PURIFICATION OF BETA-GLUCOSIDASE FROM APRICOT SEEDS TO REDUCE THE TOTAL VICINE –CONVICINE CONTENT IN FABA BEANS (*VICIA FABA L.*)

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ABSTRACT

This research was aimed to purified beta-glucosidase from apricot seeds using ammonium sulfate precipitation, followed by gel filtration chromatography, then ion exchange chromatography. Ion exchange chromatography resulted in the enzyme sample with a specific activity of 41.3 U/mg with a purification fold of 15 and a yield of 30.3%. The apricot beta-glucosidase had an optimum pH of 4 with good stability between pH 3 and 6. The optimum temperature was 60°C and there was good stability between 5 and 40°C. Faba beans treated at pH 5.5 containing beta-glucosidase showed higher total vicine removal than with only water at pH 5.5 after 4 h. Additionally, treatment at 40°C showed higher total vicine removal that treatments at 25°C. However, treating faba beans at pH 5.5 with and without beta-glucosidase resulted in a lower total vicine content for the enzyme treatment before cooking but the total vicine content after cooking was the same for the water treated compared to the enzyme treated. As an industrial by-product, apricot seeds are a rich source of beta-glucosidase that may be used to treat faba beans to increase their nutritional composition.

Keywords: Ion exchange, gel filtration, molecular weight, optimum temperature.

تنقية انزيم البيتا كلوكسيديز من بذور المشمش لاختزال كمية الفايسين والكونفايسين في الباقلاء (*VICIA FABA L.*)

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المستخلص

هدف البحث إلى تنقية انزيم البيتا كلوكوسيديز من بذور المشمش بالترسيب بكبريتات الامونيوم، ثم تلاها تنقية إضافية من خلال كروماتوكرافيا الترشيح الهلامي وكروماتوكرافيا التبادل الأيوني. حققت خطوة التنقية للمبادل الايوني فعالية نوعية بمقدار 41.3 وحدة / مجم وعدد مرات تنقية 15 مرة وحصيلة انزيمية بواقع 30.3% ، بلغ الرقم الهيدروجيني الأمثل لا إنزيم البيتا كلوكوسيديز المنقى 4 وبثباتيه تراوحت بين 3–6، وكانت درجة الحرارة المثلى لفعالية الانزيم 60م وبثباتيه تراوحت بين 5–40م. أظهرت الباقلاء المعاملة بأنزيم البيتا كلوكوسيديز عند رقم هيدروجيني 5.5 إزالة للفايسين أعلى مقارنة مع الماء فقط عند رقم هيدروجيني 5.5 بعد مرور 4 ساعات. بالإضافة إلى ذلك، أظهرت المعاملة عند درجة حرارة 40م إزالة للفايسين أعلى من المعاملة عند 25 م. ومع ذلك، فإن معاملة الباقلاء عند رقم هيدروجيني 5.5 مع أو بدون البيتا كلوكوسيديز أدت إلى انخفاض محتوى الفايسين قبل الطهي ولكن محتوى الفايسين بعد الطهي كان هو نفسه بالنسبة للمياه المعاملة مقارنة بالإنزيم باعتبارها منتجًا ثانويًا صناعيًا، تعد بذور المشمش مصدرًا غنيًا للبيتا كلوكوسيديز الذي يمكن استخدامه لمعاملة الباقلاء لتحسين تركيبها الغذائي.

الكلمات المفتاحية: التبادل الأيوني، الترشيح الهلامي، الوزن الجزيئي، درجة الحرارة المثلى.

Received:22/11/2023, Accepted:25/2/2024

INTRODUCTION

The bean *Vicia faba* L. is a legume with high protein content, it considers as a source of protein in many areas around the world. In addition to the 24 to 32% protein, the legume seeds have other important nutritional compounds including fiber, minerals and vitamins (9). In spite of this positive nutritional content, faba beans still remain an underutilized crop due to the antinutritional compounds which include the pyrimidine glycosides vicine (V) and convicine(C) (14,27). These compounds are hydrolyzed by beta-glucosidases in the intestinal tract to produce isouramil plus divicine which are responsible for favism disease which leads to severe hemolytic anemia (9, 15, 17). The amount of V and C in the seeds is about 5 mg/g and 2mg/g of dry weight respectively (20, 27). The concentrations of vicine and convicine in faba beans can be reduced by roasting, boiling or soaking. Abd Allah *et al*. (2) showed that faba beans soaked in water for 72 hours resulted in the elimination of 33% of total vicine while soaking at pH 11.3 and pH 3.2 resulted in the elimination of 79 and 78.5% respectively of total vicine after 72 h. Cardador-Martinez *et al*, (11) showed that roasting at 120°C for 10 min reduced the amount of vicine by 1 to 12% and reduced the amount of convicine by 3 to 30% depending on the variety of faba bean. While boiling at 121°C for 20 min reduced the amount of vicine by 1 to 40% and convicine by 13 to 61% depending on the variety of faba bean. Some related researches found that using tap water or alkaline , acid , or neutral aqueous solutions for soaking are active in removing some of the total vicine from faba bean seeds and flour but can also have a negative impact on the other important properties (4,27). A study investigated the use of raw almond fiour, which is a potent source for beta-glucosidase (EC 3.2.1.21) activity which can hydrolyze vicine and convicine on the reduction of these glycosides in faba beans (3). The degree of hydrolysis differs from complete to partial according to the duration of treatment (1 to 24 h), the temperature (1 to 30°C) and pH (6.4 to 4.6) with the highest hydrolysis occurring at 30°Cat pH 4.9 for 24 h. Fungal beta-glucosidase produced by *Aspergillus oryzae*, *Fusarium graminearum* and *Lactobacillus plantarum* efficiently hydrolyzed the pyrimidine glycosides in faba bean suspension and flour (27). The recent study designed to optimize the extraction and purification conditions for BGS enzyme from apricot seeds and some of its characteristic and to evaluate the potency of the enzyme in decreasing or removing the total vicine and convicine from faba beans (*Vicia faba L*.).

MATERIALS AND METHODS

Preparation of crude enzyme extract

Apricot seeds were obtained from apricots bought from a local marketplace in Baghdad, Iraq. The seeds were defatted according to (18). Briefly, the seeds were sopped in water for 2 h, hulled, air dried then ground in cold ethyl acetate with a blender. The powder was defatted by an additional 3 washes of cold ethyl acetate then left to air dry in a hood. The extraction method used sodium phosphate buffer (0.2 M, pH 7.0) containing 2% sodium chloride in a ratio of (1: 5) (solid: liquid) under refrigerated condition (4°C) and the blend was agitated for 4 h. by a magnetic stirrer. The sample was then filtered using a Whatman filter No.1 then centrifuged at 6000 x g for 20 min. to remove the solid residues. The supernatant was used as crude enzyme extract (CEE), then its volume, enzymatic activity, and protein content were determined and the specific activity & total activity were calculated as described below. The protein content was determined using protein determination kit (Pierce Chemical Co., USA).

Beta-glucosidase activity

A 70 µL aliquot of the crude enzyme was added to the same volume of citrate phosphate buffer (0.1 mM, pH 5.2 containing the substrate 0.63 mM p-nitrophenyl-β-D-glucopyranoside (pNPG, Sigma-Aldrich, USA)), and incubated at 37 °C for 30 min. The reaction terminated by adding 70 µL of 0.4 M Na_2CO_3, and the samples were centrifuged at 15490 ×g for 15 min. The absorbance at 410 nm was read (BioSpeec-1601 dual bean spectrophotometer, Shimadzu) to determine the amount of p-nitrophenyl produced. One unit of BGS activity was defined as the amount of enzyme requisite to cause a 0.01 variation in absorbance under experiment conditions, according to the method described

by Blondin *et al*, (9). The kinetic parameters including maximum reaction rate (V_{max}) and Michaelis-Menten constant (K_m) were calculated using the initial rates of dilutions of pNPG in 0.2 M sodium citrate buffer, pH 5.2 at 37°C. The Lineweaver-Burk plot was used to determine V_{max} and K_m by linear regression analysis of the reciprocal of the initial velocities graphed versus the reciprocal of the substrate concentration and the best-fit line was obtained.

Beta-glucosidase purification

Ammonium sulfate saturation (20, 30 40, 50, 55, 60, 65, and 70 %) were set up to fractionate the CEE. The crude enzyme precipitated after about 4 h. from the salt addition while mixing, then the sample was centrifuged at 10,000 x g for 30 min. The supernatant was decanted off and the pellets dissolved in proper amount of 0.2 M sodium phosphate buffer (pH 7). The entire process was done at 4°C. The ammonium sulfate precipitated enzyme was dialyzed against sodium phosphate buffer (pH 7, 0.2 M) for 24 h. with three changes of dialysis buffer (1,26). The enzyme activity and protein concentrations of both the supernatants and dialyzed pellets were determined and the specific activity was calculated. The dialyzed ammonium sulphate precipitation pellet at 65% ammonium sulfate were dissolved in running buffer (0.2 M phosphate buffer pH 7) and loaded onto a Sephadex G-100 column (70 cm x 2 cm, Sigma-Aldrich, USA) and fractionated at flow rate of 1 ml/min(5, 13). Fractions volumes of 3 ml each were collected and estimated for protein content via absorbance at 280 nm and the enzyme activity using pNPG as the substrate. Fractions 13 to 24 from the G-100 column containing β-glucosidase activity, were pooled and fractionated by ion exchange chromatography (2.5×30 cm column) with DEAE-cellulose (Sigma-Aldrich, USA) equilibrated with 10 mM phosphate-citrate buffer(pH 6.8). The column was eluted with same buffer at a flow rate of 0.5 mL/min and linear gradient of phosphate-citrate buffer containing NaCl (0 to 80 mM) . Fractions of 5 mL were collected and the protein concentration estimated via absorbance at 280 nm and β-glucosidase activity using pNPG as the substrate were determined. Fractions 9 to 18 with β-glucosidase activity were pooled and used for further study.

Polyacrylamide gel electrophoresis

The extent of purification of BGS was followed by SDS-polyacrylamide gel electrophoresis according to (16,30). SDS-PAGE was conducted using a 5% stacking gel and a 12% running gel. Samples containing (20 µg) of protein in 20 µl buffer were mixed with (10 µl) of SDS sample buffer (3X=1M Tris HCL pH 6.8, 10% SDS, 0.3% Bromophenol blue, 1% β-mercapto-ethanol, and 30% glycerol) and heated at 90 °C for 5 min. Running buffer 0.1% (w/v) SDS dissolved in 25 mM Tris and 192 mM glycine(pH 8.3) was used at 60 V for the first 15 min. and 100 V for an extra 90 min using a Mini-Protean Tetra Cell (Bio-Rad Laboratories, USA). Gels were stained using (0.1% w/v) Coomassie Brilliant Blue R-250, 45% (v/v) methanol plus 10% (v/v) acetic acid for 20 min.. Molecular range protein standards were used as molecular weight markers (GenScript and Thermo Scientific Prestained Protein Molecular Weight Marker, USA). The migration distances of the standards was plotted vs the log molecular weight of the standards. The migration distances of the assumed BGS band were determined and the molecular weight was calculated.

Determination of the optimum pH and pH stability of BGS: The optimum pH for the BGS hydrolytic activity was identified using pH values ranging from (3-10). Sodium citrate buffer solution, 0.2 M, was used to prepare buffers with pH values ranging from 3-5, sodium phosphate buffer solution(0.2 M) was used to prepare buffers with pH values ranging between 6-7, and Tris-HCL buffer solution, 0.2 M, was used to prepare buffers with pH values between 8-10. The substrate, pNPG was added to each buffer at a concentration of 0.63 mM and the activity at each pH value was determined. The reactions were conducted by incubating the substrate with enzyme at each pH for 30 min. in a water bath at 37 ° C, followed by cooling the samples in an ice bath to terminate the enzymatic reaction and the enzyme activity tested as mentioned previously after that the relationship between the enzyme activity and the pH values was

plotted. For testing of pH stability, an equal amount of the purified enzyme solution and the above mentioned buffers solutions with pH values of 3-9.0 were mixed and incubated in water bath at 37 ° C for 30 minutes , then cooled in an ice bath and the activity was measured. The remaining BGS activity was calculated as a percentage of the activity relative to the maximum activity which was at pH 5.

Determination of the optimal temperature and temperature stability of BGS

To determine the optimal temperature of BGS, activity was tested in sodium citrate buffer, 0.2 M pH 4, warmed to a range of temperatures (20 to 90°C) and activity measurements at 410 nm with pNPG were done. To determine the temperature stability, BGS was incubated at temperatures from 5 to 90 °C for 30 minutes in 0.2 M sodium citrate buffer, pH 4. Samples were cooled in an ice bath before activity measurements at 410 nm with pNPG at 20°C. The residual enzyme activity was determined as compared to the activity at 5°C and the relationship between the remaining activities at different temperature values were graphed to determine the thermal stability.

Determination of total V-C in faba beans

The Collier (13) spectrophotometric method was used to determine vicine plus convicine concentrations in experimental samples. One g of the fava bean (purchased at a local market in Baghdad, Iraq) sample with (100 ml) of freshly prepared solution of (4%) meta phosphoric acid was homogenized in a blender for 5 min. The samples were centrifuged at 2000 xg. for 30 min. and the supernatant was filtered through a Whatman No. 1 filter paper. The absorbance at 273 nm of the filtrate was determined. This method estimates the concentration of convicine plus vicine or total vicine in samples.

Impact of soaking solution temperature and beta-glucosidase (BGS) on V-C removal from fava beans

Dry fava beans, were soaked in tap water (pH 7.2) at 25°C for 12 h. then rinsed in tap water before cooking. Beans, 50 g, were placed in a container with water at pH 5.5 at ratio of 1:5 (beans: water) and another 50 g was placed in water at pH 5.5 at the same ratio containing BGS (0.1 ml/2 g beans or 32 U). This sample was left for 4 h at 25°C. The last 100 g beads were scratched with a knife so there were several scratches on each bean then prepared as described above in pH 5.5 water and pH 5.5 water containing 32 U of BGs and incubated at 40°C for 4 h. All experiments were done in duplicate. Then all samples were cooked by boiling in water for 75 min and vicine and convicine were estimated as described above before and after the cooking process.

Impact of soaking solution pH and BGS treatment duration on V-C removal

Dry fava beans, 300 g, were soaked in tap water (pH 7.2) at 25°C(room temperature) for 12 h.. Soaked samples were drained and distributed into three 50 g samples in duplicate. The 1st 50 was kept in tap water (pH 7.2) and the 2nd 50g sample was placed in an aqueous solution at pH 5.5, the 3rd 50g sample was put in an enzymatic solution at pH 5.5 containing beta-glucosidase (32 U/2 g beads) and all samples were held for 12 h. at 40 °C. Then one gram of each treatment was taken after 1.5, 3, 4.5, 6, 7.5, 9, 10.5 and 12 h for Vand C determination. All bean samples were then cooked by boiling in water for 75 min and the concentration of vicine and convicine were determined again.

RESULTS AND DISCUSSION

Beta-glucosidase purification

Results of the BGS purification from defatted apricot seed powder are shown in Table 1. The crude extract contained 3.5 U/ml, a specific activity of 2.8 U/mg and a total activity of 422.4 U. Ammonium sulfate precipitation of this sample is shown in Figure 1A. BGS precipitated from solution with ammonium sulfate concentrations of 30 to 70%. The precipitate with the highest amount of BGS was obtained with 65% ammonium sulfate. This sample showed a specific activity of 12.81 U/mg resulting in a 5-fold purification. Bhalla *et al*, (8) found that the BGS can be obtained from wild apricot (*Prunus armeniaca L.*) by $(NH4)_2SO_4$in the 20-70% saturation range. Ašić *et al*, (6) demonstrated that BGS from Agaricus bisporus (white button mushroom) precipitated out between 40- 80% of ammonium sulfate saturation. The pellet from ammonium sulfate precipitation at 65% was dialyzed and subjected to Gel filtration chromatography (Figure 1 B). There are

several protein peaks but fractions 13 to 24 contained BGS activity. The specific activity, enzyme yield and the purification fold for this step were 33 U/mg, 78.8% and 12 respectively. Fractions that showed BGS activity were then pooled and subject to ion-exchange chromatography. Figure 1 C shows the ion-exchange profile of the pooled fractions. Two major protein peaks are seen and one of them coincided with BGS activity. This step resulted in the lowest protein concentration of 0.31 mg/ml but the highest specific activity of 41.3 U/mg. The overall purification fold was 15 with a yield of 30.3%. The Michaelis-Menten constant (K_m) and the maximum velocity (V_{max}) using pNPG was 1.11 mM and 26.27 U/min/mg. A comparison of the kinetic values of BGS from other sources is shown in Table 2. The K_m is similar to that reported by apple seed BGS (31) and Brassica BGS (7). The V_{max} is also in the range reported for apple seed BGS (31) and grape BGS (29). The SDS-PAGE profile of the purification steps is shown in Figure 2. The crude apricot seed protein profile is seen in Figure 2, lane 1. This sample contains many bands in the molecular weight range of 20 to about 90 kDa. With the ammonium sulfate precipitation, size exclusion chromatography and ion-exchange chromatography, a band of approximately 30 kDa becomes the most prominent band. A comparison of the characteristics of BGS from other sources is shown in Table 2. The molecular weight of commercial almond BGS is 130 kDa and is a homodimer of two 65 kDa subunits. BGS exists as a homodimer also in apple seeds (2 x 60 kDa subunits) and chayot (2 x 58 kDa subunits) (24, 31). BGS exists as heterodimers in Brassica (80 and 50 kDa subunits) and Agaricus (46 and 62 kDa subunits) (6, 7). BGS exists as monomers in olive (65 kDa) and barley (58 kDa) (22,28). Bhalla purified BGS from wild apricot seeds and showed that it had a molecular weight of 66 kDa which is twice the weight as reported here. The molecular weight of the BGS purified here is similar to that purified from tea plant which had an estimated molecular weight between 34 to 41 kDa (23)

Table 1. Purification of β-glucosidase from defatted powder of apricot seed

Purification step	Enzyme units U/ml	Volume ml	Protein con. mg/ml	Specific Activity U/mg	Total units U	Purification fold	Activity recovery (%)
Crude enzyme extract	**3.52**	**120**	**1.26**	**2.8**	**422.4**	**1**	**100**
Ammonium sulfate	**23.7**	**15**	**1.85**	**12.81**	**12.81**	**5**	**84.16**
Gel filtration	**18.5**	**18**	**0.56**	**33.01**	**333**	**12**	**78.8**
Ion-exchange	**12.8**	**10**	**0.31**	**41.29**	**128**	**15**	**30.3**

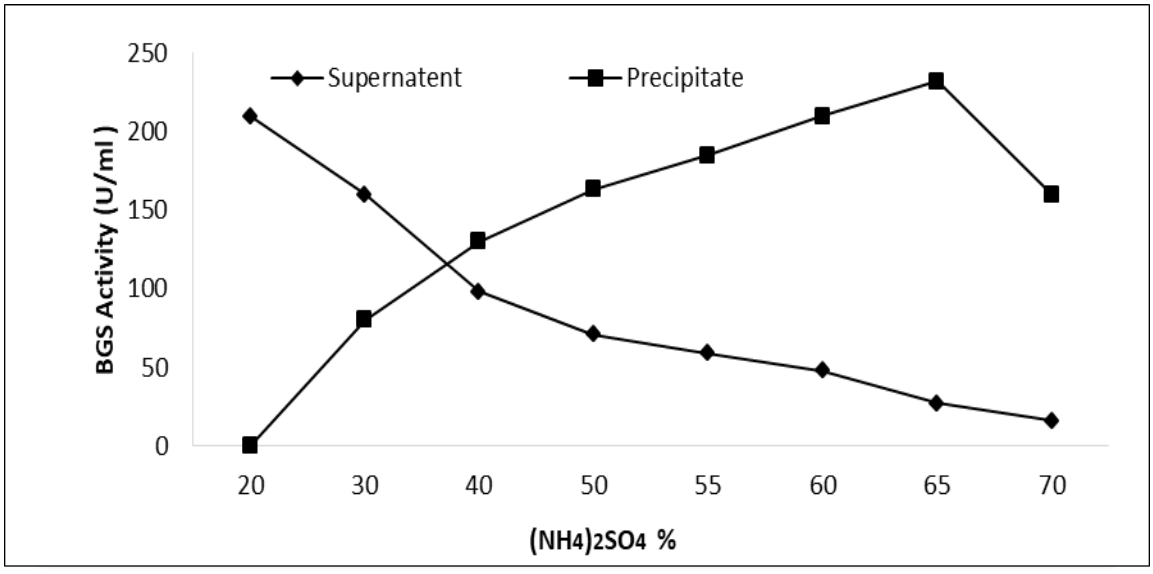

Figure 1. A. Ammonium Sulfate precipitation of crude BGS extract

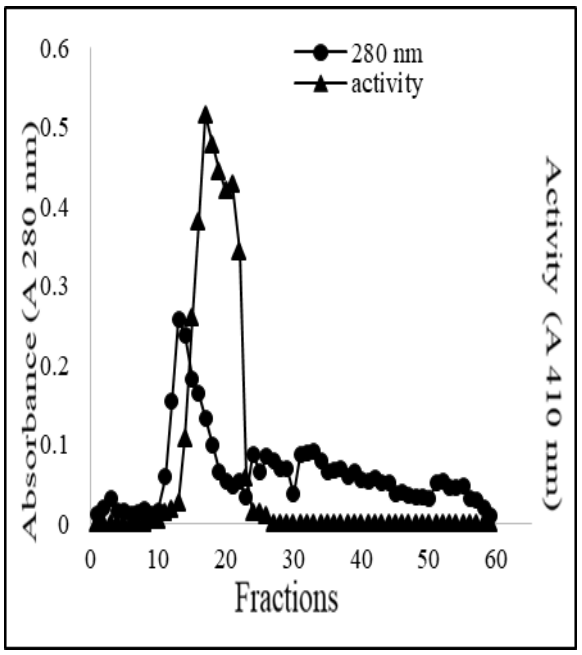

Figure 1. B. Gel filtration chromatography using Sephadex G-100 (2 × 70 cm) equilibrated by 10 mM phosphate citrate buffer (pH 6.8) at a flow rate of 1 ml/ 1 min

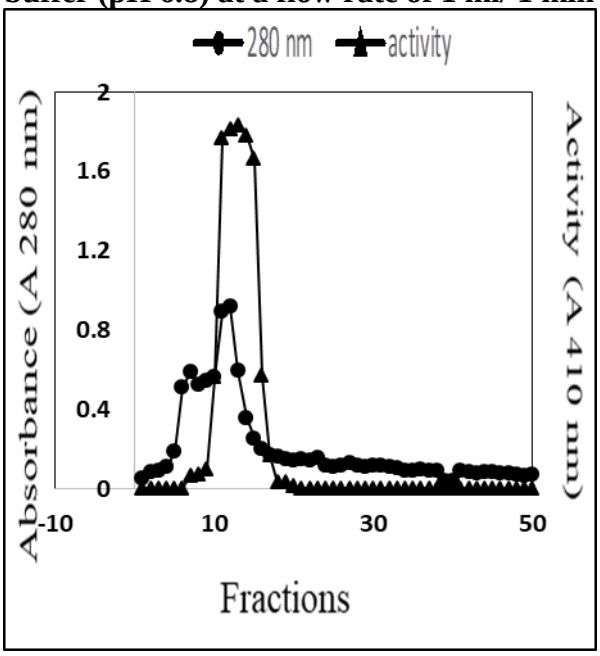

Figure 1. C. Ion exchange chromatography using (DEAE- Cellulose) equilibrated using 10 mM phosphate citrate buffer (pH 6.8) at a flow rate of 0.5 ml/ 1 min

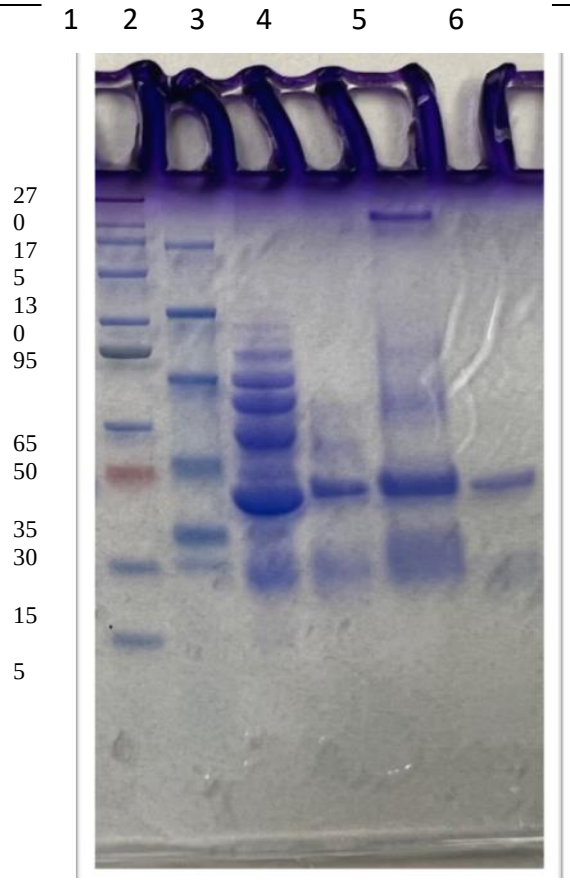

Figure 2. SDS-PAGE analysis of BGS purification. Lane 1 molecular weight markers as displayed; Lane 2 molecular weight markers (120, 90, 50, 34, 26, and 20 kDa); Lane 3 crude sample; Lane 4 after ammonium sulfate precipitation; Lane 5 after gel filtration chromatography; Lane 6 after ion exchange chromatography

The optimum pH and pH stability for β -glucosidase activity: Figure 3A illustrates the pH pattren of the BGS activity at a pH range from 3 to 10. The optimum pH for the activity of the enzyme was 4. The enzyme activity decreased by 68% at pH 3 and at pH 6 there was a 71% decrease in activity with no activity shown at pH 8 or greater. The optimum pH of BGS differs based on the source. Other BGS enzymes with an optimum pH at 4 were characterized from Agaricus and Chayote (6,24) while BGS characterized from apricot, Brassica and apple seeds (7, 8, 31) showed pH optimum at pH 6 (Table 2). Figure 3 B shows the stability of the apricot seeds BGS over the pH values of 4 to 10. BGS was stable at pH values from 3 to 6 retaining over 80% of its activity at pH 3 and 100% of its activity at pH values from 4 to 6. BGS retained more than 70% of its total activity at pH 7 but lost 65% of its activity at pH 8 and higher. These findings are similar to (7) who found that the

purified BGS enzyme from cabbage (Brassica oleracea) was stable at pH values ranged from 4 to 7.

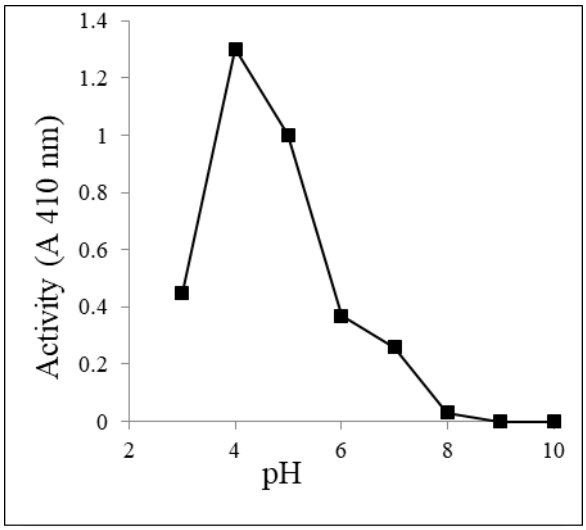

Figure 3. A Optimum pH of beta-glucosidase purified from apricot seeds.

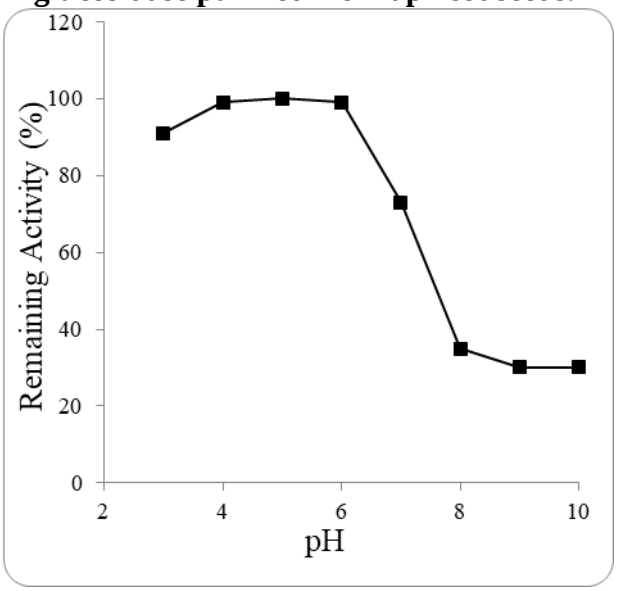

Figure 3. B pH stability of beta-glucosidase purified from apricot seeds.

Temperature optimum and thermal stability of beta-glucosidase: The influence of temperature on the activity of the apricot seed BGS was studied over different temperatures that ranged between 20 to 90 °C. The results in Figure 4A show that the optimal temperature is 60°C. BGS lost 60 % of its activity at 25 and 90°C but showed good activity (64 to 67% of maximum activity) at temperatures between 40 and 80°C. Other BGS enzymes with optimum activities at temperatures of 55 to 60 °C were purified from prunes (12) grapes (29), agaricus (6) and barely (28). BGS enzymes with optimum activities at 35°C were purified from apricot (8) and Brassica (7). The dissimilarity in the optimum temperatures of the enzyme in different published studies is due to the difference in the source of the enzyme, although it is unclear as to why the BGS purified from apricot seeds by (8) is very different from the results presented here. The temperature stability of BGS as incubated for 15 min. at different temperatures (20 to 90°C) is illustrated in Figure 4B. It was obvious that the enzyme reserved more than 90% of its total activity when incubated up to 30°C for 15 minutes and more than 80% of its original activity at 40°C. Although its activity decreased above 40°C but it still retained more than 50% and 60% of its total activity at 50°C and 60°C respectively. The BGS activity continually decreases as the temperature increased above 50°C and there was minimal activity greater than 70°C.=

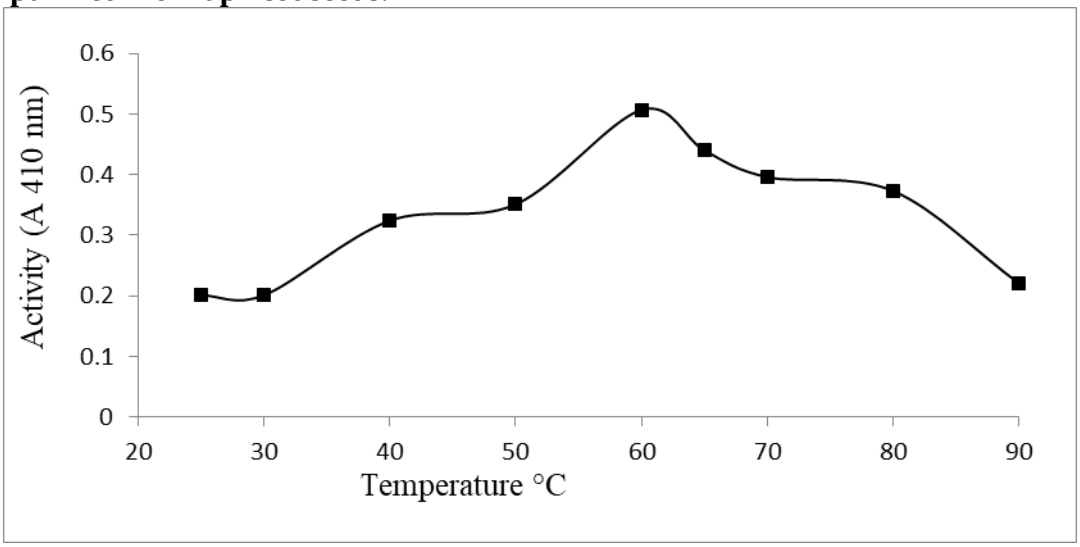

Figure 4. A Optimum temperature of β -glucosidase purified from apricot seed

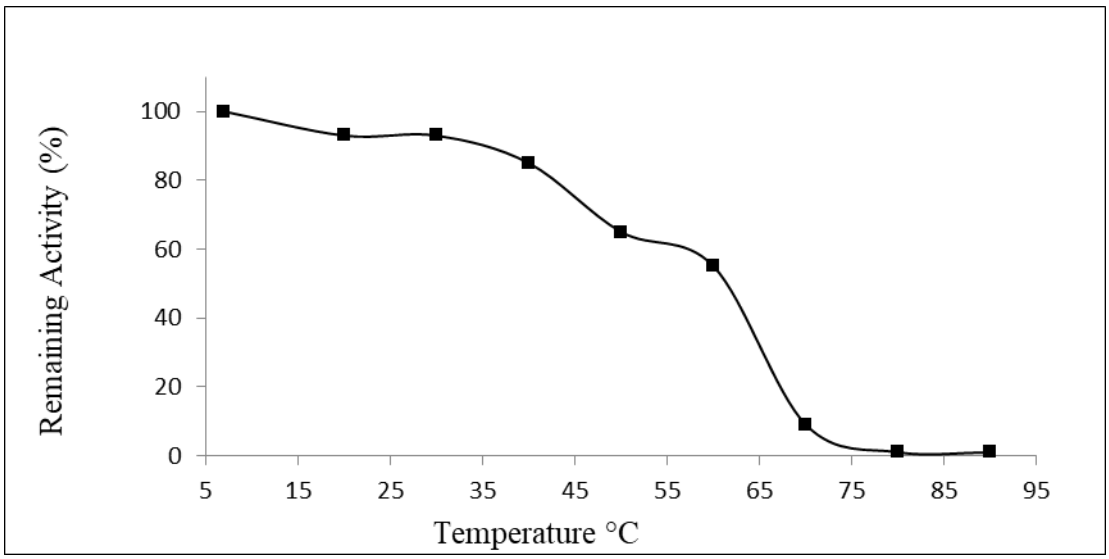

Figure 4. B Temperature stability of β -glucosidase purified from apricot seeds

Table 2. Characteristics of β - glucosidases from various sources

	MW kDa	pH	pH stability	Temp (°C)	K_m pNPG mM	Vmax U/mg	
Apple seeds	**2 x 60**	**6**	**5-9**	**70**	**1.2**	**52.4**	**27**
Tea plant	**34 to 41**	**5.5**	**4-7**	**50**			**20**
Valencia fruit	**55 to 64**	**4.5-5**		**40**	**0.115**		**8**
Olive fruit	**65**				**2.2**	**370.4**	**19**
Prune seeds	**54 to 61**	**5.5**		**55**	**3.09**	**122.1**	**10**
Grapes		**5**	**4.5-6**	**55**	**2.97**	**10.1**	**25**
Brassica oleracea	**80 and 50 subunits**	**6**	**4-7**	**35**	**0.755**	**604**	**5**
Agaricus bisporus	**46 and 62 subunits**	**4**		**55**	**1.75**	**833**	**4**
Chayote	**2 x 58 subunits**	**4**		**50**	**4.88**		**21**
Barley	**58**	**5**		**60**			**24**
Wild apricot	**66**	**6**		**35**		**281**	**6**
This research	**30.5**	**4**	**4-6**	**60**	**1.11**	**26.27**	

Effect of β -glucosidase, temperature and cooking on the removal of V and C from faba beans: Table 3 display the impact of adding β -glucosidase enzyme (16 U/g beans) to the fava bean soaking media (pH 5.5) over 4 h. incubation at two temperatures degrees 25 and 40°C. The obtained results revealed that soaking at 40 °C was more applicable in removing both (V-C) from faba beans than that at 25 °C, and the similar trends were noticed after cooking process. This may be because the treatment temperature is closer to the optimal activity temperature of BGS which is 60°C. At 40°C, there was a 17% increase in V and C removal with BGS than without. In samples that were cooked, there was a 21% increase in V-C removal with BGS than without. The highest V-C removal seen in samples with the beans which scratched with a knife before treatment. With cooking and added BGS, there was a 63% removal of V-C in this sample. Mona *et al.,* (25) reported that V and C are heat-resistant compounds, and eliminating these compounds by heat treatment is hard at a low temperature compared to high temperatures.

Table 3. Effect of β -glucosidase, temperature, and cooking on the removal of V-C from faba beans after 4 h. soaking

% Removal of vicine and convicine				
	Before Cooking		After Cooking	
Treatment	**pH 5.5**	**pH 5.5+ Enzyme**	**pH 5.5**	**pH 5.5+Enzyme**
25 °C	**15**	**24**	**27**	**35**
40° C	**26**	**43**	**34**	**55**
40 °C scratched	**35**	**55**	**44**	**63**

Figure 5. explains the effects of soaking solution pH, treatment time, and the cooking process on V-C content in faba beans at 40°C. For each treatment, there was in increase in V-C removal over time. In general, the removal of V-C was greater at treatments close to the optimum pH and temperature of BGS activity. For the before cooking samples, treatment at pH 5.5 with BGS resulted in the highest V-C removal of 67%, while the lowest removal of 36% was observed with pH 7.2 water after 12 hrs. After cooking, the highest removal, 71%, was observed was with pH 5.5 water with or without BGS while the lowest removal of 48% was observed with water at pH 7.2. In this case, BGS did not add additional effectiveness in V-C removal than the use of pH 5.5 water for a duration of 12 hrs. This may have been due to the inactivation of the enzyme over the course of the treatment (Figure 5). These results are different from Jamalian and Ghorbani, (21) who found that extraction of V and C compounds from faba beans were proportional to the increase in extraction temperature and soaking duration. Vicine removal in water acidified to pH 3.5 was 31% compared to water at pH 6.5 (17%) or water adjusted to pH 11.5 (41%). For convicine pH 3.5 removed 23% while water at pH 6.5 removed 26% and pH 11.5 resulted in 42% removal. In this study, we achieved higher V-C removal with a combination of β - glucosidase and a pH of 5.2. Cardador-Martinez *et al.,* (11) applied the boiling and roasting treatments for Vicia *faba* to elimenate these compounds, the results assured that the boiling process was more efficient in elimination process as compared to roasting process, as it resulted in elimation of 30 and 60%, while roasting resulted in eliminating only 12 and 40% of V and C, respectively. This could be attributed to the fact that the glycosidic compounds are water-soluble componants, which makes them easy to remove at a greater rate compared to roasting process. Hegazy and Marquardt., (19) suggested that there is a direct relationship between the removal percentages of these glycosidic compounds (V - C) and the soaking duration. Abd Allah *et al,* (2) reported that the acidic environment improves the permeability of vicine and convicine from the bean. Although there was not complete removal of total vicine in the experiments conducted future experiments using pH 4 and higher concentrations of BGS may eliminate total vicine from faba beans.

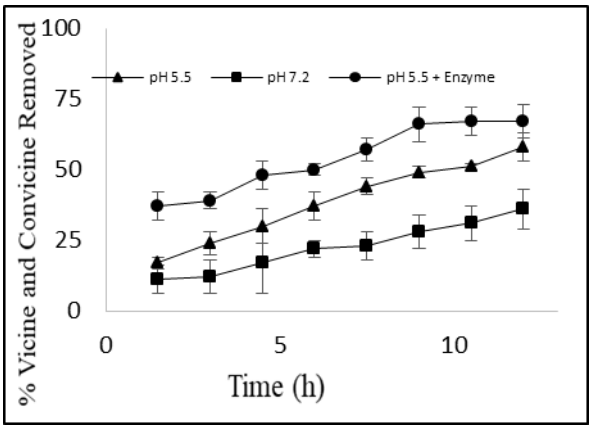

Figure 5. A. Effects of soaking solution pH and beta-glucosidase treatment time (at 40°C) on V-C removal from fava beans before cooking

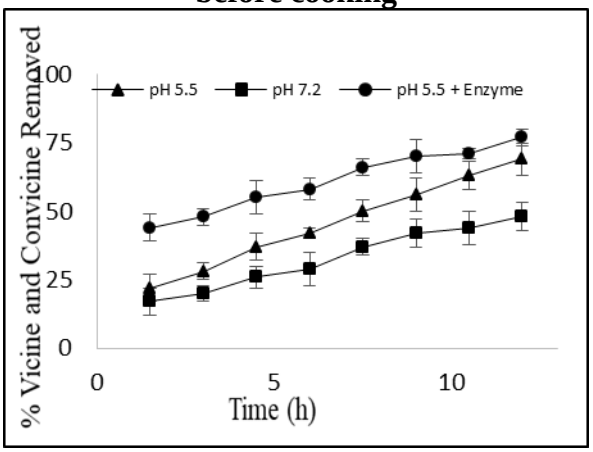

Figure 5.B. Effects of soaking solution pH and beta-glucosidase treatment time (at 40°C) on V-C removal from fava beans after cooking

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