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Mitigating acrylamide formation in potato products through the use of a thermophilic L-asparaginase: Impact of processing conditions and colour parameters

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Research Article

Keywords: Acrylamide, Thermophilic L-asparaginase, Potato processing, Spunta, HPLC quantification

Posted Date: March 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9002969/v1

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Additional Declarations: The authors declare no competing interests.

Mitigating Acrylamide formation in Potato Products through the use of a Thermophilic L-Asparaginase: Impact of Processing Conditions and Colour Parameters

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SUMMARY

Research background. Acrylamide is a heat-induced process contaminant formed predominantly in carbohydrate-rich foods subjected to high-temperature cooking through the Maillard reaction between reducing sugars and free L-asparagine. Due to its toxicological relevance, strategies to mitigate acrylamide formation without compromising product quality are of increasing industrial interest. Thermophilic L-asparaginases offer potential advantages for integration into thermal processing conditions such as blanching, avoiding the need for process cooling.

Experimental approach. This study evaluated the effect of different cooking methods (atmospheric frying, vacuum frying, and baking) and potato cut geometry

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(batonnets vs. slices) on acrylamide formation. Additionally, the effectiveness of a thermophilic L-asparaginase extract from *Thermus oshimai* was assessed in roasted potato slices, the condition showing the highest baseline acrylamide levels. Potatoes (Spunta variety) were blanched under controlled conditions (80 °C, 11 min) prior to cooking. Acrylamide content was quantified by HPLC-PDA, and surface colour was evaluated using the CIELAB system.

Results and conclusions. Significant differences ($p < 0.05$) were observed among cooking methods. Vacuum frying resulted in the lowest acrylamide levels (122.2 ± 15.6 µg/kg), whereas baking generated the highest concentrations (527.9 ± 52.3 µg/kg in slices). Enzymatic pretreatment reduced acrylamide formation by up to 77.3% compared to untreated controls. Treated samples exhibited improved color parameters (higher L*, lower a*, higher hue angle), indicating reduced Maillard reaction progression. These results demonstrate that thermophilic L-asparaginase effectively mitigates acrylamide formation under blanching-compatible conditions.

*Novelty and scientific contribution***.** This work demonstrates the applicability of a thermophilic L-asparaginase under industrially relevant thermal conditions without process modification. The study integrates processing variables, geometry effects, and enzymatic mitigation, contributing practical biotechnological evidence for acrylamide reduction in potato products and supporting safer food manufacturing strategies.

Keywords: Acrylamide; Thermophilic L-asparaginase; Potato processing; Spunta; HPLC quantification.

INTRODUCTION

Acrylamide (also known as 2-propenamide) is a chemical compound that can naturally form when certain foods, particularly those rich in starch, are cooked at temperatures above 120 °C in an atmosphere with very low water content. The optimal temperature range for its formation is between 160 and 180 °C [*1,2*]. This low-molecular-weight organic compound, which is highly soluble in water [*3*], predominantly forms in the hottest areas of the food.

The primary mechanism of acrylamide formation is the Maillard reaction, a complex chemical process involving the amino group of free asparagine and the carbonyl groups of reducing carbohydrates such as glucose and fructose [*4,5*]. This reaction proceeds through multiple stages, including the formation of a Schiff base, Amadori rearrangement, and Strecker degradation, ultimately producing acrylamide [*6*].

Potatoes (*Solanum tuberosum L.*) are the fifth most produced staple food worldwide for human consumption. Their culinary versatility leads to the application of

various cooking methods, with baking and frying being the most common [*7*]. Potato-based products are significant contributors to dietary acrylamide intake because tubers naturally contain substantial amounts of carbohydrates and precursors such as reducing sugars and specific amino acids [*4,8*]. Among these, potato crisps and French fries stand out as major sources of acrylamide exposure, widely consumed by the population [*9*].

In 2002, the Swedish National Food Authority and Stockholm University first detected elevated acrylamide levels in a broad range of commonly consumed fried and roasted food products [*1*]. Acrylamide is classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer [*10*].

Globally, the control of acrylamide in food has become a regulatory priority. The European Commission Regulation (EU) No. 2158/2017 established mitigation measures and benchmark levels for acrylamide in different food categories, while the U.S. FDA issued guidance documents encouraging industry monitoring and reduction strategies. These frameworks underscore the growing need for efficient mitigation technologies that maintain sensory quality while minimizing acrylamide formation.

Several studies have investigated possible mechanisms to reduce acrylamide formation without affecting the sensory properties of food products, given that the Maillard reaction generates a variety of compounds responsible for aroma, flavour, and colour [*11*]. Among these strategies, blanching and controlled frying or roasting have been recognized as industrially feasible interventions that influence the kinetics of acrylamide formation.

The use of L-asparaginase (L-ASNase; EC 3.5.1.1) has emerged as one of the most effective enzymatic mitigation strategies. This enzyme catalyses the hydrolysis of L-asparagine into aspartic acid and ammonia, thereby reducing the availability of asparagine as a precursor in the Maillard reaction and, consequently, acrylamide formation [*12,13*]. Commercial mesophilic L-asparaginases, however, exhibit limited thermal stability, which restricts their use in high-temperature processes. In contrast, thermophilic L-asparaginases, such as those derived from *Thermus oshimai*, exhibit high thermostability and activity under industrial processing conditions, providing a cost-effective alternative that does not require changes in equipment or process parameters. Moreover, their use has shown no detrimental impact on nutritional or sensory properties of heated products [*14*].

Blanching potatoes in a hot water bath is one of the most widely applied thermal pretreatments in industrial processing, primarily aimed at inhibiting enzymatic browning caused by polyphenol oxidase (PPO) and related endogenous enzymes [*15,16*]. This operation is traditionally carried out at temperatures between 65 and 80 °C for 10 to 30 minutes [*17*]. Beyond enzyme inactivation, blanching also modifies tissue structure,

influencing mechanical integrity, moisture migration, and heat transfer in subsequent cooking steps—factors that ultimately affect the kinetics of Maillard reaction products such as acrylamide. Establishing suitable blanching conditions is therefore essential to balancing enzymatic control, structural preservation, and chemical reaction pathways during frying or roasting. Within this technological context, blanching represents a key pretreatment step that facilitates enzyme diffusion into the potato matrix.

Although thermostable L-asparaginases have been previously described, limited information is available regarding their integration under industrial blanching conditions without process adjustment, particularly in relation to geometry-dependent acrylamide formation in potato matrices.

In this study, we characterised the asparaginase produced by a thermophilic bacterium isolated from a volcanic island in Antarctica and assessed the acrylamide levels formed under controlled thermal and enzymatic processing conditions. To this end, we aim to: (i) determine the acrylamide content in potatoes subjected to different processing methods and cutting styles, (ii) evaluate the effect of the thermophilic L-ASNase enzymatic treatment on acrylamide formation in the sample with the highest baseline concentration.

MATERIALS AND METHODS

L-asparaginase producing strain

To search for an L-ASNase enzyme that is active at high temperatures, thermophilic bacterial strains were isolated from the volcanic regions of Caviahue-Copahue, Neuquén, Argentina (37°49′6″S, 71°5′47″W) and Deception Island, Antarctica (62°58'27″S 60°42'03.6"W). The hot spring soil samples were suspended in sterile 0.85% saline solution and streaked on plates containing DSMZ 158 or DSMZ 878 culture media (https://mediadive.dsmz.de/) gelled with Phytagel™, Sigma-Aldrich®, Merck, and incubated at 60°C. The different isolated colonies were re-streaked in R2A medium (Neogen®) and incubated at 80 °C, obtaining 32 thermophilic bacterial isolates, subsequently cultured in R2A broth (Neogen®) for 24 hours at 80 °C and 150 rpm. Then, cells were separated by centrifugation at 8,500 rpm for 10 min and subjected to mechanical disruption by sonication (80% power, 10-second pulses, and 5-second pauses) for 3 min in a Vibracell Ultrasonic Processor (Sonics®). The cell extract was preserved after centrifuging at 16,000 rpm and 4 °C for 10 min, discarding the cell debris pellet.

L-ASNase activity was assayed on the culture supernatants and cell extracts by adding 80 µL of a 10 mM L-asparagine solution in 20 mM sodium phosphate buffer (pH 7.0) to 120 µL of the sample. After incubating for 30 min at 80 °C, the reaction was

stopped by adding 0.5 volumes of 0.2 M trichloroacetic acid (TCA) and centrifuged for 5 min at 12,000 rpm. Next, 180 µL of the supernatant was mixed with 20 µL of Nessler's reagent [*18*]. After 10 min, L-ASNase activity was qualitatively recorded according to the intensity of the colour developed by the reaction of the ammonium resulting from the hydrolysis of L-asparagine.

Taxonomic identification of bacterial isolates relied on partial sequencing of the 16S rRNA gene and was performed as described in Kuc et al. [*19*], comparing the obtained sequences against the specialised databases Silva and EzBioCloud.

Production and characterisation of a thermophilic L-asparaginase

The enzyme was produced in batch cultures incubated at 80 °C using R2A broth (Neogen®). Cells were harvested, disrupted by sonication, and the soluble fraction was collected by centrifugation. The crude extract was subjected to ammonium sulphate precipitation (45–65% saturation) and subsequently purified by hydrophobic interaction chromatography on HiTrap Phenyl FF (GE Healthcare, Sweden) column pre-equilibrated with 1 M ammonium sulphate in 10 mM sodium phosphate buffer (pH 7). The active fractions were pooled and desalted by overnight dialysis at 4 °C using cellulose membranes (MWCO 12,000; Sigma, St Louis, USA) against 10 mM sodium phosphate buffer (pH 7), and the resulting preparation was used for further biochemical characterisation. In each fraction, protein concentration was determined using the Bradford method [*20*] and L-ASNase activity was measured as previously described, and quantitatively recorded according to the absorbance measured at 450 nm. A calibration curve was prepared using an ammonium chloride standard. In a microplate, 180 µl of varying concentrations (0 to 1 mM) of NH_4Cl were added to 20 µl of Nessler's reagent per well, in triplicate. After 10 min, the formation of the colored ammonium-Nessler reagent complex was measured at 450 nm using a microplate reader (EZ Read 800 Research, Biochrom). One enzymatic unit (U) was defined as the amount of enzyme required to release 1 µmol of ammonia per minute under the assay conditions.

The optimal temperature and pH for L-ASNase activity were determined by incubating the enzyme with the substrate under standard assay conditions at varying temperatures and pH values. Temperature-dependent activity was assessed at 4, 20, 37, 60, 80, and 95 °C, and relative activity (%) was calculated by assigning 100% to the highest observed value. To evaluate the effect of pH, assays were performed at pH 4–11 using the following 100 mM buffers: sodium acetate (pH 4–5), sodium phosphate (pH 6–7), Tris-HCl (pH 8–9), and sodium carbonate (pH 10–11).

Samples

Potatoes (*Solanum tuberosum L.*, variety Spunta), the most consumed in Argentina and characterised by their elongated shape, light yellow flesh, smooth skin, and high yield, were purchased from a local retail market in Gualeguaychú, Entre Ríos, Argentina. This variety was selected because of its moderate reducing sugar content and typical dry matter composition, making it representative of studies on acrylamide formation in thermally processed potato products. The potatoes were stored at 10 ± 2 °C and 80–85% relative humidity for no longer than two weeks before use to prevent sugar accumulation.

Fresh potato tubers were selected for uniform size, firmness, and absence of mechanical or microbial damage. After washing under running water, the samples were peeled and divided into two groups. One group was sliced to approximately 1.8 ± 0.2 mm thickness and 50 mm diameter using a stainless-steel slicer (V5, Börner, Germany). The other group was cut into batonnets (10 × 10 × 100 mm) with a vertical potato cutter (Nativa Gastronomy, Argentina). All samples were rinsed with distilled water for 1 minute to remove surface starch, then drained and processed immediately.

These two geometries were selected because they differ in surface-to-volume ratio, a critical factor influencing heat transfer and acrylamide formation kinetics during thermal processing. The initial moisture content of the fresh tubers was 77.4 ± 0.8% (w.b.), determined by air oven method (AOAC 925.10) [*21*].

Determination of the blanching time

A blanching pre-treatment was applied prior to cooking to determine the optimal time that maintains potato firmness while avoiding tissue disintegration. Potato samples (both slice and baton geometries) were immersed in a water-bath at 80 °C for 2, 5, 7, 9, 11, and 15 minutes. These blanching times were selected to simulate the extended thermal exposure required for subsequent enzymatic pre-treatment with thermophilic L-asparaginase. The potato-to-water ratio (1:10, w/v) ensured adequate heat transfer. Temperature and time were precisely controlled using a digital thermocouple (±0.1 °C) connected to a thermostatic water-bath (Roner, Turbosaver, TS-SV60WI, Argentina).

Immediately after blanching, samples were cooled in ice-water for 1 minute until the internal temperature was below 25 °C, then drained and placed on a stainless-steel rack. Excess surface moisture was removed with absorbent paper. For each blanching time, five independent samples were tested in triplicate. The optimal blanching duration was determined as the shortest time producing a significant decrease in penetration force without tissue disintegration, providing a standardised texture baseline for subsequent processing stages.

Texture evaluation

The texture of blanched potato samples was evaluated through an indentation test using a texture analyser (Instron 3342, Norwood, MA, USA) equipped with a 2 mm flat-ended cylindrical probe attached to a 500 N load cell. The instrument was calibrated before each measurement according to the manufacturer's specifications. Tests were carried out under controlled environmental conditions (20 ± 2 °C, ambient humidity).

The probe penetrated 5 mm into the potato strip at a constant crosshead speed of 5 mm min^{-1}. Three indentations were performed per sample: one at the centre and two approximately 10 mm from each end to account for possible textural heterogeneity. The maximum indentation force (Fmax) was determined from the force–deformation curve and used as an indicator of tissue firmness.

This test was selected to assess structural integrity and softening of potato tissue during blanching, as these changes can influence enzymatic diffusion and acrylamide formation in subsequent cooking processes. Each blanching condition was analysed in five replicates.

Cooking methods

The Regulation of the European Commission (EU) 2158/2017 established mitigation measures and reference levels to reduce acrylamide levels in food, recommending that potatoes be fried at 160–175 °C and roasted at 180–220 °C. Following these recommendations, preliminary trials were conducted to establish optimal time–temperature combinations for each cooking method, ensuring a visually comparable golden-yellow appearance, defined as the typical endpoint colour in industrial potato processing.

Two frying methods were carried out using sunflower oil (Cañuelas, Molino Cañuelas, Argentina) at a potato-to-oil ratio of 1:7 (w/v).

Atmospheric frying (AF) was performed at normal atmospheric pressure in a domestic deep-fryer (Moulinex, AF102551, China). Once the oil reached 170 ± 2 °C, the blanched potato samples were immersed and fried for 11 min (batons) and 7 min (slices) until a golden-yellow colour was obtained.

Vacuum frying (VF) was conducted under sub-atmospheric pressure using vacuum-cooking equipment (Gastrovac, International Cooking Concepts, Spain). The oil temperature was maintained at 140 ± 2 °C, and once the internal pressure reached –0.80 bar (boiling point ≈ 60 °C), the samples were immersed and fried for 15 min (batons) and 11 min (slices). After frying, samples were manually centrifuged for 2 min to remove surface oil before vacuum rupture [*22*]

Roasting (R) was performed by spraying both sides of the potato samples with sunflower oil to ensure uniform heat transfer and colour development. The samples were placed on a stainless-steel mesh and roasted in a conventional oven (Morelli, Country 900, Argentina) at 200 °C for 28 min (batons) and 26 min (slices). Temperature was monitored using a digital multimetre tester (Megalite, M890G, China).

After cooking, all samples were cooled on stainless-steel racks at room temperature, vacuum-sealed individually in polyethene bags (VacPack, International Cooking Concepts, 80016™, Spain), and stored at –18 °C until analysis.

These cooking techniques were selected because they represent the most common industrial and domestic methods for potato preparation, each associated with distinct thermal and pressure profiles that influence Maillard reaction kinetics and acrylamide formation.

Enzymatic treatment with thermophilic L-asparaginase

To evaluate the effect of thermophilic L-ASNase on acrylamide mitigation, roasted potato slices were selected that exhibited the highest acrylamide concentrations; samples were subjected to an enzymatic blanching pre-treatment before roasting. All samples were subsequently analysed under identical chromatographic conditions to ensure comparability among treatments.

Fresh potatoes were washed, peeled, and sliced into 2 mm thick rounds with a diameter of 30 mm. The samples were then divided into three groups: a control without enzymatic treatment (C0), and two treatments containing 0.225 U per gram of potato (C1) and 0.450 U per gram of potato (C2) of enzymatic extract, respectively. The enzymatic extract exhibited an activity of 1.49 U/mL.

Blanching was carried out at 80 °C for 11 minutes, maintaining a potato-to-water ratio of 1:4 (w/v), using an orbital shaker incubator (TOU-120, MRC, Israel) to ensure uniform enzyme distribution. The selected temperature and time correspond to the optimal activity range of the thermophilic enzyme and reflect conditions feasible for industrial application. The pH was not adjusted to reproduce conditions typical of industrial blanching processes.

Following enzymatic blanching, the potato slices were roasted in a conventional oven (Morelli, Country 900, Argentina) with forced-air convection at 200 °C for 10 minutes. A shorter roasting time was applied due to the smaller sample size, ensuring comparable surface browning. The process temperature was monitored using a digital multimetre tester (Megalite, M890G, China).

After roasting, samples were cooled to room temperature, vacuum-packed individually in polyethylene bags (VacPack, International Cooking Concepts, Spain), and stored at −18 °C until analysis.

This enzymatic pre-treatment aimed to determine whether thermophilic L-ASNase effectively reduces acrylamide formation during roasting while maintaining industrially viable processing conditions.

Colour analysis

The surface colour of blanched and roasted potato samples, with and without enzymatic treatment, was determined using a Hunter Lab MiniScan EZ 4500L portable colourimeter (Hunter Associates Laboratory Inc., Reston, VA, USA). Measurements were performed in the CIELAB colour space, recording lightness (L*), chromaticity from red to green (a*), and chromaticity from yellow to blue (b*). The instrument was calibrated with a standard white tile prior to measurement. Colour readings were taken on the upper surface of the samples after cooling to room temperature (20 ± 2 °C). Three measurements were recorded per sample, and mean values were used for analysis. The CIELAB system was selected because it provides a precise and reproducible description of colour changes in thermally processed potato products [2,23]. Chroma (C*) and hue angle (h°) were calculated from the obtained L*, a*, and b* values, using the following Eqs. 1 and 2:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad /1/$$

$$h^\circ = \arctan(b^*/a^*) \quad /2/$$

Chroma (C*) expresses the colour intensity or saturation, whereas the hue angle (h°) represents the dominant perceived colour, expressed in degrees.

Acrylamide extraction and quantification

Acrylamide extraction was performed following the procedure described by Zou et al., with minor modifications to optimise recovery efficiency [*17*]. Fried and roasted potato samples were finely ground using a food processor (Liliana, Aliada AM 323, Argentina). One gram of each sample was weighed into a 50 mL polypropylene centrifuge tube, and 10 mL of n-hexane was added. The mixture was shaken for 15 min on a 3D angular shaker (DLab, SK-D3309-Pro, China), followed by centrifugation at 8500 rpm for 10 min at 4 °C (Heal Force®, Neofuge 15R, China). The supernatant was discarded, and the samples were air-dried at 25 °C for 3 min to remove residual solvent.

Subsequently, 3 mL of distilled water, 500 µL of Carrez I solution (15 g potassium hexacyanoferrate/100 mL), and 500 µL of Carrez II solution (30 g zinc sulphate/100 mL) were sequentially added. The mixtures were shaken again for 15 min under the same

conditions to extract acrylamide. The homogenates were centrifuged at 8,500 rpm for 20 min at 4 °C, and the supernatant was filtered through a 0.22 µm membrane (Millipore, USA) prior to chromatographic analysis.

Acrylamide quantification was carried out by High-Performance Liquid Chromatography (HPLC-PDA, Waters 1525/2707, Milford, MA, USA). Separation was achieved on a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm) using 10 mM H_2SO_4 in water as the mobile phase under isocratic conditions, at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL, and acrylamide was detected at 196 nm over an 8-minute run time.

Quantification was based on an external calibration curve prepared from acrylamide standards (0.1–10 mg L^{-1}, Sigma-Aldrich, USA), which exhibited excellent linearity (R^2 = 0.999; data not shown). Method validation included determination of the limit of detection (LOD), limit of quantification (LOQ), and recovery efficiency (> 90%), confirming the accuracy and reproducibility of the analytical procedure. All measurements were performed in triplicate, and results were expressed as, and results were expressed as µg of acrylamide per kg of dry product after correction for moisture content.

Statistical analysis

The results were expressed as mean ± standard deviation. Statistical differences among treatments were determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) using Statgraphics Centurion XVIII (StatPoint Technologies, Warrenton, VA, USA).

RESULTS AND DISCUSSION

Isolation and qualitative selection of the best L-ASNase-producer

Sixteen of the 32 thermophilic bacterial isolates obtained showed asparaginase activity in the cell extract. No L-ASNase activity was detected in any culture supernatant, suggesting that none of the isolates can export the enzyme to the extracellular medium.

The three isolates with the highest L-ASNase activity (TCA 10-20, TCA 10-21, and TCA 10-22), isolated from Deception Island, Antarctica, were taxonomically identified as *Thermus oshimai*. The partial 16S rRNA gene sequences obtained were 684, 846, and 416 nucleotides long, respectively, and shared 99.56%, 99.88%, and 99.76% identity with the homologous sequence of *Thermus oshimai* DSM 12092 (T), the closest type strain. Due to the taxonomic identity and common environmental habitat of the three bacterial isolates, TCA 10-21 was chosen for further trials.

Characterisation of the thermophilic L-asparaginase

L-asparaginase (L-ASNase) reached its maximum activity at 80 °C, while a complete loss of activity was observed at 95 °C. At temperatures below the optimum, enzyme activity decreased progressively to 40 °C, although 10% of maximum activity was retained at that temperature. These findings are consistent with the structural features described for a thermophilic enzyme [*17,24*] and may be associated with the ionic interactions and additional hydrogen bonds in its tertiary structure, characteristic of thermophilic proteins. The enzyme retained high catalytic efficiency under thermal conditions that usually inactivate mesophilic asparaginases, which represents a significant advantage for its potential application in high-temperature food processing.

Concerning the effect of pH on L-ASNase, it exhibited its maximum catalytic activity at pH 9, maintaining 40-60% of its maximal activity at pH 8, 10 and 11, indicating a remarkable tolerance to alkaline media. At lower pH values, a marked decrease in activity was observed, attributed to conformational changes in the active site. This behaviour is consistent with the characteristics reported for thermophilic enzymes of the genus *Thermus* and agrees with previous studies on *T. thermophilus*, whose L-ASNase showed an optimum reaction at pH 9.2 and 77 °C [*24*].

Determination of the blanching time

Bleaching is an essential preliminary step to inactivate polyphenol oxidase (PPO) and stabilise the colour and texture of potato matrices prior to further processing. In the present study, prolonged blanching times were evaluated not for enzyme inactivation, since PPO is completely denatured within one minute at 80°C [*25*], but rather to assess their effect on tissue firmness under the extended thermal exposure required for the action of thermophilic asparaginase. Similarly, pectin methylesterase (PME), an enzyme known to influence textural integrity, is reported to be inactivated within five minutes at the same temperature [*26*]. Therefore, enzyme inactivation was considered negligible under the present conditions, and the study focused exclusively on texture modification during blanching.

As shown in Table 1, potato firmness decreased progressively with blanching time, consistent with partial disruption of the middle lamella and pectin solubilisation. Beyond 9 minutes, no significant differences ($p > 0.05$) were observed between treatments, suggesting that the softening process reached an equilibrium state. The 11-minute treatment was therefore selected as the optimal blanching duration, providing sufficient softening to enhance enzyme diffusion while preventing structural collapse. This condition also aligns with previous findings indicating that moderate bleaching at 80 °C preserves cell integrity and minimises excessive tissue degradation. [*26*]. Establishing

this baseline texture ensured reproducibility in subsequent enzymatic and thermal processing steps.

Effect of processing conditions (cutting geometry and cooking method) on acrylamide formation.

Table 2 summarises the acrylamide content in potato samples as affected by the cooking method and type of cut. Statistically significant differences ($p < 0.05$) were observed in both variables. Among the evaluated cooking methods, vacuum-fried potatoes exhibited the lowest acrylamide levels regardless of the type of cut, being the batonnets-type cut containing 122.2 ± 15.6 µg/kg. Considering the benchmark level established by the European Union for fried potatoes (500 µg/kg; [*3*]), these results indicate a substantial reduction of acrylamide formation under vacuum conditions. This reduction is attributed to the lower thermal load achieved through decreased temperature and pressure during frying, effectively limiting the Maillard reaction and, consequently, the generation of acrylamide without compromising product quality [*27*]. It may also be attributed to the reduced oxygen availability and the lower boiling point of water under subatmospheric conditions, which limit the Maillard reaction rate [*28*]. In this context, vacuum frying emerges as a promising technology for the mitigation of acrylamide in vegetable-based products, as the process operates at significantly milder thermal conditions compared with atmospheric frying [*12*]. In this sense, some studies have shown that vacuum frying may be considered an option for producing healthier novel snacks [*29*].

Samples subjected to atmospheric frying showed intermediate acrylamide content compared with the other cooking methods. Furthermore, they showed significant differences ($p < 0.05$) depending on the type of cut, with potato slices exhibiting the highest acrylamide content. When compared with vacuum frying, atmospheric frying promotes greater oxygen exposure, enhancing lipid peroxidation and acrolein formation. These compounds contribute to reactive carbonyl species that, in the presence of free asparagine, facilitate acrylamide synthesis via the Maillard pathway [*30*].

Regarding the samples that were baked, they exhibited the highest acrylamide content, above 300 µg/kg, and the slice cut type presented the highest value. Although roasting is generally considered a healthier alternative to frying due to its lower fat content [*27*], the combination of higher surface-to-volume ratios, extended cooking times, and elevated temperatures increases exposure to oxidative and thermal stress. This, in turn, enhances the Maillard reaction and promotes the accumulation of undesirable compounds [*3, 31, 32*]. The acrylamide concentrations observed in roasted slices are

within the range reported in previous studies for similar products (400–2000 µg/kg) [*27, 33*], confirming the critical role of product geometry in acrylamide generation.

Interestingly, these results contrast with those reported by Palazoğlu et al. [*34*], who found that atmospheric frying led to higher acrylamide formation than roasting, and that potatoes roasted at 170 °C exhibited higher acrylamide contents than those cooked at 180 °C or 190 °C. Such discrepancies may arise from differences in potato cultivar, slice thickness, pretreatment conditions, or roasting duration, all of which can significantly affect acrylamide variability even within the same food matrix. These findings emphasise the need to consider varietal and processing parameters when designing mitigation strategies applicable under industrially relevant conditions.

Effect of thermophilic L-asparaginase pretreatment on acrylamide reduction

Enzymatic treatments, such as blanching potato slices with L-ASNase, emerge as an effective strategy to reduce the levels of acrylamide formed during food processing [*30*]. In particular, L-ASNase plays a key metabolic role by hydrolysing L-asparagine, one of the main precursors in acrylamide formation, into L-aspartic acid and ammonia, thereby limiting its availability to participate in the Maillard reaction and effectively inhibiting acrylamide generation during thermal processing [*17*].

Based on the results obtained from the experiment on cooking methods, where roasted potato slices exhibited the highest acrylamide concentrations among all evaluated cooking methods and cut geometries (Table 2), this condition was selected as the critical scenario for evaluating the enzymatic treatment. This decision follows an optimisation approach, as focusing on the most severe case enables a more precise assessment of the mitigation potential of thermophilic L-ASNase under realistic and industrially relevant processing conditions.

The results presented in Fig. 1 indicate that the enzymatic treatment significantly reduced acrylamide levels during the roasting of potato slices. Specifically, samples C1 and C2, which underwent a blanching pretreatment with enzymatic extract, exhibited reductions of up to 77.3% compared with sample C0 (368.4±2.85 ug/Kg), which was blanched without the enzymatic extract ($p < 0.05$). However, no statistically significant differences were found between samples C1 (125.9±2.90 ug/kg) and C2 (136.0±0.20 ug/kg) ($p > 0.05$), suggesting that increasing the concentration of enzymatic extract within the tested range did not yield additional reductions in acrylamide content.

This trend confirms the dose-dependent efficacy of the treatment and is consistent with previous research reporting reductions of more than 80% in potatoes treated with commercial asparaginases [*12, 14*]. However, this study used an enzyme of

thermophilic origin, whose stability at high temperatures makes it a technological tool with great potential, as it maintains its catalytic activity during the blanching process (80 °C), a stage in which mesophilic asparaginases tend to lose functionality. This aspect represents a significant advantage from an industrial point of view, as it allows the enzymatic treatment to be integrated without modifying existing operating conditions.

Colour parameters and correlation with acrylamide content

Since colour is of paramount importance when deciding when to stop frying, the range of colour variations within consumers' quality preferences will influence the risk of acrylamide exposure [*7*].

The sample without enzymatic treatment exhibited the lowest L* value and a higher a* value compared with samples treated with the enzymatic extract. This result indicates reduced lightness and a greater shift toward reddish hues; characteristics commonly linked to non-enzymatic browning. Correlation of these colour parameters with acrylamide concentration revealed that the untreated sample contained the highest acrylamide levels, indicating a direct relationship between colour intensity and acrylamide formation. These results align with the findings of Pedreschi et al. [*23*], who reported a strong correlation between the colour parameters of French fries and their acrylamide content. Specifically, decreased lightness (L*) and increased red component (a*) were associated with greater Maillard reaction progression and elevated acrylamide levels [*7*]. Comparison of the enzymatically treated samples revealed that sample C1, which received a lower concentration of enzymatic extract, exhibited higher L* and a* values than sample C2, treated with a higher enzyme concentration. The elevated L* value in C1 indicates a lighter colour, suggesting that moderate enzymatic treatment partially reduced asparagine availability, thereby limiting but not entirely preventing the Maillard reaction. In contrast, the lower L* and a* values observed in C2 indicate a more effective suppression of browning, likely resulting from a greater reduction of acrylamide precursors at higher enzyme concentrations. These findings suggest that increasing the concentration of thermophilic asparaginase extract produces a lighter and less reddish appearance in roasted potato samples, consistent with enhanced inhibition of acrylamide formation.

In studies conducted by Mesías et al. [*7*], the chromatic parameter a* was identified as the most reliable predictor for classifying acrylamide levels above or below the benchmark value (500 µg/kg) established by the European Union Regulation for fried potatoes, regardless of the frying method employed. Consistent with these findings, the results of the present study demonstrated a direct relationship between the a* value and acrylamide content. Specifically, samples with higher acrylamide concentrations

exhibited increased a* values, reflecting a stronger shift toward reddish hues associated with a greater degree of browning.

Analysis of the Hue angle (h°) showed marked differences between treatments: the untreated sample had the lowest values, corresponding to redder tones, while the treated samples showed a shift towards higher h° values, characteristic of yellow tones. This change suggests that treatment with thermophilic asparaginase not only reduces the formation of brown pigments derived from browning but also helps to maintain the bright golden colour typical of roasted goods with good visual quality.

Regarding the chroma (C*) values, the highest value was observed in the control sample (C0), whereas the samples treated with the enzymatic extract exhibited lower values. Significant differences were found among the enzymatically treated samples, with the sample containing the highest enzyme concentration showing a slightly higher chroma value. When correlated with the h° parameter, the control sample displayed the lowest value, indicating a more reddish tone compared with the enzymatically treated samples. In fried and roasted potato products, a high C* value generally reflects an intense golden colour; however, when accompanied by low h° values (reddish tones), it may indicate excessive browning and a higher likelihood of acrylamide formation. Therefore, the lower chroma and higher h° values observed in the enzymatically treated samples suggest that the enzymatic pretreatment effectively reduced the browning intensity, in agreement with the lower acrylamide content reported in Table 3.

CONCLUSIONS

This study evaluated the effect of different thermal processing methods and cutting geometries on acrylamide formation in potato products, as well as the effectiveness of pretreatment with thermophilic L-ASNase obtained from *Thermus oshimai* as a mitigation strategy. The results demonstrated that both processing technique and cut type significantly influenced acrylamide levels. Vacuum-fried samples contained the lowest concentrations, whereas roasted potatoes exhibited the highest levels, particularly in slice-cut samples, likely due to their greater surface-to-volume ratio and enhanced thermal exposure.

The enzymatic pretreatment prior to roasting effectively reduced acrylamide content by up to 77.3% compared with the control sample without enzyme addition, with no statistically significant differences between the two tested extract volumes. This outcome confirms that the hydrolysis of L-asparagine is an efficient mechanism to limit acrylamide formation through interruption of its Maillard-reaction precursor pathway. Furthermore, the thermophilic L-ASNase displayed optimal catalytic performance and excellent thermal stability under conditions compatible with industrial blanching (80 °C),

indicating that its implementation could be compatible with conventional blanching conditions. The positive correlation observed between colour parameters (L*, a*, b*, C*, and h°) and acrylamide content further supports its applicability as a complementary quality indicator.

Overall, the findings highlight the technological feasibility and industrial potential of thermophilic L-asparaginases as a promising strategy for reducing acrylamide formation in potato-based products, in line with increasingly stringent international regulatory standards. Future research should focus on optimising enzyme dosages, evaluating the kinetics of L-asparagine conversion in real food matrices, appraising the sensory and shelf-life impacts of treatment, and exploring scale-up to pilot and industrial systems, as well as the applicability of this enzymatic strategy to other starchy food categories.

ACKNOWLEDGEMENTS

The authors thank Dr Silvia Coria from the Argentine Antarctic Institute, Buenos Aires, Argentina, for her collaboration in obtaining and preparing the bacterial isolates, and Dr Lucas Ruberto from the same institute, for his management and dispatch of the same.

FUNDING

This research was funded as part of Project PID 9114 of the National University of Entre Ríos and Project PICT-2021-I-GRF1 of the National Agency for the Promotion of Research, Technological Development, and Innovation, Argentina.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

G.L. and V.L. conceived, designed, and supervised the project. W.M.C. and S.V. procured the volcanic soil samples, isolated and provided the thermophilic bacterial strains. A.G., A.B. and G.M. were responsible for sample preparation. A.G., A.B., V.A., G.M. V.L. and G.L. performed the experiments and contributed to data analysis. A.G., A.B., G.M., V.A., V.L., S.V. and G.L. contributed to the interpretation of the results and manuscript drafting and critical revision. All authors reviewed and approved the final version of the manuscript.

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TABLES AND FIGURES SECTION

Table 1. Effect of blanching time at 80 °C on the maximum penetration force (N) of potato slices and batonnets.

Blanching time (min.)	Maximum force (N)	
	Batonnets	Slices
2	7.68±0.64^{a}	7.41±1.52^{a}
5	8.27±0.00^{a}	9.84±0.33^{b}
7	8.13±0.21^{a}	9.51±0.15^{b}
9	8.71±0.44^{a}	8.92±1.05^{ab}
11	8.62±0.01^{a}	8.20±0.03^{ab}
15	8.45±1.35^{a}	8.28±0.06^{ab}

Values represent the mean ± standard deviation (n = 3). Different letters in the rows indicate significant differences according to Fisher's LSD test ($p < 0.05$).

Table 2. Acrylamide content (ug/kg, dry basis) in potato samples as affected by cooking method and cut type.

	Acrylamide (ug/kg)		
Samples	Atmospheric frying (AF)	Vacuum frying (VF)	Roasted (R)
Slices	269.1±11.1^{bB}	160.8±14.6^{bA}	527.9±52.3^{bC}
Batonnets	225.3±22.7^{aB}	122.2±15.6^{aA}	336.1±26.5^{aC}

Values represent mean ± standard deviation (n = 3). Different uppercase letters indicate significant differences between cooking methods within the same cut type ($p <$

0.05). Different lowercase letters indicate significant differences between cut types within the same cooking method ($p < 0.05$).

Table 3. CIELAB color parameters of roasted potato slices with and without enzymatic pretreatment.

Samples	L*	a*	b*	C*	h°
C0	43.59±0.02^{a}	5.57±0.01^{c}	27.96±0.02^{c}	28.51±0.03^{c}	78.8±0.02^{a}
C1	50.02±0.02^{c}	-1.35±0.00^{b}	19.10±0.02^{a}	19.14±0.02^{a}	94.0±0.00^{b}
C2	49.62±0.02^{b}	-1.52±0.02^{a}	20.32±0.03^{b}	20.37±0.02^{b}	94.2±0.04^{c}

Values represent the mean ± standard deviation. Different letters in the columns indicate significant differences according to Fisher's LSD test ($p < 0.05$). C0: control without enzyme; C1: 0.225 U/g potato; C2: 0.450 U/g potato.

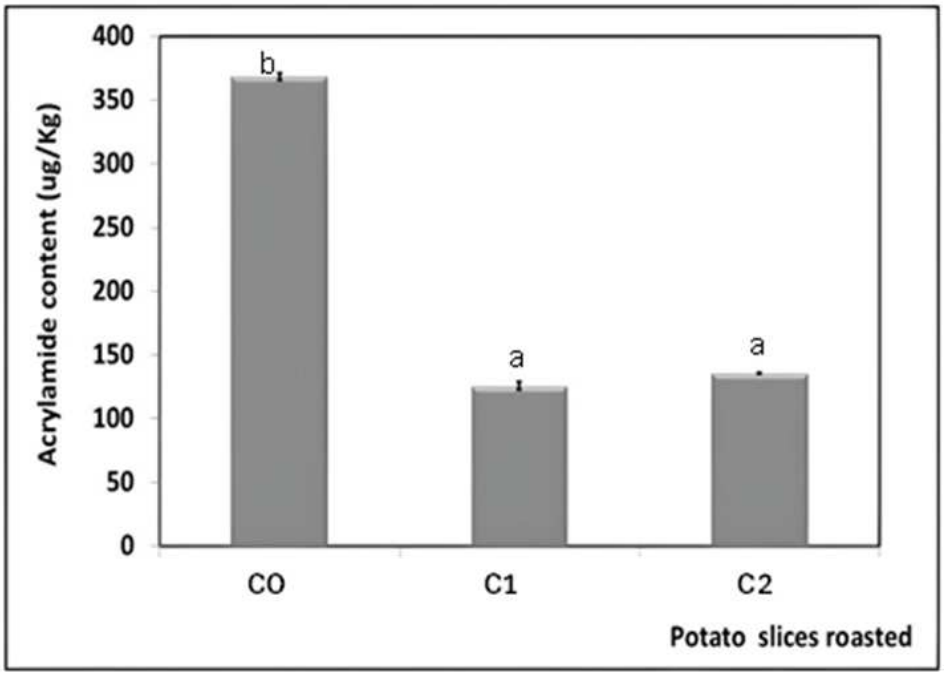


Fig. 1. Acrylamide content in potato slices roasted with L-ASNasa pretreatment. Values represent the mean ± standard deviation (n = 3). Different letters in the columns indicate significant differences according to Fisher's LSD test ($p < 0.05$). C0: control without enzyme; C1: 0.225 U/g potato; C2: 0.450 U/g potato.