VICINE AND CONVICINE LEVELS IN DRY AND FRESH BEANS DURING THE GROWTH STAGES AND THE EFFECT OF ENZYMATIC TREATMENT AND PROCESSING CONDITIONS ON THEIR REMOVAL.

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ABSTRACT

Beans (*Vicia faba*) contain some anti-nutritional compounds, especially vicine and convicine and their non-sugar derivatives, divicine and isouramil, which are responsible for the occurrence of Favism in people with hereditary deficiency of Glucose-6-phosphate dehydrogenase (G6PD). These study objective was estimation of vicine and convicine content and their distribution in dry and green bean parts during the growth stages, and the effect of beta-glucosidase from defatted apricot seed powder and some home-based processing conditions on elimination of these compounds from the bean. The results showed that the content of vicine & convicine in bean ranged from 22.7 mg/100 g in the seedling stage to 46.8 mg/100 g in the pods stage at 139 days. As for vicine, it ranged from 12.6 mg /100g to 29 mg/100g at the above stages respectively. While for convicine it ranged from 10.1 mg/100g to 17.8 mg/100 respectively. The enzymatic treatments showed significant elimination of those compounds as compared to non-enzymatic treatments. Additionally, other factors such as soaking temperature, pH, and time as well as cooking process had a positive effect on the elimination of those glucosides.

Key words: divicine ، isouramil ، faba bean، favism، β-glucosidase
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تقدير مستوى الـ Vicine وConvicine في الباقلاء الجافة والطازجة خلال مراحل النمو وتأثير المعاملة الانزيمية وظروف التصنيع في ازالتها

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المستخلص

تحتوي الباقلاء على بعض المركبات المضادة للتغذية وبخاصة الكلايكوسيدات البيرميدينية وهما الفايسين (Vicine)والكونفايسين (Convicine) ومشتقاتهما غير السكرية الداي فايسين (Divicine) والايزوياورامیل(Isouramil) المسؤولة عن حدوث ظاهرة تكسر كريات الدم الحمراء(Favism). لدى الأشخاص اللذين يعانون من نقص وراثي في نشاط انزيم Glucose–6–phosphate dehydrogenase(G6PD). هدفت الدراسة الحالية الى تقدير محتوى الفايسين والكونفايسين وتوزيعهما في اجزاء ثمرة الباقلاء الجافة والطازجة اثناء مراحل نموها وتأثير انزيم بيتا كلوكوسيديز المستخلص من مسحوق بذور المشمش مزالة الدهن وبعض الظروف التصنيعية المتبعة منزليا في إزالة هذه المركبات من الباقلاء. أظهرت النتائج ان محتوى الباقلاء من هذه الكلوكوسيدات اثناء تطور نضجها تراوح من 22.7 ملغم /100 غم باقلاء في مرحلة البادرات الى 46.8 ملغم /100 غم باقلاء في مرحلة القرنات بعمر 139 يوما. اما بالنسبة للـ Vicine فقد تراوحت من 12.6 ملغم /100 غم الى 29 ملغم /100 غم في المراحل المذكورة أعلاه على التوالي. بينما بالنسبة للـ Convicineفقد تراوحت بين 10.1 ملغم /100 غم الى 17.8 ملغم/100 غم على التوالي. أظهرت المعالجات الانزيمية إزالة معنوية لهذه المركبات مقارنة بالمعالجات غير الانزيمية. بالإضافة الى ذلك، كان لعوامل أخرى مثل درجة الحرارة ودرجة الحموضة والوقت لعملية النقع وكذلك لعملية السلق تأثير إيجابي في إزالة هذه الكلوكوسيدات.

الكلمات الافتتاحية: داي فايسين، ايزوياورامیل، بيتا كلوكوسيديز، الباقلاء، الفافزم.
البحث جزء من أطروحة الدكتوراه للباحث الأول

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INTRODUCTION

Faba bean (*Vicia faba L.*) is an ancient pulse crop, and one of the first domesticates, grown at least from the early Neolithic period (6800 to 6500 B.C.E.) (21**)**. It is considered as one of the main sources of cheap protein and energy in Africa, parts of Asia and Latin America, where most people cannot afford meat sources of protein (4,34). Faba beans contain a large amount of proteins, carbohydrates, B-group vitamins and minerals. Faba beans are not only a good source of protein, fiber and energy, but diets rich in faba beans have noted to lower LDL cholesterol levels in plasma (15,33). Faba bean, like most legumes, contains anti-nutritional compounds, of which the most important are vicine and convicine (v&c). Normally v&c comprises about 1% of the seed dry matter. The amount differs between cultivars and varieties (13). Faba beans also contain other anti-nutritional compounds like tannins, Protease inhibitors but these are usually removed by dehulling the seeds. Since v&c content is the highest in the cotyledon, dehulling does not affect the v&c content (12). vicine and convicine are compounds belong to the group of pyrimidine glycosides, which are composed of one molecule glucose linked to one pyrimidine nucleoside (aglycones), these aglycones, divicine and isouramil respectively (8,10). v&c are pyrimidine glucosides that hydrolyze to divicine and isouramil during digestion, these two compounds are powerful oxidants and harmful or deadly to humans that have a gene mutation for glucose-6-phosphate dehydrogenase (G6PD) deficiency (9). In G6PD-deficient individuals, the v&c hydrolysis products divicine and isouramil cause glutathione (GHS) in the plasma to oxidise. Normally GHS is regenerated in metabolic cycle where G6PD is used. In G6PD-deficient individuals, GHS regeneration cannot be done, which leads to the oxidized GHS altering several functions of red blood cells (RBC). This leads to hemolysis, meaning that RBC are destroyed 24 – 36 h after v&c indigestion. During this a small amount of the RBC breaks down, but majority is removed by macrophages (2, 3). In the worst case, Favism can cause the destruction of 80% of RBC and be fatal, but usually Favism cases are milder and do not require blood transfusion, which is the only cure for Favism (11). The non-carbohydrate moiety of vicine or convicine, i.e. the pyrimidine aglycones (divicine and isouramil) is attached to D-glucose via a β-glycosidic bond at position 5. The glucose unit can be released enzymatically or by treatment with strong acid at high temperature. Several studies have demonstrated acid hydrolysis to aglycones by treatment with sulphuric acid or hydrochloric acid (HCl) at 100 °C (35). Enzymatic hydrolysis using β -glucosidase is more relevant for food systems, and the enzyme can originate from several sources (20). vicine and convicine are water-soluble and heat-stable compounds, which makes them a challenge to remove. Reduction in the contents of vicine and convicine has been investigated in a few studies that mainly focused on soaking (17) cooking, heating and roasting (8), and fractionation (12,32). Certain processing methods, such as enzyme treatments (19), germination (14) and fermentation (12, 29), may induce hydrolysis of the β -glycosidic bond. Hydrolysis causes the disappearance of vicine and convicine, but the simultaneous release of the aglycones. So the current study aimed to study the potency of beta-glucosidase enzyme extracted from apricot seeds in reducing and/ or removing these compounds from dry and fresh beans.

MATERIALS AND METHODS

vicine and convicine extract

Vicine and convicine were extracted by mixing 10 g of dried bean flour with (100 ml total volume of 0.1 N sodium hydroxide solution for 30 minutes using a magnetic stirrer. The mixture was filtered through Whatman No. 4, the pH was adjusted to 4.2 by 0.1 N hydrochloric acid, then the mixture was filtered through Whatman No. 4 and the obtained filtrate was lyophilized (28).

Extraction of convicine from bean

Convicine was extracted from the experimental bean samples according to method mentioned by (6) by mixing 1 kg of whole mature green bean seeds with 800 ml of (95%) ethyl alcohol for 30 minutes and then left for three days at room temperature. The obtained suspension was filtered through Whatman No. 4 filter paper and the filtrate was extracted with ether in a ratio of 1:1 and

the aqueous layer was concentrated under vacuum to a volume of 350 ml at 40 °C. The concentrated sample was kept under refrigeration for three days until the convicine crystals were formed, which were separated using Whatman No. 4.

Determination of total vicine and convicine in faba beans: Vicine and Convicine were determined by the method of (22), One g of the bean sample was homogenized in a blender with 100 ml of freshly prepared solution of 4% meta phosphoric acid for 5 min, centrifuged for 30 min. at 2000 r.p.m. and the supernatant was filtered through a Whatman No. 1 filter paper. The obtained filtrate is taken and the absorbance is read at a wavelength of 273 nm.

Effect of beta-glucosidase enzyme(BGS) concentration on glycoside compounds: Different concentration {50, 100 and 150 µl (15.4 units/ml)} of Beta-glucosidase enzyme was added to 2ml of glycosidic compounds (Vicine and Convicine extract) which prepared by dissolving 0.1 mg v-c extract/ml water w/v) and to Convicine extract (0.1 mg/ml) separately and left for 60 min. at 40 °C. After 15, 30, 45 and 60 minutes aliquot of samples were taken for vicine and convicine determination by reading their absorbance at a wavelength of 273 nm for vicine and convicine mixture and at 271 nm for convicine.

Effect of BGS concentration and enzymatic treatment time on vicine and convicine removal from dry beans: A sample (100 g) of dry bean was soaked in tap water (pH 7.2) at room temperature (25 C) for 12 h., then the soaked bean was taken and divided into two portions, the first portion was soaked in an enzymatic solution [tap water with pH 5.5 and 0.1 ml of the experimental beta-glucosidase (15.4 units/ml) per 2 g of bean sample at ratio of 1:5 w/v. The same experiment was repeated with 0.15 and 0.2 ml/ 2g BGS concentration. All treatments were held at 40 °C for different periods. The second portion was soaked under the same condition except in the absence of the BGS. A portion of each treatment was taken after 2,4,6,8 and 12 h. for (Vicine and Convicine) estimation. After then all samples were cooked and the vicine and convicine content were determend again.

Effect of soaking temperature on enzymatic removal of vicine and convicine from dry beans:200 gm of dry bean was soaked in tap water (pH 7.2) at room temperature (25°C) for 12 hours. 100 gm of soaked beans were taken and divided into two halves. The first Half was placed in an enzyme solution [tap water with pH 5.5 and the extracted beta-glucosidase enzyme 0.1 ml / 2 g (15.4 units / ml) in a ratio of 1: 5 and the other half was left without enzyme and both treatment were left for 4 hours at room temperature (25 C). As for the other 100 g of bean sample, same thing was done but the samples were left at 40 °C. Then all samples were cooked and (Vicine and Convicine) were estimated before and after cooking process.

Effect of treatment time on enzymatic removal of vicine and convicine from fresh beans: A sample (100 g) of fresh beans (139 days old) was taken and divided into two portions, the first portion was soaked in an enzymatic solution [tap water with pH 5.5 and 0.2 ml of the experimental beta-glucosidase per 2 g of bean sample at ratio of 1:5 w/v.] All treatments were held at 40 °C for different periods. The second portion was soaked under the same condition except in the absence of the BGS. A portion of each treatment was taken after 2,4,6,8 and 12 h. for (Vicine and Convicine) estimation. After then all samples were cooked for 30 minutes, then (Vicine and Convicine) were estimated. before and after cooking process.

Effect of soaking solution pH on vicine and convicine removal from dry beans : 150 g of dry beans were soaked in tap water (pH 7.2) at room temperature (25°C) for 12 hours. 100 gm of soaked beans was taken and divided into three parts. The first part was placed in tap water (pH 7.2) and the second part was placed in an aqueous solution (pH 5.5), as for the third part, was placed in an enzymatic solution (tap water with pH 5.5 plus BGS enzyme of 0.1 ml / 2 g bean) at 40 ° C and the samples were left for 12 hours, then a small portion of each treatment was taken after 2,4,6,8, 10 and 12 h. for (Vicine and Convicine) estimation. Then all samples were cooked for 75 minutes, then (Vicine and Convicine) were estimated.

Separation and characterization of lycosides (Vicine and Convicine) in dry and fresh

bean extracts using HPLC technique: Vicine and convicine were extracted with 7% perchloric acid (PCA) according to method mentioned by (28). Water extracts were adjusted to pH 4 with 1 N HCl to precipitate proteins. A 0.5 g sample of flour was placed in a centrifuge tube and 1 ml of uridine (8 mg/ml), was added as an internal standard. The extraction solution was added either once (15 ml) or in two (2 × 7.5 ml) or three cycles (3 × 5 ml) to test the extractability. The flour was extracted by mixing with a vortex mixer for 1 min and then centrifuging at 13000 g for 10 min. The extracts were collected, filtered (0.45-µm GH Polypro) and injected into the HPLC system on the optimum selected condition.

Statistical Analysis

Statistical Analysis System have been used to analysis the data to study the effect of various condition. The significant difference has been compared with the means by using least significant difference (30).

RESULTS AND DISCUSSION

Effect of beta-glucosidase enzyme on glycoside compounds level: Table 1 shows the effect of treating faba beans with different concentrations of beta-glucosidase enzyme 50, 100 and 150 µL (15.4 units/ml) on the glyosidic compounds (Vicine and Convicine) and on extracted Convicine degradation. The results showed that these compounds were reduced by 14, 24, 46 and 68% within 15, 30, 45 and 60 minutes, when 50µL of beta-glucosidase was used. While higher percentages were removed in case of convicine extract being 30, 53, 76 and 90% for same reaction period respectively. As the enzyme concentration increased, the percentages of removed glycosidic compound were increased, 21, 66 and 91% were removed within 15, 30 and 45 minutes when 100µL was added to vicine and convicine mixture solution. Complete removal was achieved after one hour of treating. In case of using Convicine alone, 57 and 90% were reduced after 15 and 30 minutes respectively. The extracted convicine completely removed after 45 and 60 minutes respectively, when the enzyme concentration increased to 150µL. A complete decomposition for vicine and convicine mixture was noticed after 45 minutes of reaction starting point, and after 30 min. for the convicine alone. Similar results were found by (27), they stated that only 11% of the vicine was degraded after 60 minutes with a low concentration of the enzyme (0.2 units/ml), and the rate of degradation was increased as the enzyme concentration increased. Using (3 units/ml and 6 unit/ml) of the enzyme increased the degradation of vicine to 47% and 65% within 15 minutes and 96.97% after 90 and 60 minutes respectively. As for Convicine, the removal rates were greater, it reached to 72, 91 and 99% within 15, 30 and 60 minutes, respectively when (3 units/ml) was used. several methods were used to remove these compounds, such as boiling and roasting, but these compounds are relatively stable to heat, so removing them completely is a challenge (23). However, the use of enzymatic method is the only way that has achieved the complete degradation, as the vicine and convicine are analyzed using the endogenous or additive beta-glucosidase enzyme or by fermentation with microbes capable of synthesizing this enzyme. Marquardt *et al*. (19) was reported that the toxic aglycones resulting from the decomposition of vicine and convicine (Divicine and isouramil) are released, they are supposed to react and decompose quickly due to their instability and thus lose their toxic

Table 1. Effect of beta-glucosidase enzyme concentration 50,100 and150 µL (15.4 units/ml) and the time of enzymatic reaction on vicine and convicine mixture (0.1mg/ml) and convicine elimination (%) during 15, 30, 45 and 60 minutes at 40C

elimination(%)						
Convicine			Vicine +Convicine			Enzyme(units)
150 µl	100 µL	50 µL	150 µL	100µ L	50 µL	min.
81	57	30	68	21	14	15
100	90	53	93	66	24	30
100	100	76	100	91	46	45
100	100	90	100	100	68	60

Bean content of pyrimidine glucosides (Vicine and Convicine) during growth and maturation stages: The content of pyrimidine glucosides (Vicine and Convicine) was estimated through four stages of bean ripening, starting from the seedling up to the mature bean and in the local dry bean as well, in order to determine the riskiest ripening stage in terms of toxicity and effect on hemolysis of blood red cell. Where it was found that the severity of allergy to beans depends on the content of these compounds in green or dry beans (5). The results in table 2 showed that the glucosides content of the experimental beans during its ripening development ranged from 22.7 mg/100g in the seedling stage to 46.8 mg/100g in the pods stage at 139 days. Regarding the bean content of Vicine, it ranged from 12.6 mg/100g in the seedling stage to 29 mg/100g in the pods stage at the age of 139 days per 100 g, and for Convicine it ranged from 10.1 mg/100g in the seedling stage to 17.8 mg/100g in the pods stage at the age of 139 days (Table 2). From the same table, it has been noticed intensive synthesis of these pyrimidines during the first period of fruit and seed formation (green pods 139 days), after then the concentration of these compounds started to decline through the development of fruit ripening. The formation of Vicine and Convicine was most intense during the stage of green pods at the age of 139 days, as it reached to 29 mg and 17.8 mg per 100 gm of bean, respectively, after that those values decreased during the development of ripening of the fruit and reached to 14.1 mg and 10.8 mg / 100 gm of bean at the age of 182 days. Formation of green pods after 160 days was one of the stages with a relatively high concentration of these compounds, where the level of vicine and convicine of pods was 20.8 mg and 13.9 mg / 100 g of bean pods, respectively. As for the stage of seedling growth and the formation of green seeds after 182 days, vicine and convicine contents were less than the aforementioned two stages of maturity. The statistical analysis revealed that there were significant differences ($p < 0.05$) in the content of Vicine and Convicine in the different stages of growth. These results are in agreement with what was stated by (7) who found that the level of vicine and convicine in young seeds of bean is low during the first two weeks and that the final formation and assembly of vicine and convicine occurs during the development of seed maturity. It also agreed with what was stated by (17), who found that the highest amount of these glucosides reached 24 mg / 100 g of dry matter in the pods that are 5-6 cm long, after which the quantity decreased to 9 mg / 100 g of dry matter in fully mature pods. It can be concluded from this study that the stage of formation of green pods may be the riskiest stage of maturity for children with high sensitivity to eating beans and the appearance of symptoms of poisoning, as stated by (5) that severe cases of bean poisoning appear when consuming fresh green beans compared to Consumption of dry beans.

Table 2. The content of green beans of Vicine and Convicine during different growth stages, as estimated by spectrophotometric method

+ Vicine(mg/100g) of bean Convicine	Broad bean
22.7	day 7
46.8	139
34.7	160
24.9	182
* 6.372	LSD : 0.05

Distribution of the vicine and convicine content in the parts of the green bean

The content of bean fruit parts of vicine and convicine was estimated for the purpose of identifying which part is the richest in these compounds. Especially since some may consume the covers of green pods and the covers of dry seed, as well as dry seeds or dry cotyledons. The results showed that these glucosides were concentrated in the green seeds and their pods (46.8 mg/100 gm of Beans) at the age of 139 days, (Table 3). The results of the statistical analysis showed that there were significant differences among the parts of green bean of the same age, as well as differences among the parts during different ages. As for the dry whole bean seeds, the pyrimidine glycosides were concentrated in a greater amount in the cotyledons (23.8 mg/100 gm of Beans. the content of cotyledons, seeds and seed coats of vicine and convicine was similar with the results mentioned by (25), and it can be said that it is possible that green seeds with their covers have a severe effect on

person's sensitive to bean based on what was stated by (5) and (17) that this sensitivity is often caused by eating unripe bean

Table 3. Distribution of vicine and convicine content on green bean parts as estimated by spectrophotometric method (mg/100g of beans)

LSD : 0.05	182	160	139	7	Age(day) / Sample
5.084 *	24.9	34.7	46.8	22.7	Seed with cover
4.921 *	19.4	26.5	40.2	18.3	seed without cover (cotyledon)
3.672 *	5.5	8.2	6.6	4.4	Seed cover
4.361 *	9	10.7	18.3	13.4	pods covers
---	3.667 *	4.501 *	4.077 *	3.291 *	0.05 : LSD

Effect of soaking time on enzymatic removal of vicine and convicine from dry beans: Table 4 shows the effect of the enzyme and the soaking duration on the content of vicine and convicine in dry beans. Soaking the dry beans in an aqueous solution with pH 5.5 containing beta-glucosidase enzyme 0.1ml/2gm bean (15.4 units/ml) for 4, 8 and 12 hours at 40°C led to a higher rate of removal of glycosidic compounds than soaking in an aqueous solution without an enzyme, as the removal percentage reached 49, 56 and 63% in the presence of the enzyme after 4, 8 and 12 hours, respectively, while it was 31, 44 and 62% without the enzyme at the same time periods. The results also show that the removal increased when the samples cooked in boiling water, whether with or without an enzyme, and that the removal was increased with the cooking progression time, and the highest removal was noticed after 8 h., but after 12 h., there are no significant differences between the aqueous solution with and without enzyme presence, whether it was before or after cooking process, and that the removal was done by the effect of acid only. The reason may be attributed to the inactivation of the enzyme after 12 h. The reduction in the amount of pyrimidine glycosides due to the use of the acidic solution can be attributed to the decomposition of these pyrimidines and their conversion to aglycones as a result of the acidic environment. Where (31) mentioned that a complete decomposition of vicine and convicine at 70, 80, and 90 °C for 2 minute using hydrochloric acid and the resulted aglycones (Divicine and isouramil), are unstable compounds and dissociates approximately within 60 minutes and completely in 120 minutes at pH 5 at temperature 37 °C (26). The reduction in level of these compounds in the beans after cooking process may be due to a partial exuding of these glycosides from the pod or seed to the cooking water, which was referred to by (7), where the amount of these compounds decreased by 20.5% of the original amount of dry beans.

Table 4. Effect of the enzymatic treatment and soaking time at 40 °C and pH 5.5 on the dry bean content of vicine and convicine

LSD :0.05	elimination(%) after cooking: Water (pH5.5) enzyme +	after cooking: Water (pH5.5)	elimination(%) before cooking: water (pH5.5) +enzyme	before cooking: Water (pH5.5)	Time / h
6.025 *	60	37	49	31	4
6.319 *	68	53	56	44	8
5.883 *	70	70	63	62	12
---	5.427 *	6.822 *	5.360 *	6.932 *	LSD : 0.05

Effect of soaking temperature on vicine and convicine removal of dry beans treated with the experimental beta-glucosidase

Table 5 shows the effect of adding beta-glucosidase enzyme 0.1ml/2gm bean (15.4 units/ml) to dry beans soaking solution (pH 5.5) over four hours' incubation at different temperatures 25 and 40°C. The results showed that soaking at 40 °C was more effective in removing both vicine & convicine from dry beans than that at 25 °C, and the same trends were seen even after cooking process, where the percentage of removal of these compounds at 40°C was 39 % and 35 % at 25°C before

cooking. While after cooking process those percentages reached to 64% at 40°C and 47% at 25°C. The statistical analysis revealed that there were significant differences between the different treatments. Mona *et al.,* (22) mentioned that vicine and convicine are heat-resistant compounds, and removing these compounds by heat treatment is difficult at a low temperature compared to high temperatures. (17) stated that extraction of vicine and convicine compounds increases with the increase in extraction temperature and the soaking period

Table 5. Effect of enzymatic treatment 0.1ml/2gm bean), temperature, and cooking process on dry beans content of vicine and convicine at pH (5.5) for 4 hours.

elimination(%)					
	after cooking		before cooking		
LSD :0.05	Water (pH5.5) enzyme +	Water (pH5.5)	Water (pH5.5) enzyme +	water (pH5.5)	Tem.
5.673 *	47	40	35	26	25C
5.287 *	64	51	39	33	40C
---	4.679 *	4.281 *	3.734 *	3.827 *	0.05 : LSD

Effect of soaking solution pH on vicine and convicine removal from dry beans

Table 6 shows the effect of soaking solution pH, the enzymatic treatment duration, and the cooking process on vicine and convicine content of dry beans. The optimum pH and optimum temperature for enzyme activity resulted a greater decrease in the content of pyrimidine glucosides in dry beans compared to untreated beans that were soaked in tap water (pH 7.2) and pH 5.5. The experimental enzyme showed positive impact in removing vicine and convicine from faba beans and the effectiveness of the enzyme was concentration and soaking temperature and duration dependent. Additionally, the percentages of vicine and convicine removal were higher after the cooking process (44, 55, 58, 70, 71, 71%) as compared to that before cooking (37, 48, 50, 66, 67, 67%) after 2, 4, 6, 8, 10, and 12 h. respectively. The statistical analysis revealed that there were significant differences among the different treatments. Cardador-Martinez *et al.,* (8) used the boiling and roasting treatments to remove the compounds, the results showed that the boiling process was more effective in removing in comparison to roasting process, as it achieved a removal rate of 30 and 60%, while roasting achieved only 12 and 40% of vicine and convicine, respectively. This may be due to the fact that the glycosidic compounds are soluble in water, which makes them available for removal at a greater rate compared to roasting. Hegazy and Marquardt., (16) mentioned that there is a direct relationship between the removal of these glycosidic compounds (vicine and convicine) and the time of soaking. Abdallah *et al* (1) indicated that the presence of acid enhances the permeability of vicine and convicine from the bean.

Table 6. Effect of enzymatic treatment (0.1ml/2gm bean) duration, soaking solution pH and the blanching process on the dry bean vicine and convicine% at 40 °C

elimination(%)							
	after cooking			before cooking			
LSD : 0.05	water (pH5.5) +enzyme	water (pH5.5)	Tab water (pH7.2)	water (pH5.5) +enzyme	water (pH5.5)	Tab water (pH7.2)	Time / h.
7.361*	44	22	17	37	17	11	2
6.558 *	55	37	26	48	30	17	4
6.502 *	58	42	29	50	37	22	6
7.238 *	70	56	42	66	49	28	8
7.194 *	71	63	44	67	51	31	10
6.557 *	71	72	48	67	58	36	12
---	6.894 *	7.118 *	5.438 *	5.920 *	4.508 *	4.392 *	0.05 : LSD

Removal of vicine and convicine from dried beans using BGS enzyme.

Table 7 shows the effect of adding beta-glucosidase enzyme 0.15 ml/2gm bean (15.4 units/ml) to the dry beans soaking solution (pH 5.5, 40°C) during 2,4,6,8 hours. The level of vicine and convicine in the presence of the BGS enzyme was decreased with the progress of soaking time, where the highest removal percentages (85%) was seen after 8 h. of soaking as compared to the sample not treated with the enzyme (44%). These values for samples before cooking process. After cooking process, the removal% reached to 90% for 8 h. soaked enzyme-treated sample in comparison to the non-enzymatic treatment sample, where the removal was 52%. The results of the statistical analysis showed significant differences among different soaking periods, whether before or after cooking, and there were no significant differences between the samples at 8 hours in the presence of BGS enzyme before and after cooking. This may be due to the fact that the boiling process inactivated the enzyme. Rizzello *et al.,* (29) stated that the complete decomposition of vicine and convicine in fababean suspensions were noticed when incubated with different types of lactic acid bacteria (LAB), including *Fusarium gramineaum* (at 25 ° C for 72 h.), *L. plantarum* (at 30 ° C for 48 h.) and *L. plantarum* B24W (at 30 ° C for 48 h.) because all of these species are capable of producing beta-glucosidase enzyme.

Table 7. Effect of BGS enzyme (0.15 ml/2gm bean) and the time of the enzymatic treatment and the cooking process on the removal of vicine and convicine from dry bean at 40 °C and pH 5.5 during soaking process

elimination(%)					
	after cooking		before cooking		
0.05 : LSD	Water (pH5.5) enzyme) +	Water (pH5.5)	Water (pH5.5) +enzyme	Water (pH5.5)	Time / h.
4.921 *	45	30	31	23	2
6.447 *	66	38	53	33	4
6.982 *	87	48	74	38	6
7.015 *	90	52	85	44	8
---	6.913 *	5.386 *	7.901 *	5.338 *	0.05 : LSD

Effect of enzymatic treatment and cooking process on vicine & convicine content in dried beans: Table 8 shows the effect of adding beta-glucosidase enzyme 0.2 ml/2gm bean (15.4 units/ml) on the level of the glycoside compounds vicine and convicine to dry beans during different time periods 2,4,6,8 hours, as the dry beans were treated with beta-glucosidase enzyme at pH 5.5 and temperature 40 C These are the optimum conditions for the enzyme to work. Samples were taken after 2,4,6,8 hour intervals to follow up the changes in the bean samples content of vicine and convicine. As it is clear from the table that there is a noticeable decrease in the level of vicine and convicine with the progression of the treatment period and that the enzymatic treatment is the best because it achieved an amount of decrease that exceeded the others and they were 51, 80, 91, and 91% after 2, 4, 6, and 8 hours in dry bean samples before boiling and 61, 85, 96, 100% after boiling, and this is due to the action of the enzyme as it works to decompose these compounds. A few samples were selected from the same table being 6 h. enzyme treated sample before cooking process and 8 h. enzyme treated after cooking for Vicine and Convicine percentages determination by HPLC technique to ensure the accuracy of the spectrophotometric method results. The removal percentages from both techniques were close, as it is listed in table 8. Pulkkinen., (27) found that the rates of hydrolysis of Vicine and Convicine depends on the enzyme concentrations and treatment times. It was found that at low concentration 0.5 units/ml) 23% and 41% of t Vicine and Convicine were removed, respectively, after 60 minutes at pH 5 5 and temperature of 37 °C. Increasing the enzyme concentration to ten-fold led to remove 59% and 84% of Vicine and Convicine respectively after 15 minutes, and 94% and 100% after 60 minutes. Using higher concentration activity of the enzyme 10 units/ml achieved73% and 93%, removal of Vicine and Convicine by respectively, within 15 minutes, and the complete removal occurred after 30-45 minutes.

Table 8. Effect of the enzyme (0.2 ml/2gm bean) and the time of the enzymatic treatment and the cooking process on the content of dry bean of vicine and convicine at 40 °C and pH 5.5 during the soaking process

Elimination (%)					
	After cooking		Before cooking		
LSD : 0.05	Water (pH5.5) + enzyme	Water (pH5.5)	Water (pH5.5) + enzyme	Water (pH5.5)	Time / h.
5.713 *	61	24	51	21	2
7.946 *	85	37	80	30	4
7.263 *	96	44	86 ● 91	39	6
6.529 *	100 ● 100	55	91	48	8
---	7.137 *	5.983 *	8.632 *	7.304 *	LSD : 0.05

● Elimination (%) for HPLC analysis

Removal of vicine and convicine from fresh beans using beta-glucosidase in soaking solution

Table 9 shows the effect of adding beta-glucosidase enzyme 0.2 ml/2gm bean (15.4 units/ml) on vicine and convicine% in fresh beans during 2,4,6, and 8h. soaking at 40 °C, pH 5.5 (the optimum conditions for the enzyme activity). Samples were taken after 2,4,6,8 h. to follow up the changes in the bean samples content of vicine and convicine. As it is obvious from the table, there is a noticeable decrease in the level of vicine and convicine with the reaction time and the enzymatic treatment was superior as compared to the treatment without the beta-glucosidase. About 72 and 91% of vicine and convicine content were eliminated after 2 &4 h. of the treating time. Complete removal was achieved after 6 hours, as these samples subjected to boiling process, 91% removal was achieved after two h., and complete removal was noticed within four h. This is due to the action of the enzyme as it works to decompose these compounds. A few samples were selected from the tables above, and the mount of Vicine and Convicine were estimated by HPLC technique to ensure the accuracy of the results obtained by the spectrophotometric method used, and the results from both techniques were similar as shown in the same table. Luzzatto., (18) pointed out that there are many sources of variation in the levels of divicine and isouramil that will attack red blood cells, including that the glycosides in the bean, when eaten raw, are primarily responsible for the release of divicine and isouramil, but when they are cooked, the glucosides are greatly inactivated. Whereas, cooking and roasting leads to the breakdown or decomposition of these glycosides, and this is the main reason that the incidence of Favism increases when eating uncooked beans more than eating cooked beans. Also, the time of harvest affects the content of glycosides, as the youngest beans have higher levels of ripe beans and also the content of these glycosides varies in different species. Cardador-Martinez *et al*., (8) stated that vicine and convicine are thermally stable (boiling points are 242 and 244, respectively), so cooking and boiling do not remove more than 50% of these compounds.

Table 9. Effect of the enzymatic treatment (0.2 ml/2gm bean) and cooking process on vicine and convicine removal in fresh bean soaked for 2,4,6 and 8 h. at 40 °C and pH 5.5.

Elimination (%)					
	After cooking		before cooking		
LSD : 0.05	Water (pH5.5) enzyme +	Water (pH5.5)	Water (pH5.5) +enzyme	Water (pH5.5)	Time / h.
6.783 *	82 ● 91	41	72	29	2
8.166 *	100	50	91	33	4
8.075 *	100	60	100	45	6
7.558 *	100	66	100	57	8
---	7.509 *	5.935 *	7.931 *	5.025 *	0.05 : LSD

● Elimination (%) for HPLC analysis

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