Impact of L-asparaginase on acrylamide content in potato products

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Summary
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 was investigated. The application of different amounts of enzyme at two temperatures (20 °C and 37 °C, resp.) were compared in a system simulating the composition of raw potatoes and subsequently in fresh and stored potatoes, respectively, and also in dried potato products. It was found out that the addition of as little as 0.2 units of lyophilized enzyme per 1 g of fresh potato mash resulted in approximately 50% reduction of acrylamide content. A larger amount of enzyme (1 $U.g^{-1}$) and higher incubation temperature (37 °C) of fresh potato sample with enzyme to still more suppression of acrylamide production (up to 97%) when exposed to the same heat regimen (180 °C, 20 min). In the case of potatoes stored at 4 °C, acrylamide content 4 times higher than in fresh potatoes was observed. The addition of enzyme (2 $U.g^{-1}$) and the following incubation of the mixture at 37 °C for 30 min led to 70% reduction of acrylamide content. A sufficient mitigation of acrylamide content (90–97%) was achieved also in products prepared from dried potato powder treated by L-asparaginase.

Keywords
acrylamide; L-asparaginase; Maillard reaction products; potato; mitigation of acrylamide

An undesirable acrylamide concentration in many heat-treated foods rich in carbohydrates was observed first by Swedish scientists in April 2002 [1]. Subsequently, a considerable public concern was attained and an extensive international research was initiated. The reason for the worldwide effort of regulating authorities, the food industry, and the research institutes is the fact, that neurotoxicity, reproductive toxicity, genotoxicity, clastogenicity, and carcinogenicity have been demonstrated to be potential human health risks that may be associated with exposure to acrylamide [2, 3]. Moreover, acrylamide has been classified by the International Agency for Research on Cancer (IARC) in 1994 as probably carcinogenic to humans (class 2A) [4]. Up to the present time several acrylamide formation pathways have been reported and some details of acrylamide formation mechanism have been described [5-10]. The acrylamide formation was found to occur during the browning process by the Maillard reaction of reducing sugars with the amino acid asparagine at temperatures higher than 120 °C. The highest amounts of acrylamide were found in French fries and potato chips [11]. Therefore, several studies examined the mechanism of formation and the factors influencing the acrylamide formation in potatoes and potato-like model systems [8-10]. In fresh potatoes, asparagine and sugar contents depend on several factors, such as potato cultivar, farming systems, fertilization, time of harvest, storage time and temperature [12, 13]. Different potato types can show an extreme variability in their composition, and so care should be taken during selection of raw materials, sample preparation and processing. Many parameters affecting the level of acrylamide in foods were investigated [14-16]. Besides mentioned heat intake and the level and type of sugars and amino acids, presence of water in system seems to be important [17]. On the basis of these observations various ways for acrylamide minimization in foods have been proposed [18-24]. Despite unquestionable advances achieved in decreasing the acrylamide content in foods, there is not enough knowledge about the possibility of using additives such as enzymes and antioxidant agents. In some cases, pre-treatment with the enzyme L-asparaginase is sufficient to reduce acrylamide content, since L-asparagine is considered to be one of the main precursors for the acrylamide

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formation in foods [2, 5, 6]. L-asparaginase, an enzyme (type EC 3.5.1.1) which catalyzes the hydrolysis of asparagine into aspartic acid and ammonia, has been used in medicine as antineoplastic drug to treat different kinds of blood cancer. Asparaginase is found in some bacteria *(E. coli, Erwinia)*, mould *(Aspergillus)*, plants and many animals, including guinea pigs but it does not occur naturally in humans [25]. High temperatures during the cooking process inactivate this enzyme by proteolysis. The first documented application of using L-asparaginase for generating asparagine and concomitant acrylamide reduction in potato matrix that of ZYZAK et al. [7]. Furthermore, the effect of asparaginase was examined in gingerbread, resulting in a decrease of 75% of free asparagine, 55% of acrylamide content and no negative effect on either taste or color [26].

The aim of this work was to find conditions for the effective application of L-asparaginase for mitigation of acrylamide formation in industrially processed and home-cooked high heat treated potato products. Moreover, the potato model system was compared with real systems using raw, fresh or stored potatoes as well as dried potato products under controlled conditions.

MATERIALS AND METHODS

Chemicals and materials

List of chemicals

Acrylamide analytical grade (Serva Feinbiochemica, Heidelberg, Germany), starch from potatoes (Fluka Chemie, Buchs, Switzerland), D-glucose monohydrate for biochemistry, D-fructose for biochemistry, sucrose for biochemistry (all from Merck, Darmstadt, Germany), L-asparagine monohydrate 99% (Sigma Aldrich Chemie, Buchs, Switzerland), L-asparaginase from *E. coli* lyophilized powder, chromatographically purified (Sigma Aldrich Chemie, Steinheim, Germany), 2,3,3-D_3 labeled acrylamide 98% (Cambridge Isotope Laboratories Inc., Andover, USA), silica gel 60 for column chromatography (Fluka Chemie, Buchs, Switzerland), sodium sulphate annealed at 600 °C for 6 h (Lachema Brno, Czech Republic), methanol and acetonitrile HPLC grade (Sigma Aldrich, Buchs, Switzerland).

Sample material

Potatoes (yellow, variety Marabel and rose, variety Rosara) were purchased from a local shop from the 2005 harvest. They were used fresh or stored at 4 °C for 8 weeks. Dried potato products (Bramborak, producer Amylon Havlickuv Brod, Czech Republic) were obtained also from a local shop, its composition was the following: wheat flour, dried potatoes, potato starch, salt, dried garlic, dried eggs, marjoram.

Preparation of model system samples

The model system was assembled such that corresponds to the average composition of fresh potatoes: starch from potatoes with initial moisture of approximately 10% was dried at 105 °C to final moisture of 0.2%. 1 g of dried starch was mechanically homogenized with 0.2 g of mixture consisting of glucose (1.46 $mmol.g^{-1}$), fructose (0.89 $mmol.g^{-1}$), sucrose (0.58 $mmol.g^{-1}$), and L-asparagine (2.33 $mmol.g^{-1}$), after that 4 ml of deionized water was added and the suspension was stirred.

Preparation of genuine potato samples

Raw potatoes, var. Marabel fresh, var. Rosara fresh and var. Rosara stored were washed, peeled, cut and mixed in a blender. An aliquot of 5.2 g of each sample mash was transferred into a 40 ml vessel. All samples were prepared in triplicate.

Application of L-asparaginase

Lyophilized enzyme powder was dissolved in deionized water to obtain a solution with enzyme concentration of 100 $U.ml^{-1}$ (1 unit is the amount of enzyme which releases 1 μmol NH_3 from L-asparagine per 1 minute at pH 8.6 and 37 °C). The solution was used for enzyme pre-treatment of samples (model or genuine) in concentrations of 0.2 U, 1.0 U, 1.5 U and 2.0 U per 1 g of sample, respectively. The samples were incubated for 30 and 60 min, resp. at room temperature or at 37 °C on a shaker or were occasionally stirred (exact conditions are given for each experiment).

Heat intake and acrylamide extraction

The sample tubes were sealed with Teflon caps and kept in the Thermochem Metal-block Thermostat (Liebisch Labortechnik, Bielefeld, Germany) for 20 min at constant temperature (180 °C). Afterwards the samples were cooled and 10 μl of internal standard (methanolic solution of deuterium-labeled acrylamide (D_3-acrylamide, 22.9 $\mu g.l^{-1}$) was added. Acrylamide was extracted with 5 ml of mixture of solvents (methanol/acetonitrile 20:80) in an ultrasonic bath (60 °C, 20 min). Subsequently, the extract was cleaned through a silica gel column with annealed Na_2SO_4. Second extraction was made with 5 ml of the same mixture of solvents, thoroughly hand-shaken at room temperature. Both extracts were combined

and analyzed by GC/MS. Three runs were performed for each sample.

Measurement of dry weight (dw)

Potatoes were homogenized using a blender and 3 × 10 g of each kind potato mash was dried in an oven at 105 °C for 5 h.

GC/MS analysis

Acrylamide without derivatization was determined by GC-MS in the NCI mode [27] using the Agilent 6890 GC equipped with MS Detector 5793 under following conditions: split/splitless inlet 250 °C, 2 μl pulsed splitless, single tapered liner with glass wool, oven: 60 °C (1.0 min), 10 °C.min^{-1} to 190 °C (0 min), 50 °C.min^{-1} to 240 °C (2 min), column: 30 m x 0.25 mm x 0.25 μm DB-FFAP, helium as a carrier gas, 0.8 ml.min^{-1} constant flow, Single Ion Monitoring mode, Interface/Source/Quadrupole: 250 °C/150 °C/150 °C, tune: NCI CH_4.U, reagent gas: methane 2 ml.min^{-1}, EM offset: 400 above tune, resolution: low, dwell time 150 ms. Signals at *m/z* 70 for acrylamide and *m/z* 73 for deuterium-labeled acrylamide were used for quantitation. All analyses were run in triplicate.

Quantitative determination

Acrylamide was determined using a linear calibration curve established with standard solutions of acrylamide dissolved in water. Concentrations used were 1.0, 2.0, 10.2, 25.5, 51.0, 76.5, 102.0, and 204.0 μg.l^{-1} respectively Each solution contained 22.9 μg.l^{-1} of D_3-acrylamide. The same concentration was used as an internal standard during sample preparation.

RESULTS AND DISCUSSION

Application of L-asparaginase in the model system

As it has been already documented in several reports, among the various free amino acids measured in potatoes, the content of free asparagine was the highest. Widely varying concentrations of asparagine, glucose, fructose and sucrose were reported recently [12-14]. This variability may explain the difference in the amounts of acrylamide that may be formed in the products during processing. The reason for this large spread in the content of asparagine and reducing sugars is probably due to multiple factors, such as potato cultivar, farming systems, field site, fertilization, pesticide/herbicide application, time of harvest, storage time and temperature. For that reason, our model system composition was designed to simulate average composition of fresh potatoes, as reported by TAYEMANS et al. [18] and TREGUBOV et al. [28] (Table 1).

Tab. 1. Average composition of fresh potatoes and an artificial model system.

Constituent	Fresh potatoes		Model system
	min.	max.	
Water [mg.g^{-1} raw potato]	632[a]	869[a]	769
Dry matter [mg.g^{-1} raw potato]	131[a]	368[a]	231
Starch [mg.g^{-1} raw potato]	80[a]	294[a]	192
Asparagine [mg.g^{-1} dw]	2.3[b]	39.4[b]	13.0
Glucose [mg.g^{-1} dw]	0.2[b]	27.1[b]	11.0
Fructose [mg.g^{-1} dw]	0.2[b]	25.0[b]	6.0
Sucrose [mg.g^{-1} dw]	1.4[b]	42.3[b]	7.7

a - according to TREGUBOV et al. [28], b - according to TAYEMANS et al. [18], dw - dry weight.

Many strategies for suppresing acrylamide formation have been suggested so far with more or less impact on the sensory quality of final products or consumer acceptance [18-24]. The application of commercial L-asparaginase for elimination of one of the mentioned precursors – amino acid L-asparagine – without compromising the sensory properties of processed products was tested in our study. This enzyme was added as a solution into the model system described above at 20 °C for 60 min or at 37 °C for 30 min in different concentrations (ranging from 0.2 U to 2.0 U per 1 g of the model mixture). The influence of enzyme application before heat treatment on the acrylamide content determined after heat treatment (180 °C, 20 min) in those mixtures was demonstrated (Fig. 1). The incubation of the enzyme solution with the artificial mixtures at room temperature caused a reduction of acrylamide by about 25% in the whole range of enzyme concentrations. Higher temperature and shorter enzyme incubation time (37 °C, 30 min) was sufficient for 90% decrease of acrylamide content after the same heat treatment at the concentration of 2.0 U of enzyme per 1 g of the wet artificial mixture (Fig. 1).

Application of L-asparaginase in potatoes

Because of the successful application of L-asparaginase in the model system, a similar enzyme pre-treatment was used in fresh potato mashes of two varieties of potatoes (Marabel and Rosara). The acrylamide content after heat treatment (180 °C, 20 min), but without enzyme pre-treatment was slightly higher in the case of the Rosara variety (6.56 μg.g^{-1} dw) in comparison with the Marabel variety (4.08 μg.g^{-1} dw). After that

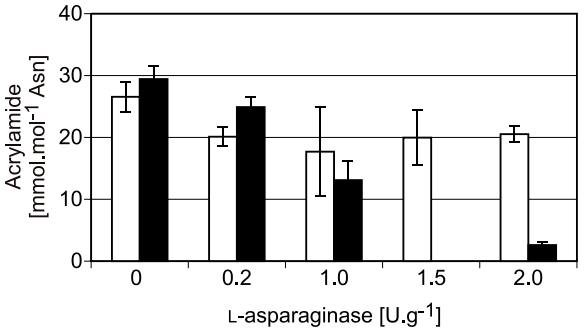

Fig. 1. Acrylamide content after heat intake (180 °C, 20 min) with L-asparaginase pre-treatment by different amounts of enzyme at 20 °C for 60 min (white bars) or at 37 °C for 30 min (black bars) in a model mixture.
Asn - L-asparagine

observation, L-asparaginase was applied at 37 °C for 30 min at three different concentrations. The effect of enzyme pre-treatment on the acrylamide content after heat treatment (180 °C, 20 min) was similar in both varieties (Fig. 2). Larger reduction of acrylamide content was achieved at higher enzyme concentration. 0.2 U of enzyme per 1 g of raw mash resulted in app. 45% decrease of acrylamide content, while 2.0 U of enzyme per 1 g caused 97% acrylamide mitigation.

It is known that the content of reducing sugars in potatoes depends on many factors, e.g. the sort and the variety of potatoes, the conditions during harvesting and storage, especially the temperature. During several days of storage at temperature below 8 °C the sugar content strongly increases which provides the possibility for a higher acrylamide formation. In the case of potatoes var. Rosara, which were stored at 4 °C for 8 weeks, the acrylamide content after heat intake increased app. 4 times at the level of 25 μg acrylamide.g^{-1} dw. When stored potatoes were used for the enzyme pre-treatment, the incubation with 2.0 U of L-asparaginase per 1 g at 37 °C for 30 min caused a decrease of acrylamide content after heat intake to the level of 7.2 μg.g^{-1} dw, which means more than a 70% reduction of acrylamide (Fig. 2).

Application of L-asparaginase in dried potato products

Dried potato products are produced for simple preparation of meals based on potatoes by heat treatment. These products usually consist of dried potato powder, dried potato starch, wheat flour, dried eggs, salt, dried garlic, and dried marjoram. Wet dough is prepared by adding water to this mixture and after roasting the final product is ready for consumption. Some of the mentioned components are considered to be the precursors of acrylamide formation (potato, starch, flour), others seem to be the inhibitors of it (salt, antioxidant components of garlic and marjoram) [11, 29, 30]. Our results showed that without enzyme pre-treatment acrylamide was formed in the samples after heating at 140 °C, 160 °C or 180 °C for 5 min (Fig. 3) and its content was similar at all used temperatures. The application of L-asparaginase in concentration of 1 U.g^{-1} of wet dough at 20 °C for 30 min before heat treatment resulted in 70–85% decrease of acrylamide level. Higher concentration of enzyme (2 U.g^{-1} of wet dough) resulted in even higher 90–97% reduction of acrylamide level (Fig. 3). Final concentration of acrylamide in potato products treated by higher concentration of enzyme was app. 2 μg.g^{-1} dw at all tested temperatures. Similar effectiveness (99%) of L-asparaginase to reduce acrylamide formation in mashed potato product heated in microwave oven was evaluated by Zyzak et al. [7].

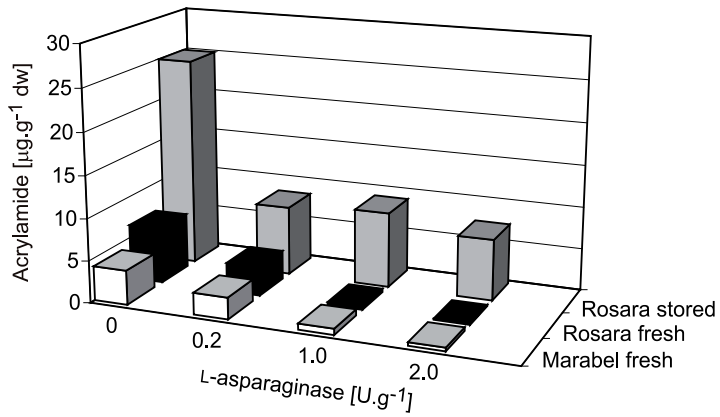

Fig. 2. Acrylamide content after heat intake (180 °C, 20 min) with L-asparaginase pre-treatment by different amounts of enzyme at 37 °C for 30 min in mashes prepared from potatoes var. Marabel fresh (white bars), Rosara fresh (black bars) and Rosara stored (grey bars).

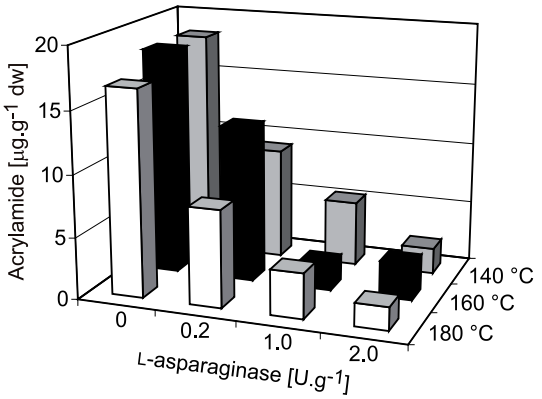

Fig. 3. Acrylamide content after 5 min heat intake at 140 °C (grey bars), 160 °C (black bars) and 180 °C (white bars) with L-asparaginase pre-treatment by different amounts of enzyme at 20 °C for 30 min in dried potato products.

CONCLUSIONS

The application of L-asparaginase was confirmed to be one of the successful ways of acrylamide mitigation in potato products. It was applied in an artificial model system as well as in a real system of fresh and stored potatoes or dried potato products. 90% reduction of acrylamide level was achieved at enzyme concentration of 2 U per 1 g of wet model mixture and incubation temperature of 37 °C for 30 min. A sufficient decrease in acrylamide level was observed also following application of enzyme at the same conditions to fresh or mashed stored potatoes, although the content of acrylamide formed in stored potatoes was much higher due to higher level of reducing sugars formed in potatoes during storage at temperature below 8 °C. The application of L-asparaginase is convenient also for pre-treatment of dried potato products, where 90% reduction of acrylamide level was attained after the incubation of 2 U of enzyme per 1 g of wet dough at 20 °C for 30 min and following heat intake (180 °C, 5 min).

Feasibility of the described way of acrylamide reduction through enzyme pre-treatment opens new perspectives of this application for many commercially produced semi finished products and home-cooked meals prepared from potato mashes. For this reason, this procedure is protected by the patent application filed with the Industrial Property Office of the Slovak Republic under the number 5027-2006.

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