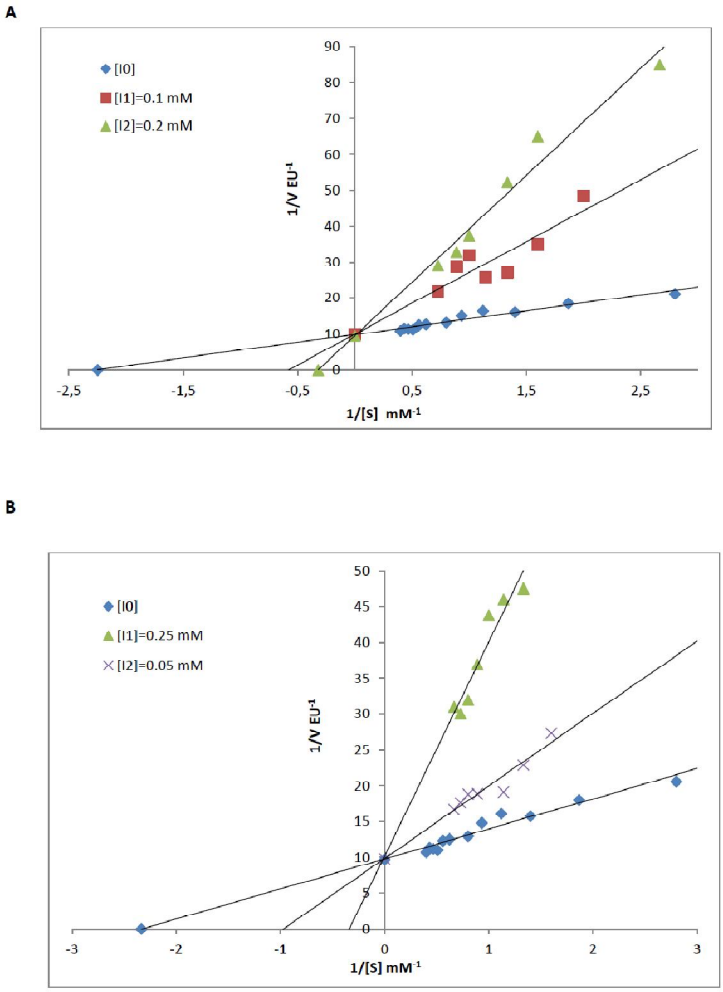

Fig. 6 Inhibition of purified β-glucosidase from mint (*Mentha piper*ita L.) by glucose and δ-gluconolactone **A.** Lineweaver-Burk plot with various substrates (*p*-NPG) and glucose as inhibitor concentrations to determine the inhibition type and K_i value **B.** Lineaveawer-Burk plot with various substrates (*p*-NPG) and as inhibitor δ-gluconolactone concentrations to determine the inhibition type and K_i value

4. Conclusion

Consequently, β-glucosidase was purified from the mint in this study, and some of its biochemical properties were determined. Determination of the biochemical properties of this enzyme, a plant with commercial and pharmaceutical importance because of its phenolic content, will benefit advanced studies such as enzyme immobilization. Considering the usage of exogenous enzymes in wine and fruit juice production, we believe our results may be helpful in biotechnology and food biotechnology. It will be safer to obtain enzymes used in food and feed processing from a source used as food rather than a microorganism.

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