PAPER

IMMOBILIZATION AND CHARACTERIZATION OF β-GLUCOSIDASE FROM GEMLIK OLIVE (*OLEA EUROPEA* L.) RESPONSIBLE FOR HYDROLIZATION OF OLEUROPEIN

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ABSTRACT

The β-Glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) enzyme was purified from Gemlik variety olive (*Olea europea* L.). The purified enzyme was immobilised onto Supermagnetic Nanoparticles in order to stabilise the enzymatic efficiency and increase usage in the food industry. The purified and immobilised enzyme was characterised by molecular weight, kinetic parameters and optimum pH and temperature values comparatively. The enzyme is a monomer with a mass of approximately 40 kDa. The Kinetic values of the immobilised and purified enzymes were 1.34 mM and 384.61 U/mg; 0.37 mM as Km and 370.37 U/mg as Vmax respectively.

Keywords: β-glucosidase, *Olea europea* L., pNPG, purification, SPMN

1. INTRODUCTION

Olive is a fruit which consists a lot of biotransformal compounds. There is a wide variety of phenolic compounds in *Olea europaea L.* which are important for sensorial properties. They also have substantial effects on human health such as nutritional, physiological and pharmaceutical effects. Unripe olive fruit has important phenolic secoiridoids causing bitter taste. These polyphenolic substances called oleuropeins have many aldehydic or dialdehydic forms such as hydroxytyrosol and tyrosol, transformed by *β*-glucosidase as a part of the defence mechanism in the plant tissue (GARCIA-RODRIQUEZ *et al.*, 2011; DE LEONARDIS *et al.*, 2015). As a result of this system, many oleuropein-related compounds are also available from olives, rearranged via aglycon by the elenolic acid ring. The quality and quantity of these substances change by variety, tissue by tissue (leave, fruit, etc.) and in terms of ripening stage (Bianchi, 2003). Olives gradually get rid of their bitterness at the ripening stage. This occurs by *β*-glucosidase gradual hydrolyzation of oleuropein and leads to changes in taste (GUIRIMAND *et al.*, 2010).

β-glucosidases are biologically active enzymes that hydrolyse 1.4 *β*-glycoside bonds between carbohydrate molecules (ÜNAL and SENER, 2017). Olive β-glucosidases (DE LEONARDIS *et al.*, 2015) (*β*-D-glucoside glucohydrolases, EC 3.2.1.21) show high substrate specificity (MAZZUCA *et al.*, 2006) as in other plant species (SAVAS *et al.*, 2018). They hydrolyse the *β*-glycosidic bonds in oligosaccharides or other glucose moieties and ester bonds in oleuropein, which is responsible for bitterness (VELÁZQUEZ-PALMERO *et al.*, 2017).

After the reaction, *β*-glucosidases lose their catalytic activities like other enzymes. Some techniques may be used in food processing systems (debittering, flavour enrichment *etc.*) to stabilise enzyme usage. Enzyme immobilisation by covalent bonding is used for stability and reuse of enzymes. For this purpose, different bulk or magnetic materials could be used as a matrix.

Superparamagnetic iron oxide nanoparticles (SPMN) that are used for high colloidal stability, magnetism and be biocompatible materials are preferred as immobilisation matrices (MA *et al.*, 2009). These are small synthetic Υ-Fe_2O_3 or Fe_3O_4 particles with a core size of < 10 nm and well dispersed in a liquid for biomedical applications. They may be removed easily with simple magnetism from the reaction medium.

Gemlik variety olives are from the north-west Turkey (UYLAŞER and ŞAHIN, 2004). This variety, which are processed olives for direct consumption has high oil content.

Considering the rare reports on the topic indicates the catalytic activity of olive β-glucosidases. The objective of this study is to show that Gemlik variety olive fruits could be used as a one β-glucosidase source for biotechnological application in the food industry. In this context, purified and immobilised forms of *β*-glucosidases (*β*-D-glucoside glucohydrolase) were investigated as a naturally occurring enzyme involved in the biotransformation of oleuropein.

2. MATERIAL AND METHODS

2.1. Materials

Gemlik variety olives that were used in our study were obtained from Balikesir at their black maturity stage. They were brought to the laboratory in cold storage conditions

(+4°C). After the washing and sorting steps, they were used for obtaining acetone powder as the enzyme source.
All chemicals that were used in our study were supplied from Sigma-Aldrich (St. Louis, MO, USA), and protein molecular weight markers were supplied from Thermo Scientific (Waltham, MA, USA). They were of the highest grade available.

2.2. Preparation of acetone powder

Acetone powder was used as the enzyme source in this study (SAVAS *et al.*, 2018). 100 g of olive pulp was homogenised for 2 min in 750 mL of cold acetone (-20°C) using a homogenizer for preparation. The homogenate was filtered by Whatmann No 1 filter paper, and retentate was extracted three times with 500 mL of acetone (−20°C) to remove oil residues. Reddish purple residues on the filter were air-dried at room conditions on blotting papers and held at -20°C for enzyme assays. 2 g of the acetone powder was homogenised in 100 mL of a cold extraction buffer (4°C) (pH 9.5) using an Ultra Torrax homogenizer. The mixtures were centrifuged at 15,000 rpm for 30 min at 4°C, and crude extracts were obtained from the supernatant.

2.3. Chromatographic Study

The further step of enzyme purification was carried out based on the method by KARA *et al*. (2011) by Hydrophobic Interaction Chromatography. As defined in the method, the solid ammonium sulphate in concentrations from 0 to 50% was added to the crude extract at +4°C for ammonium sulphate precipitation. The reaction mixture was centrifuged at 15000 rpm for 30 min (+4°C), the sediment was dissolved in 50 mM of the sodium phosphate buffer (pH 6.8), and the final saline concentration of the mixture was set to 1M ammonium sulphate.
The hydrophobic column was synthesized using 10% CNBr in a 1:1 solution of Sepharose 4B and distilled water for the second step of the purification process. The pH of the mixture was stabilised at 11 for 8–10 min. The gel obtained was filtrated and washed with a cold 0.1M $NaHCO_3$ buffer (pH 10). After the reaction mixture was combined with the saturated L-tyrosine, the solution was stirred for 90 min. After the gel, washing and diazotization of 1-naphthylamine in this complex was fixtured to the sepharose-4B-l-tyrosine. The further steps were carried out as described in the method by 3 mL of enzyme solution loaded onto the hydrophobic column. 1 mL fractions were gathered at a flow rate of 30 mL/h in a linear gradient. The fractions were collected with the highest protein content and used in next studies as the purified enzyme.

2.4. Immobilisation

Superparamagnetic nanoparticles (SPMN) were synthesized specifically for the use of enzyme immobilization (KOCKAR *et al.*, 2010). 20-100 mg of $Fe^{+2/+3}$ superparamagnetic nanoparticles (SPMN) was placed into 2 ml of a 0.003 M phosphate buffer (pH 6) with 0.1 M of NaCl, and 0.5 ml of carbodiimide solution (0.025 g/mL in buffer) was placed into the reaction medium. The reaction medium was sonicated for 10 minutes. 2 ml of purified β-glucosidase enzyme was added and sonicated for 30 minutes.

2.5. Characterisation assays and protein determination

In all steps for olive *β*-glucosidase extraction, purification and further studies, activity of the enzyme was measured at 410 nm against para-nitrophenyl-*β*-D-glucopyranosides (p-NPG) as substrate (LOWRY *et al.*, 1951). 70 μL of enzyme solution in 50 mM sodium acetate (pH 5.5) and 70 μL of substrate were added in a 96 well plate. Incubation of the well content was facilitated at 37◦C for 30 min in triplicates. 70μL of 0.5M Na_2CO_3 was added into the medium for stopping the reaction, and the absorbance values were determined by spectrophotometry. Enzyme activity was expressed as μmol p-nitrophenol composed per minute in the reaction medium under these terms. Molecular mass values of protein were estimated using a commonly used standard (bovine serum albumin - BSA).
SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to estimate the molecular weight of olive *β*-glucosidase according to the method reported by LI *et al. (*1997) using a Minigel system (Bio-Rad Laboratories, USA). After gel colorization with Coomassie brilliant blue R-250 and decolorization with 7.5% acetic acid in 5% methanol to detect protein bands, the gel was photographed with UV Light.
25 mM sodium acetate (2.0–10.0) buffers were used for the pH optima assays (KARA *et al.*, 2011). Activity measurements were achieved by using 5 mM of substrate (pH 5.5) at the temperature range of 25 to 65ºC for 30 min to determine the optimum temperature. The thermal stability was determined by incubating at 70 for 30 min and then cooling down to 4 °C.
p-NPG (concentration range from 0.12-2.38 mM) and oleuropein were studied for determining *K*m and *V*max values. Glucose, citric acid, lactic acid, sodium hydroxide and sodium chloride were studied as potential inhibitors. Activity measurements of the samples were performed, and 1.75 mM of pNPG and 1 mM of Ni^{+1}, Zn^{+1}, Cr^{+1}, Mn^{+2}, Mg^{+2} and Co^{+1} were used in the reaction medium. The effect of various concentrations (0.0029-0.0297 mM) of Deltamethrin, Chlorpyrifos and Alphacypermethrin as widely used pesticides against olive insects on olive β-glucosidase were also studied using 1.25 mM of pNPG as the substrate. Results are given as relative activity, which the enzyme activity in the non-inhibitor medium is considered 100. The inhibitor concentration, which reduces the enzymatic activity by 50% (IC_{50} values), was determined by the relative plots.
A Fourier Transform-Infrared Spectroscopy (FT-IR) analysis was performed to prove the correctness of the enzyme immobilisation after the purification step. IR spectra of Fe_3O_4 superparamagnetic nanoparticles, β-glucosidase and immobilised β-glucosidase on the superparamagnetic nanoparticles were obtained by using the KBr pellets preparation technique in ATR cells (600-4000cm^{-1}) with a Perkin Elmer-1600 Series device.

3. RESULTS AND DISCUSSION

3.1. Enzyme Extraction and Purification

Protein assays were achieved by using acetone powder as previously reported (KOUDOUNAS *et al.*, 2015). Although it is known that the use of acetone leads to mutual effects, thanks to usage of acetone, it is possible to obtain concentrated proteins without pigments derived from fresh fruits (ROMERO-SEGURA *et al.*, 2009). Furthermore, acetone powder usage also makes it possible to use as stock enzyme source in the absence of the olive fruit.

Hydrophobic interaction chromatography (SAVAS *et al.*, 2018) was used by precipitation with ammonium sulphate to separate *β*-glucosidase from the olive acetone powder. After precipitation of the *β*-glucosidase active fractions with ammonium sulphate, 97% of the activity was measured (Table 1). In this part of the process, great proteins except *β*-glucosidase were removed, and the quantity of the protein was reduced from 297 to 23 mg.
The elution pattern of enzyme activity and total protein concentrations for all fractions that were collected on the hydrophobic column are shown in Fig. 1. The fractions that had the highest enzyme activity were pooled. The enzyme was purified 163-fold from the remaining particles with clear homogeneity with an overall enzyme yield of 9.90% and a specific activity of 6291.7777 U/mg (Table 1).
The purification yield values were higher than those previously reported for olive (LI *et al.*, 2005; MAZZUCA *et al.*, 2006) and several sources (CAMERON *et al.*, 2001; LI *et al.*, 2005; VERMA *et al.*, 2011). Minimal sequential steps, matrix and ligand characteristics (hydrophobic structure of 1-napthylamine, sepharose-4B gel matrix and l-tyrosine arm) led to increased purification factors. More purification steps could result in better purification rates. However, more steps cause a dramatic decrease in enzyme activity and protein amounts.

Table 1. Purification of β-glucosidase from olive (*Olea europaea* cv. Gemlik).

Purification steps	Volume (mL)	Total Protein (mg)	Total Activity (U)	Specific activity (U/mg)	Yield (%)
Crude extract	40	297.27	11430.88	38.4528	100
Ammonium sulphate	10	23.19	11123.31	479.6597	97.30
Hydrophobic chromatography	2	0.18	1132.52	6291.7777	9.90

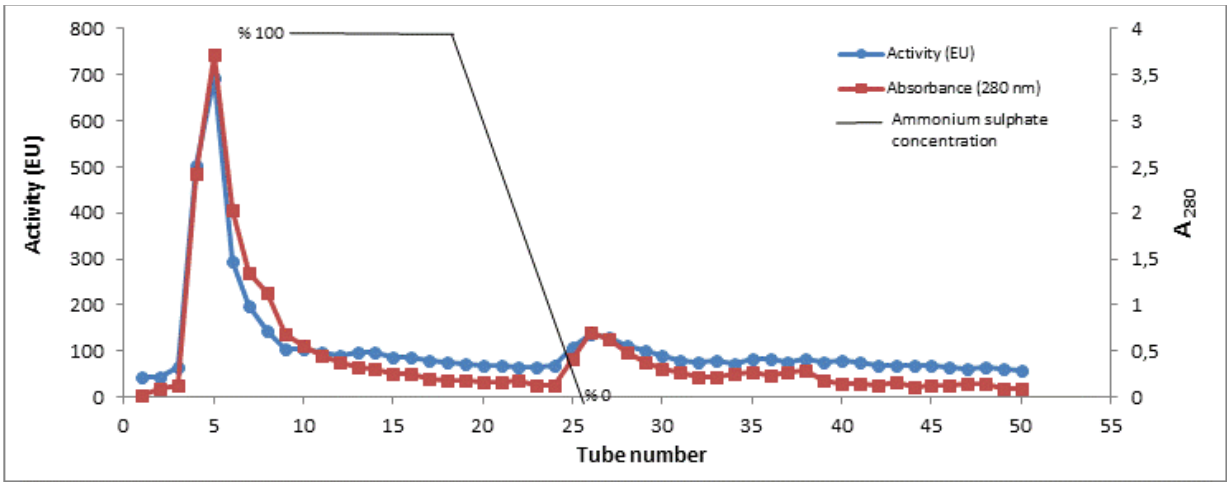

Figure 1. Purification of olive fruit β-glucosidase by hydrophobic interaction chromatography. The enzyme activity and total protein concentrations were determined from all fractions that were collected, as described in Section 2. The enzyme activity was expressed as *μ*mol of p-/o-nitrophenol liberated per minute in the reaction.

A single band with an apparent molecular mass of ca. 42 kDa was seen by standard methods of SDS-PAGE electrophoresis (Fig. 2). The β-glucosidase of the olive is a

monomer like other plant sources e.g. tea, citrus. There are many β-glucosidase results reported as monomer and oligomer from different plant sources e.g. 68 kDa from Rauvolfia serpentina, 92 kDa (VERMA *et al.*, 2011), 37 kDa from tea leaves (LI *et al.*, 2005), 65 kDa from almond (HE and WITHERS, 1997), from sweet cherry fruit (Prunus avium L.) (GERARDI *et al.*, 2001) and 55 kDa from Citrus sinen-sisvar. Valencia (KAYA, 2014). Estimated molecular mass of β-glucosidase from olive was reported previously as 55-65.5 kDa (ROMERO-SEGURA *et al.*, 2009; KARA *et al.*, 2011; KAYA, 2014). These different results indicate the molecular mass of β-glucosidases from olives depending on variety.

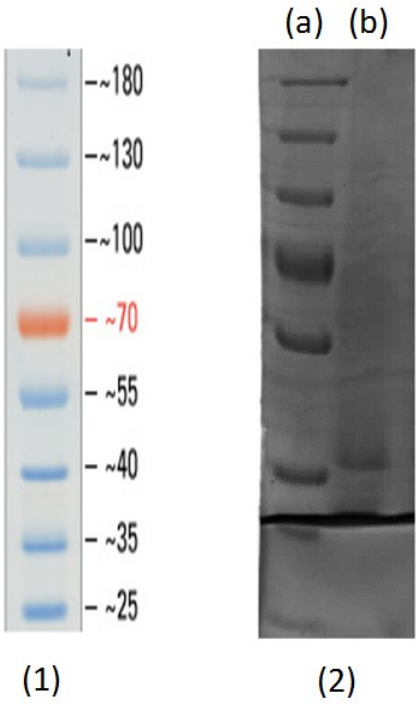

Figure 2. SDS-PAGE analysis of the β-glucosidase purified from olive (*Olea europaea* cv Gemlik) fruit. The enzyme was electrophoresed at pH 8.3 on a 12% polyacrylamide gel and stained with Coomassie brilliant blue R-250. Lane 1: molecular weight standards (β-galactosidase, 116kDa; bovine serum albumin, 66.2kDa; egg albumin, 45kDa; lactate dehydrogenase, 35kDa; Rease Bsp981 (E. coli), 25kDa; β-lactoglobulin,18.4kDa; lysozyme, 14.4kDa); Lane 2: purified β -glucosidase.

3.2. Characterisation of enzyme sources

The activities of purified and immobilised olive β-glucosidase in different pH are shown in Fig. 3. The optimal pH was found at 5.5 for both enzymes. The purified enzyme showed higher activity relatively in the range of pH 4.5-6 with any activity at pH 2 and 8.
The optimal pH values of β-glucosidases that were determined in previous studies from olive fruit (ROMERO-SEGURA *et al.*, 2009; KARA *et al.*, 2011; KOUDOUNAS *et al.*, 2015), from citrus (CAMERON *et al.*, 2001), from wheat (SUE *et al.*, 2000a) and rye (SUE *et al.*, 2000b) were similar and higher than β-glucosidases from rice (pH 4.5) (AKIYAMA et al., 1998), soybean (pH 4.5) (MASARU *et al.*, 1995), barley (pH 5.0) (LEAH *et al.*, 1995) and lower than vanilla bean (pH 6.5) (ODOUX *et al.*, 2003) and maize (pH 5.8) (CUEVAS *et al.*, 1992).

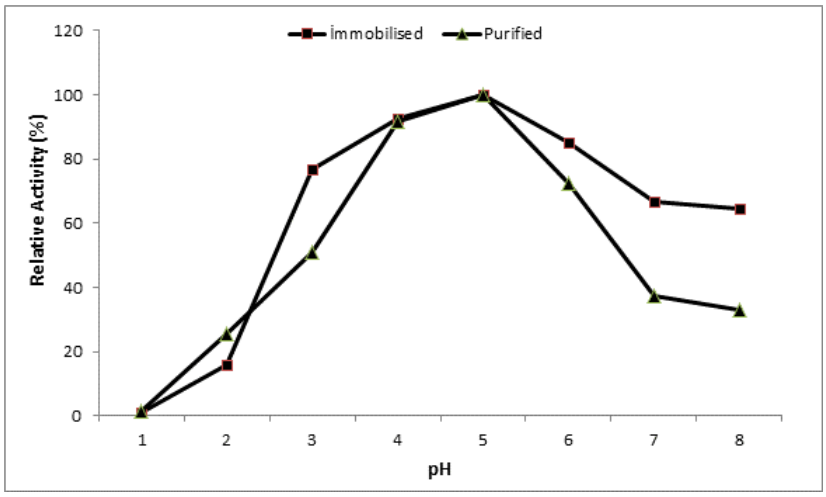

Figure 3. Effect of pH on activity of purified olive (*Olea europaea* cv Gemlik) fruit β-glucosidase. pH optima of β-glucosidase purified from olive (*Olea europea* L.) and immobilised form.

The optimum temperature was determined in the purified and immobilised enzyme sources respectively as 50 and 55°C by using p-NPG as a substrate (Fig. 4A). Temperature optima of the immobilised enzyme were higher than the free enzyme by 5°C like other immobilised enzymes (SINGH *et al.*, 2011). The enzyme became more stable after the immobilization process by means of the vineyard structures. The enzyme purified from olive fruit showed maximum activity at 50°C and 35°C with p-NPG and oleuropein as substrates, respectively (Fig. 4B). The purified enzyme lost its catalytic activity at the end of the 30th min at 70°C with p-NPG as a substrate in the 50 mM acetate buffer (Fig. 4C).
It was reported that plant β-glucosidase showed maximal hydrolytic activity towards p-NPG at 40–45°C in Citrus sinensis var. Valencia fruit (CAMERON *et al.*, 2001), 25–30°C in rye (SUE *et al.*, 2000b), 45°C in soybean (MASARU *et al.*, 2005), 50°C in rice (AKIYAMA *et al.*, 1998), 60°C in barley (LEAH *et al*, 1995) and 40°C in vanilla bean (ODOUX *et al.*, 2003). The temperature optima of our enzyme were similar to that for β-glucosidases taken from tea (LI *et al.*, 2005), rice (AKIYAMA *et al.*, 1998) and maize (CUEVAS *et al.*, 1992) using p-NPG as a substrate. The enzyme was still active by 33% at 4°C and 11% of the initial level at 25°C after 8 weeks (data not shown). This temperature-sensitive enzyme was more stable at cold conditions.

3.3. F-TIR analysis

The F-TIR charts indicated patterns of purified and immobilised olive β-glucosidase on to SPIONs, Fe_3O_4 Superparamagnetic Nanoparticles (SPMN) and processed SPIONs (Fig. 5). The peak of the enzyme at 1400cm^{-1} was lost on the SPMN activated by carbodiimide (without enzyme) and enzyme-bond SPIONs, while the new peak occurred in the range of 1000-1100 cm^{-1}. It was concluded according to the IR spectrum that, the new bond indicated SPMN-carbodiimide activation. In the enzyme bond SPMN pattern, the characteristic peaks revealed that bonding of the enzyme onto nanoparticles took place.

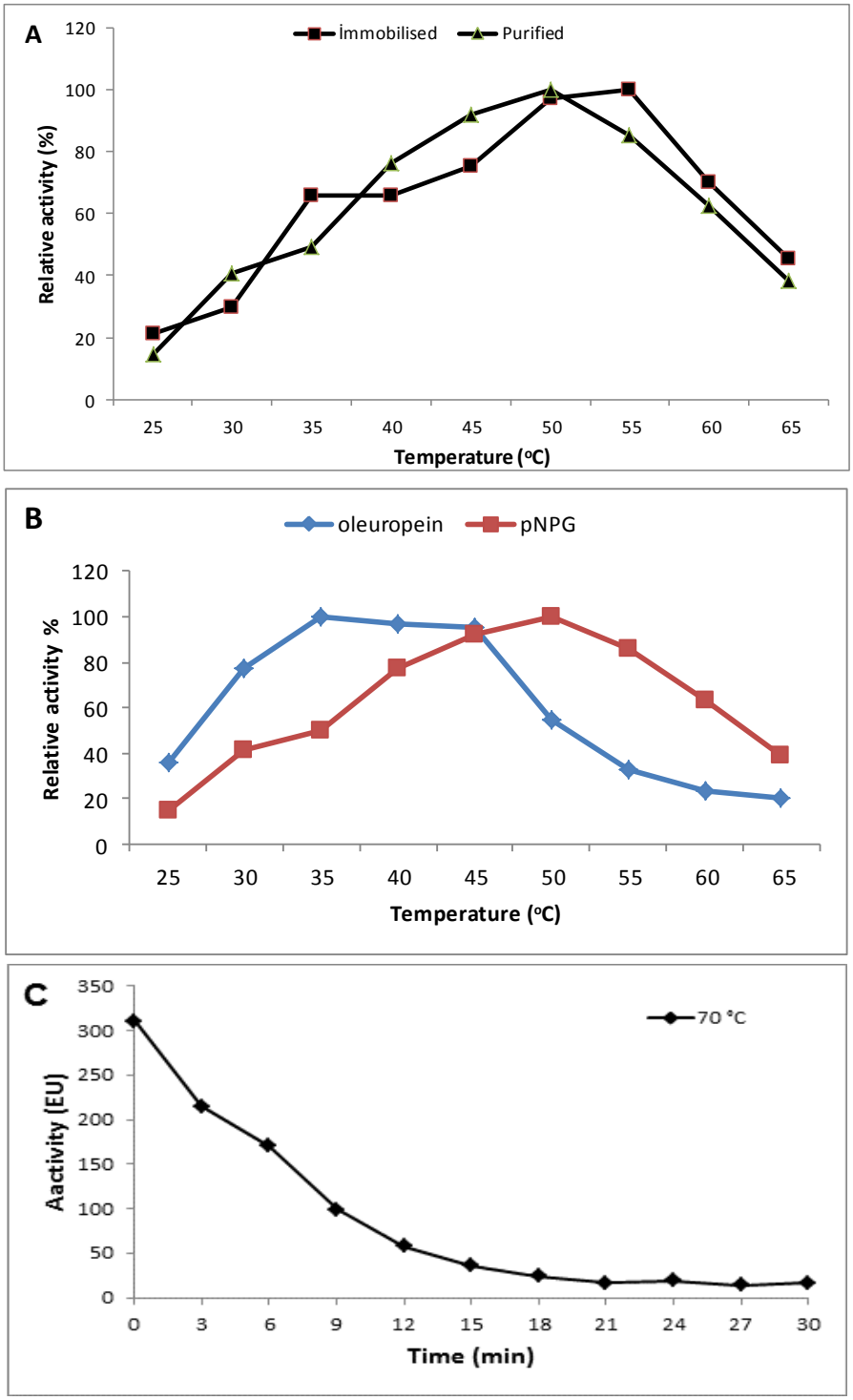

Figure 4. Effect of temperature on olive β-glucosidase activity. Temperature optima of A) β-glucosidase purified from olive (*Olea europea* L.) and immobilised form using p-NPG as the substrate, and B) purified enzyme using different substrates. C) Thermal stability of purified olive β-glucosidase by using p-NPG as the substrate.

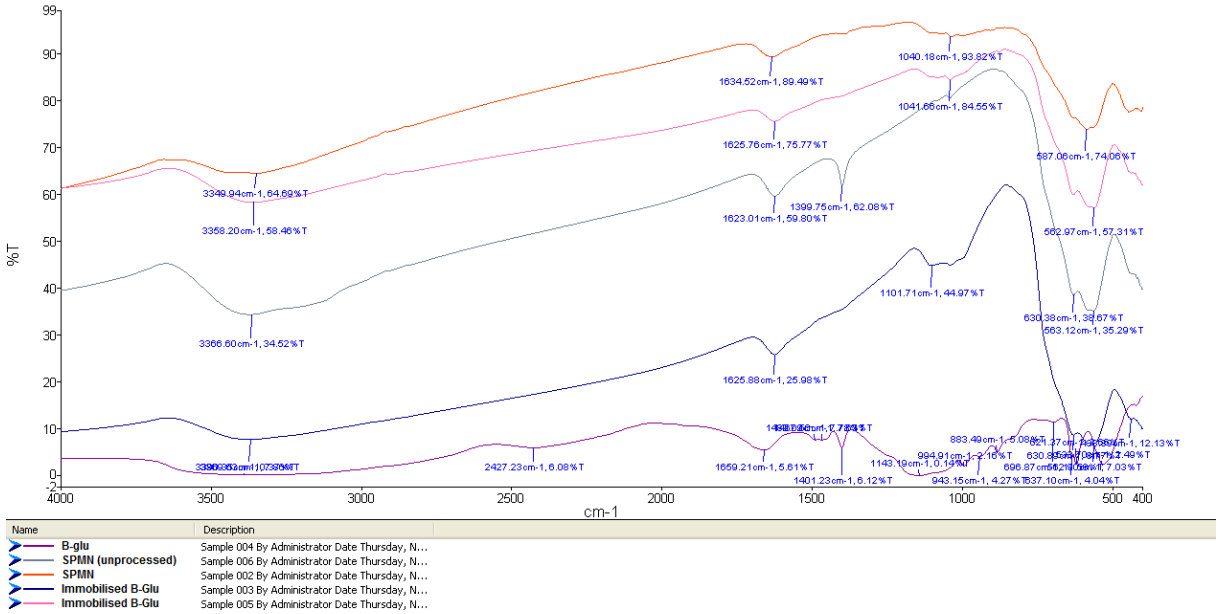

Figure 5. FT-IR spectra of immobilization support material, pure enzyme and enzyme bound nanoparticle.

3.4. Substrate specificity

Enzyme kinetics were studied using p-nitrophenyl-β-d-glucopyranosides (p-NPG) and oleuropein as substrates. The β-glucosidase from olive tissues activates oleuropein by converting the secoiridoid glucoside moiety of the oleuropein when the tissues are damaged by insects or herbivores (KONNO *et al.*, 1999). Olive fruit β-glucosidases that were able to hydrolyse olive glucosides exhibited high substrate specificity to oleuropein (ROMERO-SEGURA et al., 2009). The enzyme was effectively active on p-NPG and oleuropein with the *K*m values of 0.37 and 1.7 mM and the *V*max values of 370.3 and 1000 U/mg, respectively (Table 2). Although the affinity of the olive β-glucosidase for p-NPG was considerably higher than for oleuropein, the activity was lower. The higher β-glucosidase affinity for p-NPG was reported on Sorghum (CICEK and ESEN, 1998), tea leaves (LI *et al.*, 2005), orange (CAMERON *et al.*, 2001) and olive (KARA *et al.*, 2011).

Table 2. Kinetic parameters of purified and immobilised β-glucosidase from olive with different substrates.

β-glucosidase	Substrates	*V*max(U)	*K*m	*V*max/*K*m
Purified	p-NPG	370.37	0.37	1001
	Oleuropein	1000	1.7	588.23
Immobilised	p-NPG	384.61	1.34	287.02
	Oleuropein	2000	6	333.3

3.5. Effects of inhibitors

β-glucosidases have been researched with reversible and irreversible inactivators (HE and WITHERS, 1997; REMPEL and WITHERS, 2008). The purified enzyme was incubated in the presence of glucose, lactic acid, sodium hydroxide, sodium chloride, citric acid, some pesticides and Mg^{2+}, Mn^{2+}, Ni^{1+}, Co^{1+}, Cr^{1+}, Zn^{1+} ions using p-NPG as the substrate (1.66 mM) to determine the inhibition kinetics. Although a strong inhibitory effect could not be detected at the studied concentrations, citric acid was more effective with IC_{50} of 2.38 mM than glucose with IC_{50} of 23.33 mM and lactic acid with IC_{50} of 145.7 mM (Fig. 6A-F).

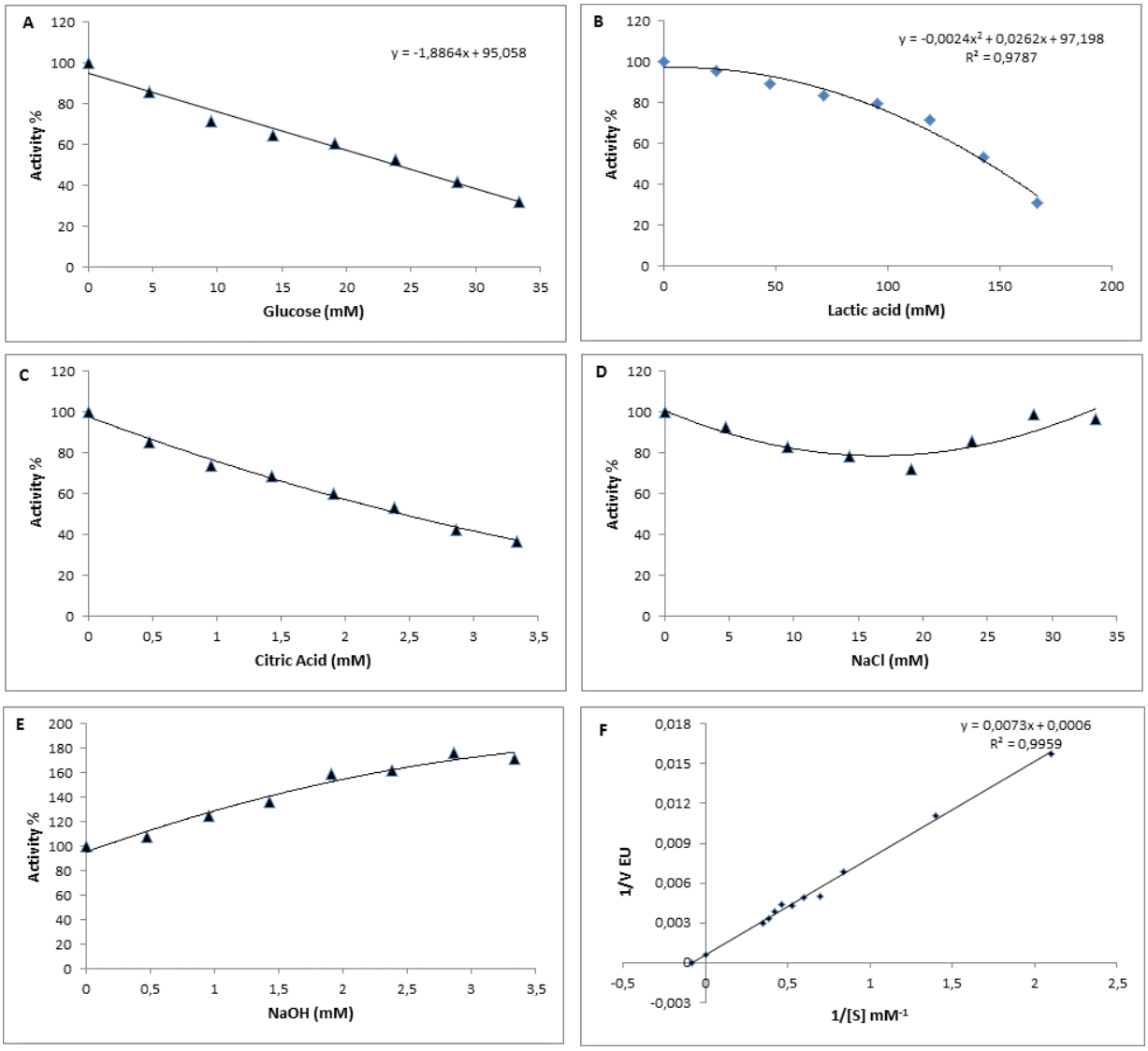

Figure 6. Inhibition of the β-glucosidase purified from olive (*Olea europaea* L.) fruit by p-NPG Activity (%) curve in the presence of different A) Glucose, B) Lactic acid, C) Citric acid, D) NaCl and E) NaOH concentrations, F) Lineweaver–Burk plot with various concentrations of p-NPG for IC_{50} .

It was reported that citric acid is the main organic acid followed by succinic acid in Turkish olive varieties (ASLAN and OZCAN, 2011). It is the most commonly used agent in

olive fermentation for initial acidity in the fermentation medium. It is contemplated that the concentration of the citric acid in the reaction medium will result in a lower pH level than the pH optima of the enzyme.
Glucose is the main compound for enzymatic saccharification of cellulolytic substrates and presents as fermentable sugar in the reaction medium. Similar inhibitory effects of glucose have been reported for β-glucosidase in olive in a previous study (KARA *et al.*, 2011; SAVAS *et al.*, 2018), whereas in another study, it was stated that no inhibitory effect of glucose was determined (ROMERO-SEGURA *et al.*, 2009). Differences in the sequence of amino acids found in the structure of plant β-glucosidases reflect observed quaternary structures and change in the properties of the active site (YU *et al.*, 2007). It is thought that, as a result of this, the kinetic parameters will be changed. These differences in olive β-glucosidases, whose molecular weights and other kinetic parameters are different from each other, are due to the differences in protein structures depending on the variety. It is necessary to study the kinetic parameters of olive β-glucosidases of different varieties and at different maturity levels for future studies.
Generally, plant origin β-glucosidases were reported to be resistant to high glucose concentrations (RAMANI *et al.*, 2012). Unlike other chemicals that are used, the observed slight activation by NaCl and NaOH may be explained by the ions' effect of Na^{+} on enzyme structure stabilization.
β-glucosidases were reported as metalloprotein and required metal ions for action (RAMANI *et al.*, 2012). The inhibition kinetics were determined in the presence of Mg^{+}, Mn^{+}, Ni^{+}, Co^{+}, Cr^{+} and Zn^{+} to verify the effects of several metal ions on olive β-glucosidase activity (Fig. 7).

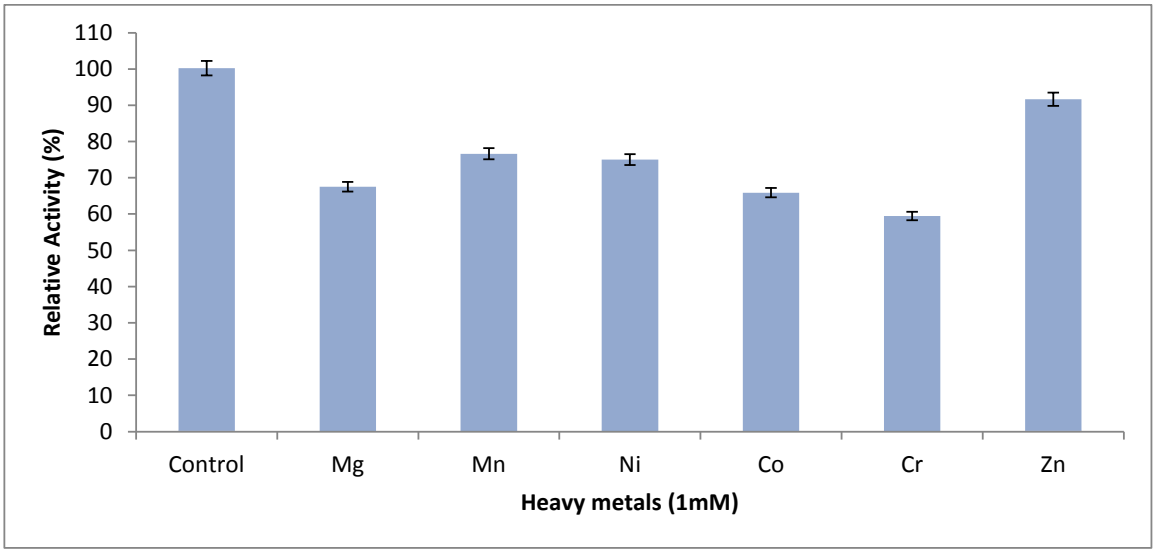

Figure 7. The effects of some heavy metal ions on the activity of β-glucosidase purified from *Olea europea L.* The crude enzyme activity was indicated as the control. The activity of the control was accepted as 100%. Trials were achieved by three replicates.

Metal ion concentration in this study was in the range from 1 to 1.75 mM. The relative enzyme activity was 67.36, 76.46, 74.85, 65.75, 59.33 and 91.4 presence of Mg^{2+}, Mn^{2+}, Ni^{1+}, Co^{1+}, Cr^{1+} and Zn^{1+} ions, respectively. The results showed that all metal ions, which were

used showed successful inhibitory effects on olive β-glucosidase. Especially Cr^{+} and Mg^{+} were more effective ions as the inhibitor onto olive β-glucosidase, while Ni^{1+} was mentioned in the literature as an inhibitor of olive β glucosidase like Cd^{2+} Pb^{2+} Cu^{2+} and Ag. 1-10 mM of $Mg2^{+}$ and Mn^{2+} were reported as strong activators for fungal β-glucosidases (MA *et al.*, 2011; CHEN *et al.*, 2012; BAI *et al.*, 2013) except for *Neocallimastix patriciarum* W5 (CHEN *et al.*, 2012). Our findings were mostly incompatible with those obtained by other studies. This is thought to be caused by the diversity of the enzyme sources. The metal ions that are mentioned above are found in the chemicals mostly used as antifungal agents.
The IC_{50} values of Deltamethrin, Chlorpyrifos and Alphacypermethrin as the most commonly used pesticides in olive farming were 0.0323, 1.25 and 13.29 mg/L respectively. Deltamethrin is used as insecticide in olive trees. As the reported optimum concentration that was used was 0.31 for olive tree, the obtained IC_{50} value was sufficient for inhibition of olive β-glucosidase.

4. CONCLUSIONS

The olive β-glucosidase was purified from Gemlik variety Turkish olives, and the free enzyme was immobilised onto $Fe^{+2/+3}$ Superparamagnetic nanoparticles to increase the stability and reusability for industrial applications. This is the main enzyme the is responsible for oleuropein hydrolysis during the maturation period. At the same time, there is also industrial use of it for aroma formation and debittering step in table olive production. In previous studies, basic characterisation of olive β -glucosidase was investigated. However, this is the first time where this enzyme was immobilised onto superparamagnetic nanoparticles and characterization of the immobilised enzyme was studied comparatively. In the study, the potential activator and inhibitor effects of the substances under the process conditions in table olive production were determined for the purpose of developing materials which can be used in the debittering process of table olive production. Because of the nanomaterial that was used is biocompatible and risk-free, it may be safely used in food production. After immobilization, the enzyme became more stable under environmental conditions.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

kDa	kilodalton
HIC	Hydrophobic interaction chromatography
SPMN	Superparamagnetic nanoparticles
EU/mg	Enzyme unit/milligram
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
UV	Ultra Violet
IR	Infra-Red
TEMED	N,N,N′,N′,- Tetramethylenediamine

*p*NPG	p-nitrophenyl-alpha-D-galactopyranoside
*o*NPG	Ortho-Nitrophenyl-Galactopyranoside
*p*NPGal	p-Nitrophenyl a-D- Galactopyranoside
*o*NPGal	Orto-nitrophenyl-*β*-d-galactopyranoside
PMSF	Phenylmethylsulfonyl fluoride
EDTA	Ethylenediaminetetraacetic acid
DTT	Dithiothreitol
BSA	bovine serum albumin
DMPD	N, N-dimethyl-p-phenylenediamine
DPPH	Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium
H_2O_2	Hydrogen Peroxide Hydrogen Peroxide
Tris	Tri hydroxymethyl)aminomethane
FTIR	Fourier transform infrared spectroscopy
KBr	Potassium bromide
PE	Purified enzyme
IE	Immobilised enzyme
[S]	Concentration of substrate
[K_M]	Michaelis- Menten Constant
[*V*max]	Maximum speed
mM	Millimolar
*μ*M	Micromolar
mg	Milligram
g	Gram
kg	Kilogram

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