

The effect of asparaginase on acrylamide formation in French fries

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Abstract

Acrylamide formation in French fries was investigated in relation to blanching and asparaginase soaking treatments before final frying. Par-fried potatoes of Bintje variety were prepared by cutting strips (0.8 × 0.8 × 5 cm) which were blanched at 75 °C for 10 min. Unblanched strips were used as the control. Control or blanched strips were then dried at 85 °C for 10 min and immediately partially fried at 175 °C for 1 min. Finally, frozen par-fried potatoes were fried at 175 °C for 3 min to obtain French fries. Pre-drying of raw or blanched potato strips did not generate acrylamide formation as expected. Partial frying of pre-dried control potato strips generated 370 µg/kg of acrylamide and the final frying determined French fries with 2075 µg/kg of acrylamide. When control potato strips were treated with a 10000 ASNU/l asparaginase solution at 40 °C for 20 min, the acrylamide formation in French fries was reduced by 30%. When blanched potato strips were treated in the same way, the produced French fries have 60% less acrylamide content than blanched strips without the enzyme treatment. Soaking of blanched potato strips (75 °C, 10 min) in an 10000 ASNU/l asparaginase solution at 40 °C for 20 min is an effective way to reduce acrylamide formation after frying by reducing the amount of one of its important precursors such as asparagine.

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1. Introduction

Large variation in suitability of potato (*Solanum tuberosum*) for processing of potato chips and French fries have special quality demands compared to ware potatoes. On the other hand, deep fat frying is extensively used in food processing both industrially and at home, and fried potato products are one of its largest applications. Frying of potato strips is based on heat transfer from the hot oil, which results in water removal and oil uptake by the piece (Aguilera & Gloria-Hernandez, 2000). French fries represent a composite structure formed by two regions: (i) an external dehydrated and crispy where oil is located and (ii) a humid and cooked core free of oil. The external crust is

very similar to the structure of a fried potato slice or potato chip (Bouchon, Aguilera, & Pyle, 2003; Pedreschi & Aguilera, 2002; Pedreschi, Aguilera, & Pyle, 2001). For French fry processing, firstly the raw potato strips are blanched in hot water and dried with hot air until reaching a moisture content of around 60 g water/100 g (total basis). Then, the dried potato strips are fried in hot oil (160–190 °C), cooled, frozen and finally packaged (Bunger, Moyano, & Rioseco, 2003). The final preparation of the par-fried frozen potatoes could accomplish by a final frying, baking or microwave cooking. Final oil and moisture content of French fries are around 15 g/100 g and 38 g/100 g (total basis), respectively (Aguilera & Gloria-Hernandez, 2000; Saguy & Dana, 2003).

It has been confirmed that a wide range of food – prepared industrially, in catering or at home – contain high levels of acrylamide. This includes staple foods like bread,

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fried potatoes and coffee as well as specially products like potato chips, biscuits, French fries, bread, and a range of other heat-processed products (Rosen & Hellenäs, 2002). Acrylamide, a compound present in potato chips, has been classified as probably carcinogenic in humans, so it is important to reduce the contaminant levels in these products (Rosen & Hellenäs, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). As acrylamide has not been detected in unheated or boiled foods, it was considered to be formed during heating at high temperatures (Becalski, Lau, Lewis, & Seaman, 2003). They attributed this fact to the higher temperatures reached in Maillard nonenzymatic browning reactions required for desirable colour, flavour and aroma production (Coughlin, 2003). It has been stated that many times, Maillard reaction is the principal mechanism for acrylamide generation. Crucial participants in Maillard reaction are amino acid compounds (e.g. asparagine for the case of potato) and reducing sugars (fructose and glucose) (Mottram & Wedzicha, 2002).

Recent findings of acrylamide in foods have focused research on the possible mechanisms of formation. Some authors presented a mechanism for the formation of acrylamide from the reaction of the amino acid asparagine and a carbonyl-containing compound at typical cooking temperatures (Zyzak et al., 2003). The confirmation of this mechanism was accomplished through selective removal of asparagine using asparaginase which resulted in a reduced level of acrylamide in a selected heated food. The potential capability of different potato varieties to form acrylamide during heat treatment correlated well with the concentration in the tubers of reducing sugars (especially glucose and fructose) and asparagine. The potato cultivars show large differences in their potential to form acrylamide which was primarily linked to their sugar contents (Amrein et al., 2003). Besides, Amrein, Schobachler, Escher, and Amadó (2004) studied the influence of asparaginase and other ingredients on acrylamide formation in gingerbread concluding that a significant reduction on acrylamide in gingerbread could be achieved by using sodium hydrogencarbonate. The application of asparaginase in dough resulted in 55% decrease of acrylamide content, in 75% degradation of asparagine, and in no detrimental effect on taste and color of gingerbread. On the other hand, Ciesarova, Kiss, and Boegl (2006) evaluated the impact of L-asparaginase on the acrylamide content reduction after high heat treatment in a model system as well as in potato based material. They found that an important mitigation of acrylamide content (90–97%) was achieved also in products prepared from dried potato powder treated by L-asparaginase.

It has been developed the production of asparaginase based on cloning of *Aspergillus oryzae*. The *A. oryzae* asparaginase has been cloned and expressed in commercial relevant yields in *A. oryzae*. The *A. oryzae* is well known for production of food enzymes. The *A. oryzae* asparaginase has pH optimum at pH 6–7 with good activity between pH 5 and 8, which may be the pH range for pro-

duction of potato products like French fries and crisps (tested by Novozymes A/S). The optimum temperature of the asparaginase activity measured at pH 7 is 60 °C (Novozymes A/S). However at temperatures above 60 °C the enzyme activity decreases rapidly (Novozymes A/S) wherefore a temperature range between 40 and 60 °C may be preferable (a linear increase in activity from 60% to 100% is seen within the temperature range 40–60 °C) (Food Standards, 2007). In general, the degradation of the asparaginase enzyme increase with increasing temperature.

A biotechnology company has introduced a commercially enzyme solution that reduces acrylamide. This enzyme is an asparaginase that can reduce acrylamide levels by up to 90% by converting asparagine into another common amino acid, aspartic acid, without altering the appearance or taste of the final product (Vang Hendriksen et al., 2006). This commercial enzyme has also received generally recognized as safe (Kuilman & Wilms, 2007) status from the US, approval from the Danish authorities and a positive evaluation from the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2007; Novozymes Switzerland AG, 2007).

A strategy to reduce the acrylamide in food products would be to reduce the precursor levels in the raw materials. The precursor levels may be reduced by choosing optimum cultivars and by modification of growth and storage conditions. For potato processing industries, e.g. industries for chips or French fries, it is possible to reduce the acrylamide precursor levels e.g. asparagine and reducing sugars either by blanching, by soaking in water or acidic solutions potato pieces, respectively (Pedreschi, Kaack, & Granby, 2006). The objectives of this research were: (i) to study the effect of enzymatic treatment with asparaginase on reducing the asparagine precursor for acrylamide formation in French fries; (ii) to study the interactions between asparaginase and other relevant pre-treatment and processing parameters (temperature for enzyme treatment, blanching and unblanching conditions, etc.); and (iii) to study the acrylamide formation during the different steps in the production of par-fried potatoes and French fries.

2. Materials and Methods

2.1. Materials

Potatoes (variety Bintje, 79 g/100 g of wet weight, length >13 cm) and vegetable oil (Fritao, Denmark) were the raw materials. Potatoes stored at 8 °C and 95% of relative humidity were washed and peeled in an industrial peeler IMC (model M591E4, England). Strips of cross sections of 0.8 × 0.8 cm² were cut from the pith of the parenchymatous region of potato tubers. A ruler and a knife were used to provide strips with a length of 5 cm. Asparaginase enzyme was given by Novozymes A/S. This enzyme has a molecular weight of approximately 36,000 Da, optimum temperature and pH of 60 °C and 7.0, respectively. Its activity ranges between pH 5 and 9.

2.2. Pre-treatments

Strips were rinsed immediately after cutting for 1 min in distilled water to eliminate some starch material adhering to the surface prior to frying. The following pre-treatments were studied after frying:

- (i) C: Control strips were considered those raw potato slices rinsed in water distilled without any treatment.
- (ii) B: Blanched strips were considered those strips immersed in hot distilled water at 75 °C for 10 min in a ratio of potato to water (g/g) of 0.5.
- (iii) E1: First case of asparaginase (Novozymes A/S, Denmark) treated samples was considered those strips immersed in a 10000 ASNU/l asparaginase solution at 40 °C for 20 min (ratio of potato to enzyme solution –g/g– of 0.5).
- (iv) E2: Second case of asparaginase treated samples was considered those strips immersed in a 10000 ASNU/l asparaginase solution at 50 °C for 10 min (ratio of potato to enzyme solution –g/g– of 0.5).
- (v) E3: Third case of asparaginase treated samples was considered those strips immersed in a 10000 ASNU/l asparaginase solution at 60 °C for 10 min (ratio of potato to enzyme solution –g/g– of 0.5).

2.3. Production of cooked par-fried potatoes

The following steps were followed to produce French fries and first, par-fried potatoes:

- (i) Drying (D): Control (C), blanched (B) or asparaginase treated strips (E1, E2 or E3) were dried in a convection oven at a dry bulb temperature of 85 °C for 10 min.
- (ii) Par-fried (P): Dried strips were partially fried in an industrial fryer containing 100 L of oil at 175 °C for 1 min. Frying temperature was maintained constant since the potato mass to oil mass ratio (g/g) was kept very low (0.001333). Par-fried potatoes were frozen and store at –30 °C for 2 days before final frying.
- (iii) Final frying (F): 30 strips of frozen par-fried potatoes were fried in an industrial fryer containing 100 L of oil at 175 °C for 3 min. Frying temperature was maintained constant since the potato mass to oil mass ratio (g/g) was kept very low (0.001333).

2.3.1. Analysis

It was used a liquid chromatography-tandem mass spectrometry analytical methodology for simultaneous analysis of acrylamide and their precursors such as asparagine and glucose (Nielsen, Granby, Hedegaard, & Skibsted, 2006). Two replicates for analytical determinations were used. French fries were homogenized using a Brand handheld mixer (type 4169) fitted with a blended-like sample com-

partment (type 4297, Braun AG, Germany). An aliquot of approximately of 4 g of homogenate was transferred to a centrifuge tube and 40 ml of deionised water was added by using a dispenser. Internal standards and maltitol were added at the following levels: 200 µl 10 µg/ml D₃-acrylamide, 100 µl 880 µg/ml ¹⁵N₂-asparagine, 400 µl 0.2% sorbitol and 200 µl 0.2% maltitol. The sample was extracted by an Ultra Turrax T25 homogenizer (Janke & Kunkel, Germany) at 10,000–12,000 rpm for 2 min. The sample was centrifuged at 500g for 20 min (Heracaus Multifuge, Osterode, Germany) and an aliquot of 4 ml was transferred to Eppendorf vials and frozen to –18 °C for 30 min or more and subsequently microcentrifugated at 12,100g for 10 min (Eppendorf Ag Minispin Centrifuge, Germany). The sample thaw during centrifugation and starch precipitated from the supernatant at this low temperature. The SPE cleanup was performed by an automated Gilson sampler (Gilson Aspec Xli, US) using LiChroLut Rp-C18 SPE-cartridges (500 mg) from Merck (Germany) conditioned with 2 ml of methanol 2 × 2 ml of water and 0.5 ml of sample lead to waste. Subsequently 1.75 ml of sample was loaded onto the cartridge and the eluate transferred to Miniprep PTFE filter HPLC vials with a pore diameter of 0.45 µm (Whatman Inc., USA).

The LC system consisted of a HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, USA) equipped with a vacuum degasser, a solvent delivery compartment with high pressure mixing, an autosampler and a column compartment. The autosampler was kept at 10 °C and the injection volume was 10 µl. Separation was performed on a Hypercarb column (dimensions 2.1 mm × 100 mm, particle size 5 µm). In front of the LC-column was a C18 ODS SecurityGuard column (dimensions 4 mm × 2 mm). from Phenomenex (Cheshire, UK). The column was eluted with 0.1% formic acid in water with a flow rate of 0.2 ml/min until acrylamide had eluted after which the flow rate was increased to 0.3 ml/min.

The MS–MS detection was performed on a Quattro Ultima triple quadrupole instrument from Micromass (UK) equipped with an atmospheric pressure ionisation (API) interface. The mass spectrometer was operated with electrospray in the positive (ESI⁺) and negative ion mode (ESI[–]). Capillary voltages of 3 kV (ESI⁺) and 2 kV (ESI[–]) were applied. The source was kept at 120 °C and the desolvation temperature was 400 °C. Nitrogen was used as cone and desolvation gas with flow rates of 150 and 500 l/h, respectively. Argon was used as collision gas and kept at a pressure of 2.4 × 10^{–3} mbar. Detection was performed by multiple reaction monitoring (MRM). Cone voltage and collision energy were optimised for each analyte. Acrylamide and sugars were detected in positive mode, while asparagine was detected in negative ion mode. Detection in positive and negative ion mode was performed separately in this study, thus at least two injections were required for a full analysis. Quantification was done using MassLynx software version 4.0 including QuanLynx.

2.4. Statistical analysis

Analysis of variance was carried out using Statgraphic Statistical Package (Statistical Graphics Corporation, Version 4, Rockville, USA) including multiple range tests ($P > 0.05$) for separation of least square means. Experiments were run in duplicate.

3. Results and discussion

Acrylamide content in raw potato strips, after pre-drying, partial and final frying could be observed in Fig. 1. Raw potatoes did not present any acrylamide. Drying of raw potato strips did not cause any acrylamide formation since it was conducted at 85 °C for 10 min. For French fry analysis the limit of detection for the acrylamide method is 20 µg/kg. The data published so far indicate that a temperature >100 °C is required for acrylamide formation (Becalski et al., 2003). Tareke et al. (2002) showed that acrylamide was formed by heating above 120 °C certain starch-based foods, such as potato chips, French fries, bread and processed cereals. Partial frying of pre-dried potato strips at 170 °C for 1 min generated 370 µg/kg of acrylamide. Final frying at 175 °C for 3 min increased acrylamide content of par-fry potatoes in almost 460% (French fry acrylamide content of 2075 µg/kg). Potato variety, field site and processing conditions (pre-treatments, temperatures and times) had a noticeable influence in acrylamide formation in fried potatoes (Pedreschi et al., 2006). For instance, in potato used for the manufacture of potato chips, the dominant free amino acid is asparagine (940 mg/kg, representing 40% of the total amino-acid content). Asparagine is present in potatoes in varying, relative high amounts of 0.5–3% of dry matter, depending on factors like variety, location, fertilization, storage and processing (Martin & Ames, 2001). Selecting cultivars for food use that contain low levels of asparagine and devising conditions to hydrolyze asparagine to aspartic acid chemically

or enzymatically with asparaginase or other amidases prior to food processing may result in low-acrylamide foods (Friedman, 2003).

Potatoes used in this research were grown in Denmark and were given by a Danish potato processing company. These raw potatoes contained 14.6 g/kg dw of asparagine and 17.6 g/kg dw of glucose, respectively. The effect of soaking raw potato strips in an asparaginase solution over acrylamide formation is shown in Fig. 2. Soaking of raw potato strips in an 10000 ANSU/l asparaginase solution at 40 °C for 20 min, reduced acrylamide formation in French fries in 30%. Increasing soaking temperature of asparaginase solution to 50 and 60 °C, diminished significantly ($P > 0.05$) acrylamide reduction in French fries in only 20% and 22%, respectively. On the other hand, Fig. 3 shows that when the potato strips are blanched at 75 °C for 10 min before the soaking treatment in asparaginase solution at 40 °C for 20 min, the acrylamide content of French fries decreased surprisingly to 483 µg/kg. Acrylamide content of the blanched French fries without asparaginase soaking treatment was 1264 µg/kg; so the asparaginase soaking treatment reduced significantly ($P > 0.05$) acrylamide formation in ~62%. It is worth to noting that when blanched strips are soaked in asparaginase solutions at temperatures higher than 40 °C (50 °C and 60 °C), acrylamide formation in French fries was reduced significantly ($P > 0.05$) to 34% and 33%, respectively. This fact is due to the fact that the optimum temperature of the asparaginase activity measured at pH 7 is 60 °C (Novozymes A/S). However at temperatures above 60 °C the enzyme activity decreases rapidly therefore a temperature range among 40–60 °C may be preferable. In general, the degradation of the asparaginase enzyme increase with increasing temperature. Hence the activity of the enzyme may last for longer time if the temperature is set to a relatively low temperature.

Acrylamide content of blanched samples (1264 µg/kg) were lower than unblanched sample (2075 µg/kg) despite

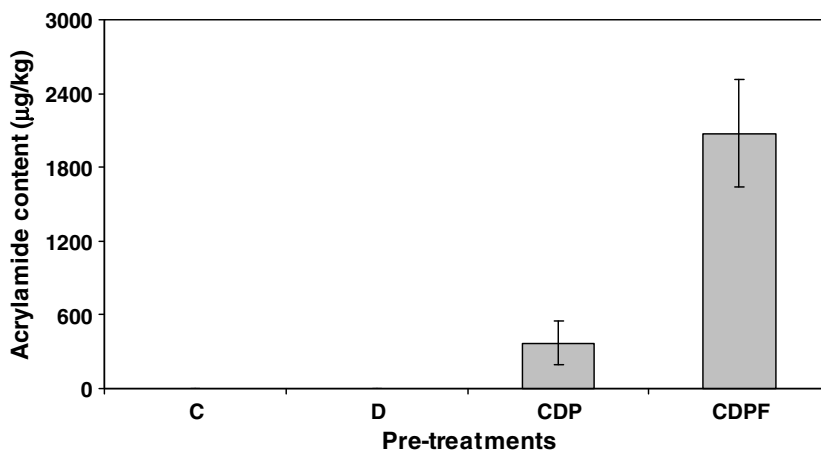


Fig. 1. Acrylamide content of potato at different stages of French fry production. C, Control or raw potato strips; CD: Control strips dried at 85 °C for 10 min; CDP: Control strips dried at 85 °C for 10 min, partially fried at 170 °C for 1 min and frozen at –30 °C for 2 days; CDPF: Control strips dried at 85 °C for 10 min, partially fried at 175 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min.

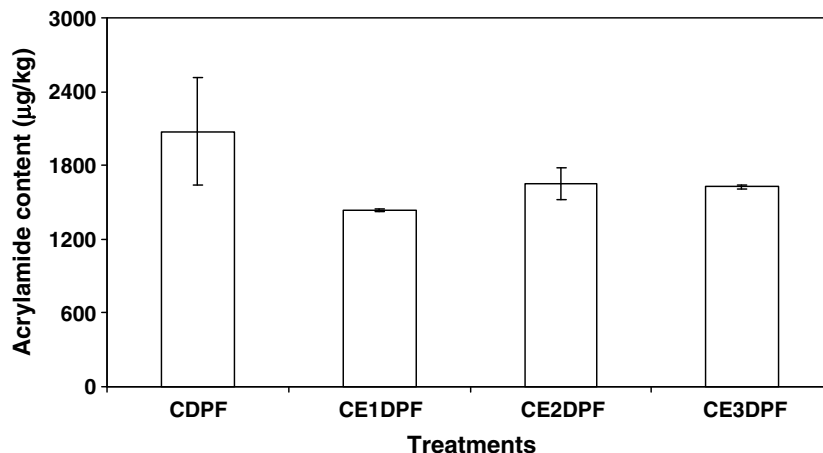


Fig. 2. Acrylamide content of French fries without blanching and treated with asparaginase. CDPF: Control strips dried at 85 °C for 10 min, partially fried at 175 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min; CE1DPF: Raw potato strips immersed in an 10000 ANSU/l asparaginase solution at 40 °C for 20 min, dried at 85 °C for 10 min, partially fried at 170 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min; CE2DPF: Raw potato strips immersed in an 10000 ANSU/l asparaginase solution at 50 °C for 10 min, dried at 85 °C for 10 min, partially fried at 175 °C per 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min; CE3DPF: Raw potato strips immersed in an 10000 ANSU/l asparaginase solution at 60 °C for 10 min, dried at 85 °C for 10 min, partially fried at 175 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min.

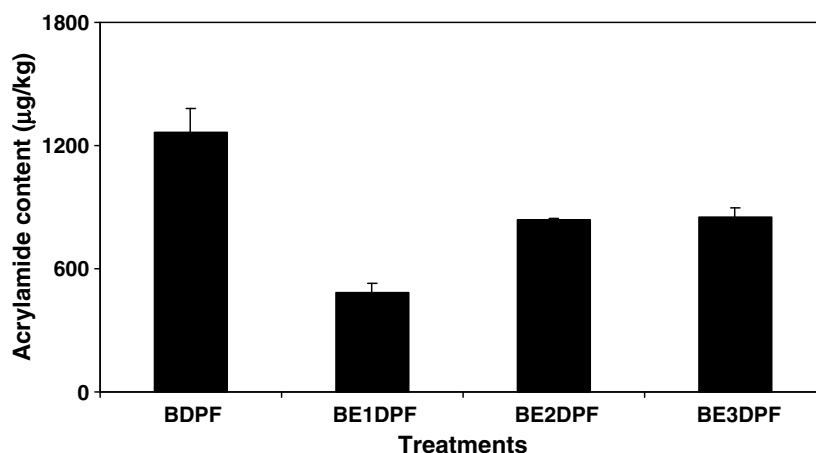


Fig. 3. Acrylamide content of blanched French fries treated with asparaginase. BDPF: Blanched potato strips at 75 °C for 10 min, dried at 85 °C per 10 min, partially fried at 175 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min; BE1DPF: Blanched potato strips at 75 °C for 10 min, immersed in an 10000 ANSU/l asparaginase solution at 40 °C for 20 min, dried at 85 °C for 10 min, partially fried at 170 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min; BE2DPF: Blanched potato strips at 75 °C for 10 min, immersed in an 10000 ANSU/l asparaginase solution at 50 °C for 10 min, dried at 85 °C for 10 min, partially fried at 175 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min; BE3DPF: Blanched potato strips at 75 °C for 10 min, immersed in an 10000 ANSU/l asparaginase solution at 60 °C for 10 min, dried at 85 °C for 10 min, partially fried at 175 °C for 1 min, frozen at –30 °C for 2 days and finally fried at 175 °C for 3 min.

that the amounts of glucose and asparagine were not significant different between both samples ($P > 0.05$). This observation could be due the large variability in the glucose and asparagine contents of different strips (which make difficult to detect small differences in glucose and asparagine content among different pre-treatments) considered in the experiments. When strips are fried the variability of acrylamide content is less than those corresponding to glucose and asparagine in raw and blanched potato strips allowing to detect clearly acrylamide differences among the different pre-treatments tested. Besides, in this research, the blanching treatment was at low temperature (75 °C) for not such a

long time (10 min) which makes the leaching out of glucose and asparagine not significant to differentiate glucose and acrylamide contents of the blanched samples from those corresponding to the control.

Fig. 4a shows that neither the blanching treatment (75 °C, 10 min) nor the asparaginase soaking treatments had a considerable effect in removing glucose, an important precursor of acrylamide formation during frying. On the other hand, the asparaginase soaking treatment had a significant effect in removing asparagine ($P > 0.05$), another important precursor of acrylamide formation during frying (Fig. 4b). Blanching conditions considered in this research

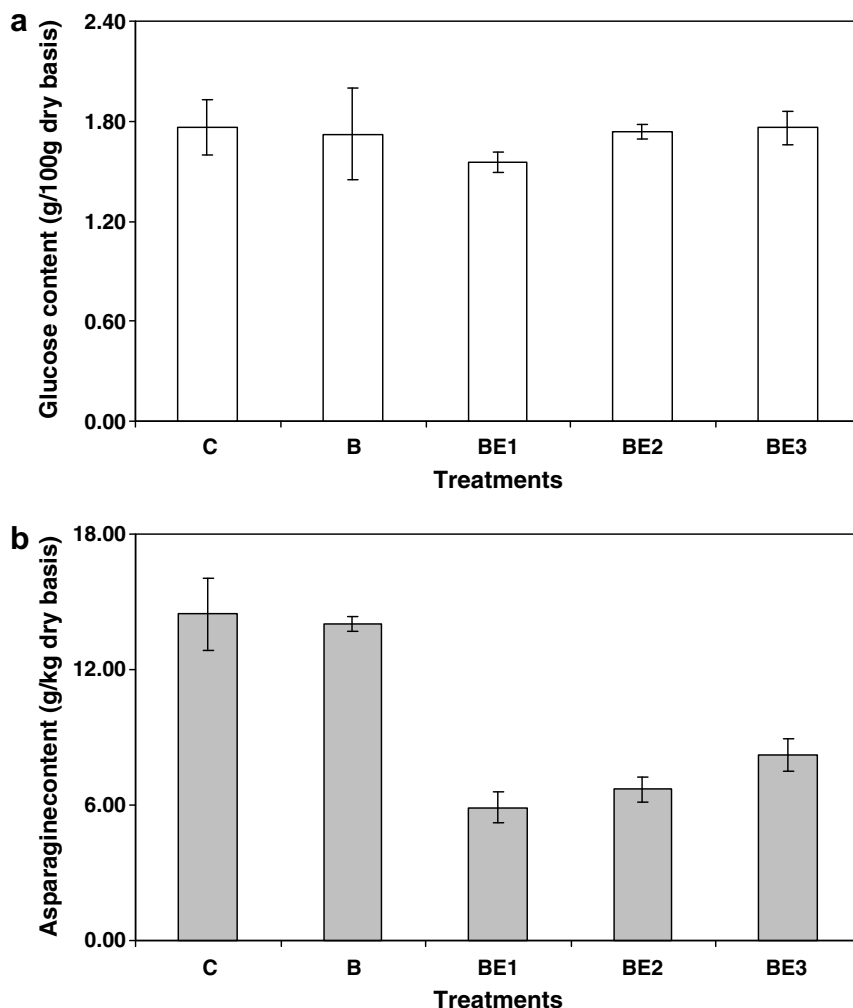


Fig. 4. Effect of asparaginase over (a) glucose and (b) asparagine content of potato strips before drying, par-frying and final frying. C: Control or raw potato strips; B: Blanched potato strips at 75 °C for 10 min; BE1: Blanched potato strips at 75 °C for 10 min and then immersed in an 10000 ANSU/l asparaginase solution at 40 °C for 20 min; BE2: Blanched potato strips at 75 °C for 10 min and then immersed in an 10000 ANSU/l asparaginase solution at 50 °C for 10 min; BE3: Blanched potato strips at 75 °C for 10 min and then immersed in an 10000 ANSU/l asparaginase solution at 60 °C for 10 min.

did not remove significant amount of asparagine from raw potato strips ($P > 0.05$). Soaking in asparaginase solution at 40, 50 and 60 °C of blanched potato strips reduced significantly ($P > 0.05$) their asparagine contents in 58%, 52% and 41%, respectively. These results are coincident with those shown in Figs. 2 and 3 for acrylamide formation in French fries. Asparaginase hydrolyzes the amide of asparagine to form aspartic acid and ammonia. Novozymes notes that asparaginase also hydrolyzes glutamine, but no other free amino acids residues within protein or peptides. Soaking of blanched potato strips (75 °C, 10 min) in a 10000 ANSU/l asparaginase solution at 40 °C for 20 min is an effective way to reduce significantly acrylamide formation after frying by previously reducing the amount of asparagine which is a crucial precursor for acrylamide generation.

Asparaginase treatment duration was the same (10 min) at 50 °C and 60 °C leading to less asparagine degradation at 60 °C. This could be due to the fact that even at 60 °C, which is the optimal temperature for asparaginase

activity, the duration of the treatment was so long that the enzyme began to inactivate. Best results were obtained when asparaginase was used at 40 °C for 20 min in this research.

Asparaginase pre-treatment used in this paper after some time/temperature/dosage optimization, is realistic. It also depends on authority view/handling of acrylamide, which will affect the producers and their willingness to introduce process changes. Heat treatment like blanching during potato processing causes a change in the texture of potato strips (*e.g.* the starch swells), that may improve the diffusion of asparagine towards the asparaginase solution surrounding the strips. Apparently, blanching produces microstructural changes in potato tissue which makes for asparagine and asparaginase to get in contact faster. Besides Lisińska, Tajner-Czopek, and Kalum (2007) stated that heat treatment like blanching during potato processing causes a change in the texture of French fries (*e.g.* starch gelatinization), that may improve the diffusion of asparagine towards the asparaginase solution

surrounding the strips. Actually, in industrial practices the texture of French fries is improved by various temperatures of the blanching water and blanching procedure, with the use of one, two or three blanchers.

Interestingly, asparaginase just right now is commercially available and it is sold today. The cost of asparaginase treatment will depend on the time/temperature/dosage optimization. Besides, the enzyme can be reused in other batches which will bring down enzyme dosage per kg final product, and thereby also costs. Finally, the content of asparaginase in the solution is low meaning that the total protein load of the water will not affect the overall quality of the process waste water.

4. Conclusions

Pre-drying of French fries did not generate acrylamide in potato strips. Partial frying and principally, final frying of potato strips increased significantly the amount of acrylamide in French fries. Soaking in an asparaginase solution of blanched potato strips was an effective method to diminish acrylamide formation after frying. Asparaginase reduced significantly the amount of asparagine, an important precursor of acrylamide formation.

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