

Occurrence of Acrylamide in selected Iraqi bakery products and mitigation by partially purified L-Asparaginase.....

Hyder N. Al Zobaidy , Khalida A. Shakir , Selma Abdulhussain , Gale M. Strasburg

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Abstract

The plenty of scientific evidence on the consequences of dietary acrylamide exposure, justify the requirement to reduce the acrylamide formation in foods. Among the mitigation strategies is the Asparaginase pre – treatment. Therefore, the following study was undertaken to investigate the Acrylamide occurrence in some Iraqi local bakery products as well as using a partially purified Asparaginase for Acrylamide mitigation. After using Ammonium sulfate in Asparaginase precipitation, from Pole beans, the Asparaginase shown to precipitate out at saturation range between 30 – 80 %. In bakery tested samples Acrylamide content were 189.25 – 222.75, 178.75 – 188 and 177.5 – 183.75 µg/Kg for Iraqi traditional Sammoun , Iraqi flat bread and Iraqi bread respectevily. In two samples of biscuit Acrylamide content was 25 and 50 µg/Kg. While no Acrylamide occurrence found to be in other three tested biscuit samples. After adding the crude enzyme and partially purified enzyme to the dough, from Farinograph record, the water absorption values were 58.6% and 59.6% respectively as well as the dough stability time (DST) was 11.5 and 10 min respectively. There were no changes in final loaf properties after enzyme treatment that was confirmed from measuring loaf volume and weight and texture. The addition of crude enzymes and dialyzed partially purified Asparaginase (300 U/kg) to the loaf recipe results in reduction the acrylamide content 39.7% and 42% respectively.

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Introduction

The presence of acrylamide in cooked foods was announced in April 2002 by a number of Swedish research projects which investigate the presence of acrylamide in roasted coffee and coffee products (19). Since that the problem of acrylamide in food became topical due to its toxicity and probability of being a human carcinogen (12). Until present, there are no legal regulations concerning the AA levels in food the only legal action assumed so far was the discard of child biscuits containing more than 1000 mg/kg from the Swiss market in 2005 (10). Acrylamide can form in some bakery products as a result of the reaction between asparagine and the carbonyl group of reduced sugar via Maillard reactions (16), (23). The development of browning in bakery products during baking decreases the bioavailability of proteins and amino acids and leads to the formation of Acrylamide (18). Many possible methods have been identified to reduce acrylamide levels in foods such as avoiding excessive browning when frying, baking or roasting (7). Amrein *et. al.* (1) believe that the concentration of precursors in the raw material along with processing heat are key factors for acrylamide formation, subsequently the control of these factors during food process is vital for the Acrylamide reduction. The latter researches based on precursor depletion like fermentation and Asparaginase pre-treatments. Asparaginase pre-treatment of raw potatoes and dough was effective to reduce acrylamide levels without altering the properties of the final product (23) (6). It is well known Asparaginase hydrolyze the asparagine into aspartic acid and ammonia, thereby specifically eliminating a key precursor for acrylamide formation (15). Allow the consumption of Asparaginase as a food additive has been proposed (2). The aim of this study is to investigate acrylamide formation in some local bakery products as well as partially purification of Pole beans Asparaginase moreover the effect of the addition of crude enzymes and partially purified Asparaginase on loaf properties and acrylamide formation during standard loaf baking.

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Materials and methods

Pole beans (*Phaseolus vulgaris* L.) pods were collected from a glasshouse at Michigan State University (USA). Healthy looking pods were taken and washed with a distilled water to remove dust and any other unwanted materials. Fresh pole beans of 250g about 10-12 cm long and about 0.8 – 1 cm thick, were blended with three volumes of 0.01 M Tris.HCl pH 8.6 with 0.1 M KCl solution, homogenized for 2 minutes, large parts filtered by Watman filter No.1 and centrifuged at 15000 xg for 15 minutes to remove the fine parts. The supernatant was designated as a crude extract (3). The Protein estimated by BCA protein assay using bicinchoninic acid (BCA). The enzymatic activity determined by Asparaginase activity kit obtained from Sigma – Aldrich Company. The activity is determined by a coupled enzyme assay, which results in colorimetric (750 nm) / fluorometric ($\lambda_{ex} = 535 / \lambda_{em} = 587$ nm) products proportional to the aspartate generated. One milliunit (mU) of Asparaginase is defined as the amount of enzyme that catalyzes the formation of 1 μ mole of aspartate per minute at 25 °C. Ammonium sulfate saturation percentages (20, 30, 40, 50, 60, 65, 70 and 80) % were set up to add to precipitate the crude enzyme. The precipitation allowed to continue for about 30 min. Subsequently, the mix was centrifuged at 10,000 xg for 10 min, carefully the supernatant poured off and the protein dissolved in appropriate volume of 0.01 M Tris.HCl buffer at pH8.6. Whole process achieved in a cold room (5). The ammonium sulfate precipitate enzyme was dialyzed against Tris – HCL buffer (pH=8, 0.05 M)for 24 hours with three substitutions of dialysis buffer. Bakery products from different regions of Baghdad province were subjected to acrylamide investigation. Iraqi flat bread (B1, B2), Sammoun hajary (B3, B4), Sammoun kahrbaee (B5, B6) and Biscuit Marry (B7) & (B8) were tested as well as some common bakery products, available in Iraqi markets biscuit Meno, cocoa biscuit and biscuit Tard (B9, B10 and B11) respectively. The acrylamide was analyzed by gas chromatography according to Perkins Elmer field application report. The following GC conditions were used in analyzing

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both acrylamide standards and food samples: Column: Elite-5MS - L 30 m, ID 250 μ m DF 1., Inj: 1.0 μ L, 0.00 min. hold, Injector temp.: 250 °C, Carrier gas: helium, constant pressure, Flow: 25 ml/min and oven temp.: initial 40 °C (hold 1.0 min.), Ramp 1: 20 degree/min. to 180 °C hold for 0.00 min. The Farinograph was conducted with the small Brabender Farinograph bowl and the constant flour weight procedure. Hard wheat flour from Mennel Milling Company, Fostoria – Ohio of 100 gm at 14% moisture basis was weighted and placed into the mixing bowl along with 3 gm of shortening and 1 gm of L – Asparagine. Yeast, sugar – salt and ascorbic acid solutions of 20, 11 and 5 ml was added to the flour. The dough placed in bowl greased with shortening and placed in the proofing cabinet for 90 min interrupted by 1st, 2ed punch and panning after 52, 25 and 13 min respectively. After the panning the dough was rolled, molded, put in greased pan and placed in the proofing cabinet for 33 min. Finally the loaf was baked for 24 min at 215 °C (9). The loaf cut to observe the color and the appearance. The texture was measured by the texture analyzer and the acrylamide formation was estimated as well. Crude enzyme and partially purified Asparaginase of 300 U/Kg were added to the experimental loaf bread recipe then the bread properties were measured as well as the acrylamide reduction. The collected data were statistically analyzed using analysis of variance (ANOVA) by GENSTAT computer software package. Differences between treatment averages were compared using Least Significant Difference (LSD) \leq 0.05 probability level.

Results and discussion

L -Asparaginase purified from pole beans, using a salt precipitation are shown in figure (1). The enzyme precipitated out between 30% - 80% of ammonium sulfate saturation. Toribio, *et. al.* (20) reported that the Asparaginase can be isolated in the 30-70% saturation fraction by using fractional precipitation with ammonium sulfate. Other study demonstrated that Asparaginase precipitated out between 40% - 60% of ammonium sulfate saturation (17).

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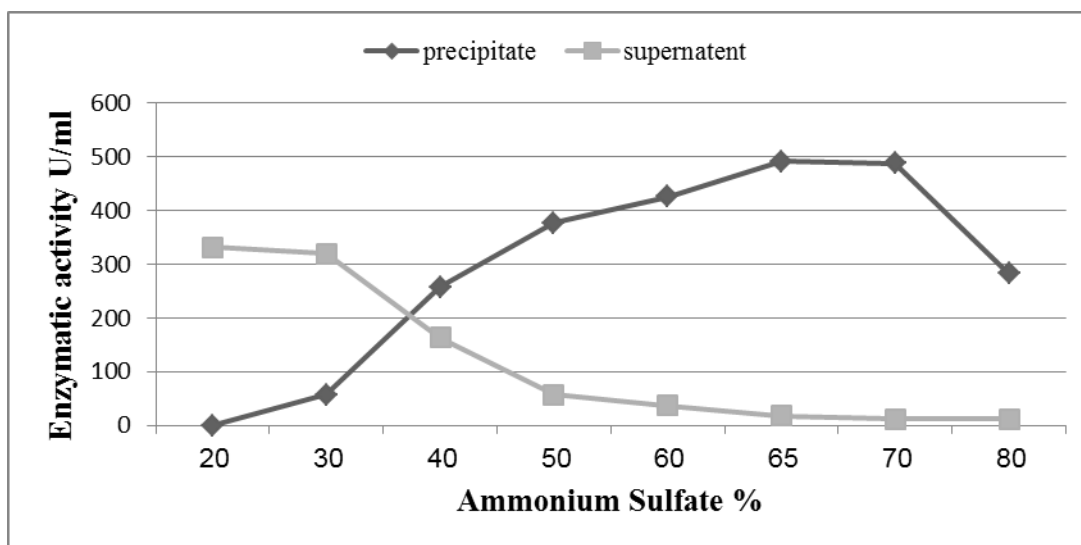


Fig. 1. Ammonium sulfate precipitation of L – Asparaginase

The investigations of the Acrylamide occurrence in the local bakery products are placed in fig. (2). The highest level of Acrylamide was recorded for Iraqi traditional Sammoun 189.25 – 222.75 $\mu\text{g}/\text{Kg}$, followed by the acrylamide content of Iraqi flat bread 178.75 – 188 $\mu\text{g}/\text{Kg}$ and Iraqi bread 177.5 – 183.75 $\mu\text{g}/\text{Kg}$ (these samples were collected from two different region of Baghdad province).

Considerable numbers of acrylamide levels were also received for two different kinds of locally available biscuit in Iraqi markets, 25 and 50 $\mu\text{g}/\text{Kg}$. While no Acrylamide occurrence found to be in other three tested biscuit samples. Some products may be relevant for certain consumers. If, for example, infants daily eat 50 g of biscuits containing 500 mg/kg_1 acrylamide, their exposure could be 25 mg/ day (10). In correlation, a report published by European Food Safety Authority (EFSA) (8) demonstrated that Acrylamide levels in biscuit samples collected in 2007 was about 30 $\mu\text{g}/\text{Kg}$.

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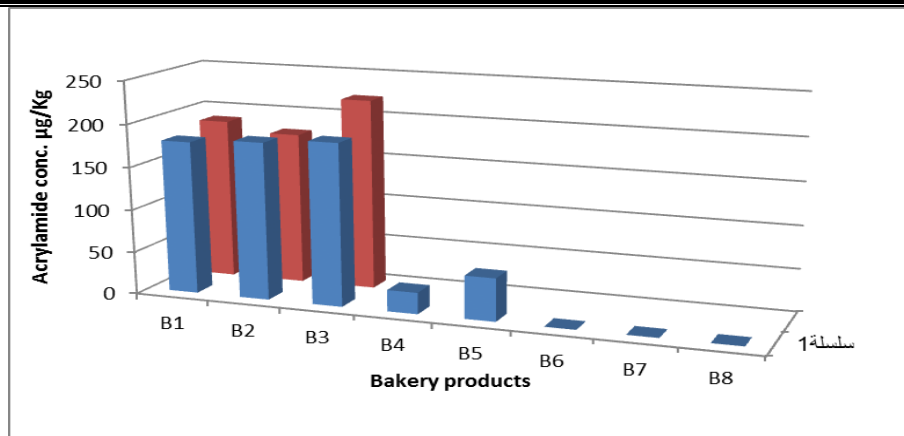


Fig. 2. Acrylamide levels in some bakery categories collected from local Iraqi markets.

The higher level of the Acrylamide was detected in the Iraqi traditional Sammoun, ranging between 189.25 – 222.75 µg/Kg (samples from two different region of Baghdad province) (fig.3). Collected data produced by European Union Member States on the acrylamide content of food published that the maximum acrylamide content reported for bread

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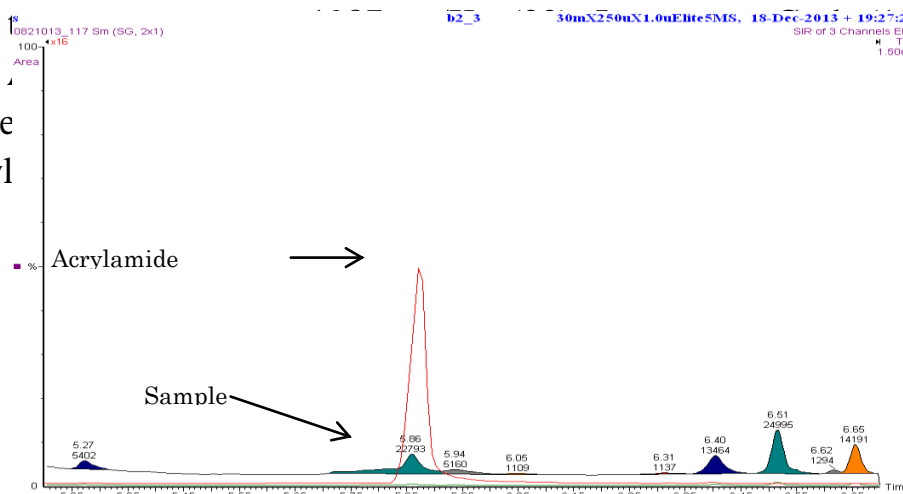


Fig. 3. GC – MS analysis of Acrylamide showing the highest tested sample vs. the standard

and 59.0% for crude enzyme treatment and partially purified enzyme respectively. The water absorption estimated when the Farinogram peak is centered on the 500 BU (Brabender Units) (fig.4). High water absorption is desired in bread baking because a less flour is needed to make a loaf. For

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yeast bread 55–65% water absorption is appropriate (13). The dough stability time (DST) was calculated from the farinograph as well (fig.4). For crude enzyme treatment and partially purified enzyme (DST) were 11.5 and 10 min respectively. Good quality dough has stability of 4–12 min (14). Previous study revealed that the Asparaginase could enzymatically hydrolyze the Asparagine and decrease acrylamide formation without modification of the recipes (10).

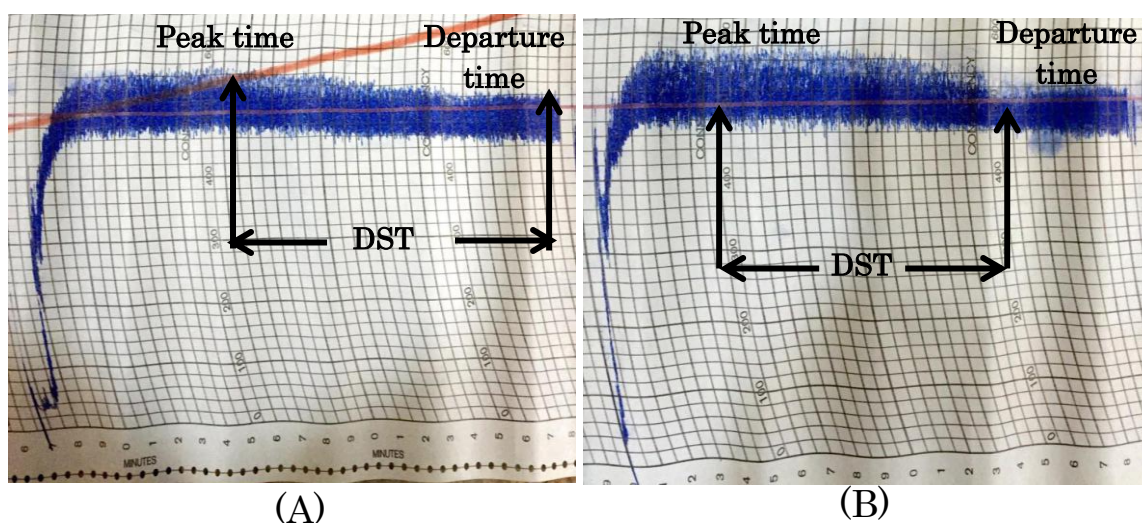


Fig. (4). Farinograph records of (A) crude enzyme treatment and (B) partially purified enzyme treatment.

Measuring the properties of Asparaginase pretreated loaf demonstrate that the volume of the control, crude enzyme treatment and partially purified enzyme treatment were 655, 620 and 655 cm³ respectively (table 1), (fig.5). Volume of the loaf is usually taken as the best single aspect on which judgment is based. Good baking quality flour has to give 500–600 cm³ loaf volumes based on 100 g flour (14).

Table 1. Volume, weight and texture of loaf from 100 gm flour dough baked with different enzyme treatments.

Treatment	Volume cm ³	Weight gm	Crumb Texture N.cm ⁻²
Control	655.7	141.3	4.23

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Crude enzyme	620.8	146.05	5.07
Partially purified enzyme	655.3	145.11	3.91
L.S.D 0.05	N.S	N.S	0.64

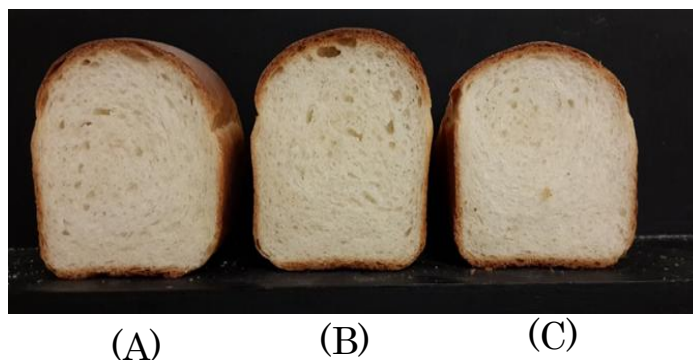
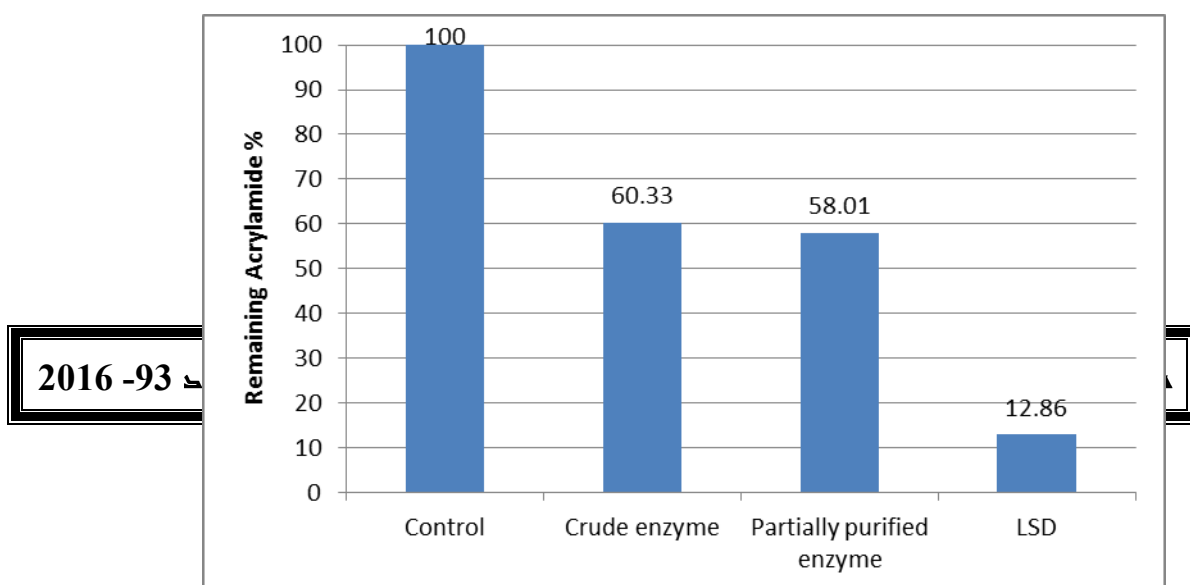


Fig. 5. Images of cross section of loaf (A) Control (B) Partially purified enzyme treatment (C) Crude enzyme treatment.

The addition of crude enzymes and partially purified Asparaginase (300 U/kg) led to a reduction in acrylamide content 39.7% and 42% respectively (fig.6). The addition of asparaginase had no effect on loaf properties as showed in table (1). Previous study revealed that Asparaginase reduced acrylamide formation up to 88% and had no effect on browning development during toasting of brad (6). Vass et al. (21) demonstrate that addition of enzyme to crackers decreased acrylamide levels by 70% and no changes in colour or flavour of the final products could be noticed. Studies have revealed that Asparaginase activity is affected by enzyme dose, temperature, reaction time and pH when the reaction occurs (11).



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Fig. 6. Remaining acrylamide after bread treatment with crude enzyme and partially purified Asparaginase.

Conclusion

The present study indicates that a considerable level of Acrylamide appeared in Iraqi bakery product but also illustrate the pretreatment of dough with crude and partially purified Asparaginase has successful result in reducing the Acrylamide content about 40 %. However loaf properties dose not affected by the enzyme treatment. Furthermore the Pole beans was a good source of Asparaginase.

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تواجد مركب الاكريلاميد في بعض منتجات المخابز العراقية و تقليله

بإضافة انزيم الاسبارجينيز المنقى جزئيا

حيدر ناجي الزبيدي

قسم الانتاج الحيواني – كلية الزراعة / جامعة واسط.

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غيل ستراسبورغ

قسم علوم الاغذية وتغذية الانسان / جامعة ولاية ميشيغان - امريكا.

المستخلص

وفرة العديد من الادلة العلمية حول عواقب استهلاك مركب الاكريلاميد من خلال الغذاء استوجب الحاجة الى العمل على تقليل تواجد هذا المركب في الاغذية. من بين هذه الطرق المستخدمة لتقليل تواجد الاكريلاميد , هو المعاملة الاولى بانزيم الاسبارجينيز. لذلك عنيت الدراسة الحالية بالتحري عن تواجد مركب الاكريلاميد في بعض منتجات المخابز العراقية, اضافة الى استخدام انزيم الاسبارجينيز المنقى جزئيا في تقليل تكون هذا المركب. بعد استخدام ملح كبريتات الامونيوم في ترسيب الاسبارجينيز من نبات الفاصولياء ظهر ان هذا الانزيم يترسب في مديات من التشبع بملح كبريتات الامونيوم تراوحت بين 30 - 80 %. محتوى المخبوزات المفحوصة من مركب الاكريلاميد كان 189.25 - 222.75 و 178.75 - 188 و 177.5 - 183.75 مكغم / كغم لكل من الصمون الحجري العراقي و الخبز العراقي والسمون الكهربائي على التوالي. كما وجد ان محتوى الاكريلاميد في عينتان من البسكويت 25 و 50 مكغم / كغم. بينما ثلاث عينات اخرى من البسكويت كانت خالية من مركب الاكريلاميد. بينت قراءة الفارينوكراف ان الامتصاصية المئوية للعجينة بعد اضافة الانزيم الخام والمنقى جزئيا الى العجينة 58.6 % , 59.6% على التوالي و وقت الثبات للعجينة 11.5 و 10 دقيقة على التوالي. لم يكن هناك اي تاثير على خصائص خبز اللوف الناتج بعد المعاملة الانزيمية هذا ما بينته قياسات المنتج النهائي كالحجم والوزن والنسجة. ادت اضافة الانزيم الخام والمنقى جزئيا و بواقع 300 وحدة / كغم الى اختزال مركب الاكريلاميد بنسبة 39.7% و 42% على التوالي.