XVII[th] World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

ACRYLAMIDE MITIGATION IN FRIED POTATO SLICES BY USING COMMERCIAL ASPARAGINASE

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CSBE100336 – Presented at Section VI: Postharvest Technology and Process Engineering Conference

ABSTRACT Acrylamide is a suspected carcinogen that is formed by heat-induced reaction between sugar and an amino acid called asparagine. Asparaginase converts free asparagine into aspartic acid, another amino acid that does not form acrylamide. The nutritional properties of the products are unaffected, and so are the browning and taste aspects. In this research acrylamide formation in potato chips was investigated in relation to blanching and commercial asparaginase (Acrilaway) immersion treatments before final frying. Potatoes slices (Verdi variety, diameter: 40 mm, width: 2.0 mm) were fried at 170 °C for 5 min (final moisture content of ~ 2.0 %). Prior to frying, potato slices were treated in one of the following ways: (i) Rinsing in distilled water (control I); (ii) Rinsing in distilled water plus blanching in hot water at 85 °C for 3.5 min; (iii) Rinsing in distilled water plus immersion in an aspaginase solution (10000 ASNU/L) at 50 °C for 2 min; (iv) Rinsing in distilled water plus blanching in hot water at 85 °C for 3.5 min plus immersion in an asparaginase solution (10000 ASNU/L) at 50 °C for 20 min; (v) Rinsing in distilled water plus blanching in hot water at 85 °C for 3.5 min plus immersion in distilled water at 50 °C for 20 min (control II). Blanching in hot water was almost as effective as asparaginase potato immersion in order to diminish acrylamide formation in potato chips (% of reduction was almost 17%). When potato slices were blanched before aspariganase immersion, the acrylamide content of the resultant potato chips was reduced considerably by almost 90 %. It seems to be that blanching of the potato slices before the immersion in the enzyme solution facilitates the diffusion of asparaginase in the potato tissue leading to potato chips with considerable lower contents of acrylamide.

Keywords: Potato chips, Frying, Acrylamide, Asparaginase, Blanching, Acrylaway.

INTRODUCTION 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, 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, & Hellenas, 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.

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., 2002). Besides, Amrein et al. (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, Ciesarová, Kiss & 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.

The production of asparaginase has been developed based on cloning of *Aspergillus oryzae*. The *Aspergillus oryzae* asparaginase has been cloned and expressed in commercial relevant yields in *Aspergillus oryzae*. The *Aspergillus oryzae* is well known for production of food enzymes. The *Aspergillus oryzae* asparaginase has pH optimum at pH 6-7 with good activity between pH 5 and 8, which may be the pH range for production 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 40-60 °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 decreases with increasing heat.

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 percent 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). A strategy to reduce the acrylamide in food products would be to reduce the precursor levels in the raw materials. The objective of this research was to study the effect of enzymatic treatment with asparaginase on reducing acrylamide formation in potato chips.

METHODOLOGY Potatoes slices of Verdi variety (diameter: 40 mm, width: 2.0 mm) were fried at 170 °C for 5 min until reach a final moisture content of ~ 2.0 %). Prior to frying, potato slices were treated in one of the following ways: (i) Rinsing in distilled water (control I); (ii) Rinsing in distilled water plus blanching in hot water at 85 °C for

3.5 min; (iii) Rinsing in distilled water plus immersion in an aspaginase solution (10000 ASNU/L) at 50 °C for 2 min; (iv) Rinsing in distilled water plus blanching in hot water at 85 °C for 3.5 min plus immersion in an asparaginase solution (10000 ASNU/L) at 50 °C for 20 min; (v) Rinsing in distilled water plus blanching in hot water at 85 °C for 3.5 min plus immersion in distilled water at 50 °C for 20 min (control II). Asparaginase solution was made from Acrilaway which is the commercial version of asparaginase (Novozyme, Denmark).

It was used a liquid chromatography-tandem mass spectrometry analytical methodology for analysis of acrylamide (Pedreschi et al 2008). Potato chips were homogenized using a Brand handheld mixer (type 4169) fitted with a blended-like sample compartment (type 4297, Braun AG, Germany). An aliquot of approximately of 1.5 g of homogenate was transferred to a centrifuge tube and 30 ml of deionised water was added by using a dispenser. Internal standards and maltitol were added at the following levels: 150 μl 10 μg/ml D_3-acrylamide. 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 500 x *g* for 20 min (Heraeaus Multifuge, Osterode, Germany) and an aliquot of 2 ml was transferred to Eppenderf vials and frozen to -18 °C for 30 min or more and subsequently microcentrifugated at 12,100 x *g* 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 Isolute Multimode SPE-cartridges (300 mg) from Int. Sorbent Technol. (Hengoed Mid Glamorgan, UK) conditioned with 2 ml of methanol 2 x 2 ml of water and 0.5 ml of sample lead to waste. Subsequently 0.4 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 x 50 mm, particle size 5 μm). In front of the LC-column was a C18 ODS SecurityGuard column (dimensions 4mm x 2mm) from Phenomenex (Chesire, UK). The column was eluted with 0.1% formic acid in water with a flow rate of 0.2 ml/min.

The MS-MS detection was performed on a Quattro Ultima triple quadropole instrument from Micromass (UK) equipped with an atmospheric pressure ionisation (API) interface. The mass spectrometer was operated with electrospray in the positive (ESI^+).Capillary voltages of 3kV (ESI^+) and 2kV (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. Quantification was done using MassLynx software version 4.0 including QuanLynx.

RESULTS AND DISCUSSION Figure 1 shows the effect of different pre-treatments in mitigating acrylamide formation during frying of potato slices. The precursors in

acrylamide formation in fried potatoes are reducing sugars and the aminoacid asparagine. In this research, we tested the enzyme asparaginase in order to reduce or eliminate asparagine from the potato tissue in order to diminish acrylamide formation during the frying process.

Blanching at 85 °C for 3.5 min reduced the acrylamide in content of potato chips with ~ 17 %. This fact has been explained previously by some authors since not only reducing sugars but also asparagine are leached out during blanching (Pedreschi et al. 2004). On the other hand, immersion of potato slices in asparaginase solution at 50 °C for 20 min did not mitigate considerable acrylamide formation in potato chips (only 15 %, quantity very similar to that mitigated by blanching). However, when the enzyme immersion treatment is done after blanching potato slices at 85 °C for 3.5 min, acrylamide formation could be diminished in 92 %. Approximately 40 % of this effect could be attributed to the action of the asparaginase enzyme. This enzyme worked very well in reducing acrylamide content in French fries as suggested by Pedreschi et al. (2007).

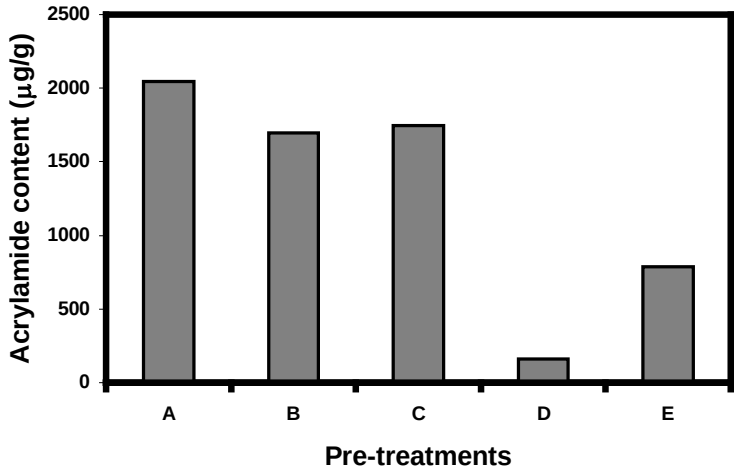

Figure 1. Acrylamide content potato slices treated with asparaginase. **A**. Raw potato slices (control I); **B**. Blanched potato slices at 85 °C for 3.5 min; **C**. Immersion in a 10000 ASNU/L asparaginase solution at 50 °C for 20 min; **D**. Blanched potato slices at 85 °C for 3.5 min plus immersion in a 10000 ASNU/L asparaginase solution at 50 °C for 20 min; **E**. Blanched potato slices at 85 °C for 3.5 min plus immersion in a 10000 ASNU/L asparaginase solution at 50 °C for 20 min (control II).

CONCLUSION Blanching improved the action of asparaginase in removing asparagine and leading to potato chips with lower acrylamide contents. When asparaginase acts directly over potato slices (without a previous blanching), its effect in reducing acrylamide content in the resultant potato chips is significantly lower.

Acknowledgements Authors acknowledge financial support from FONDECYT Project N° 1070031. Collaboration of Novozymes A/S, Kims A/S and Aarhus Oil

(www.AAK.com) are highly appreciated for supplying us with the asparaginase, the potatoes and the oil, respectively.

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