J Food Sci Technol (September 2023) 60(9):2358–2369
https://doi.org/10.1007/s13197-023-05757-5

ORIGINAL ARTICLE

Strain improvement, artificial intelligence optimization, and sensitivity analysis of asparaginase-mediated acrylamide reduction in sweet potato chips

Ernest Akwagiobe[1] · Maurice Ekpenyong[2,3] · Atim Asitok[2,3] · Andrew Amenaghawon[4] · David Ubi[1] · Eloghosa Ikharia[3] · Heri Kusuma[5] · Sylvester Antai[2,3]

Revised: 2 January 2023 / Accepted: 26 March 2023 / Published online: 3 May 2023

Abstract In recent times, L-asparaginase has emerged as a potential anti-carcinogen through hydrolysis of L-asparagine in the blood for anti-leukemic application, and in carbohydrate-based foods, for acrylamide reduction applications. In this study, *Aspergillus sydowii* strain UCCM 00124 produced an L-asparaginase with a baseline acrylamide reduction potential of 64.5% in sweet potato chips. Plasma mutagenesis at atmospheric pressure and room temperature (ARTP) was employed to improve L-asparaginase production while artificial neural network embedded with genetic algorithm (ANN-GA) and global sensitivity analysis were used to identify and optimize process conditions for improved acrylamide reduction in sweet potato chips. The ARTP mutagenesis generated a valine-deficient mutant, Val$^-$Asp-S-180-L with 2.5-fold L-asparaginase improvement. The ANN-GA hybrid evolutionary intelligence significantly improved process efficiency to 98.18% under optimized conditions set as 118.6 °C, 726.37 g/L asparagine content, 9.92 µg/mL L-asparaginase, 4.54% NaCl, and soaking time of 15 h without significant changes in sensory properties. The sensitivity index revealed initial asparagine content as the most sensitive parameter to the bioprocess. The enzyme demonstrated significant thermostability with Arrhenius deactivation rate constant, K_d, of 0.00562 min^{-1} and half-life, $t_{1/2}$, of 123.35 min at 338 K. These conditions are recommended for sustainable healthier, and safer sweet potato chips processing in the food industry.

Keywords ARTP mutagenesis · ANN-GA optimization · Sensitivity analysis · L-asparaginase · Acrylamide mitigation · Sweet potato chips

Introduction

Acrylamide is a *bis*-functional monomeric water-soluble organic compound with a highly reactive electrophilic double bond and an amide functional group. The compound, also called 2-propenamide or acrylicamide (C_3H_5NO), is formed by the Maillard reaction involving free amino acids especially L-asparagine and reducing sugars in carbohydrate-based foods, and other compounds with carbonyl groups during high temperature (> 120 °C) processing (Wen et al. 2016). It can also be formed from 3-carbon compounds like acrylic acid and acrolein derived from fat oxidation during frying (Nematollahi et al. 2021).

Several starch-based food products including potato chips or crisps, crackers, French fries, cakes and cookies are among the foods with high concentration of acrylamide (Negoita et al. 2022). As the world continues to move at high

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13197-023-05757-5.

✉ Maurice Ekpenyong
maurygg2002@yahoo.com;
mauriceekpenyong@unical.edu.ng

1 Food and Industrial Microbiology Unit, Department of Microbiology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria

2 Environmental Microbiology and Biotechnology Unit, Department of Microbiology, University of Calabar, Calabar, Nigeria

3 University of Calabar Collection of Microorganisms (UCCM), University of Calabar, Calabar, Nigeria

4 Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Nigeria

5 Department of Chemical Engineering, Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Yogyakarta, Indonesia

pace, with little time for consumption of normally processed foods, potato and cereal snacking tends to dominate major global diet. Owing to increased concerns about the neurotoxicity, genotoxicity and carcinogenic potentials of acrylamide, several strategies have been developed to reduce, if not completely eliminate, acrylamide presence in these snacks (Yeo et al. 2021; Liyanage et al. 2021).

A number of variables have been identified as contributing to acrylamide formation in high temperature processed foods and these include initial L-asparagine content, nature of frying oil (in terms of their smoke points), volume of frying oil, frying temperature, duration of frying, duration of soaking, and NaCl concentration (Lingnert et al. 2002). These have formed, in singles and/or in combinations, the basis for several investigations towards acrylamide reduction (Munir et al. 2019; Negoita et al. 2022). However, one of the trendy acrylamide mitigation strategies is the use of L-asparaginase; an enzyme that hydrolyzes L-asparagine into L-aspartic acid and ammonia; thereby making asparagine unavailable for the Maillard reaction (Yassin et al. 2022).

All biological systems produce L-asparaginase; however, bacterial L-asparaginases are most copiously documented. Owing to cross-reactivity with L-glutamine and urease by prokaryotic L-asparaginases and the attendant untoward events during applications, research has, in recent times, concentrated efforts on bio-prospecting eukaryotic microbioresource, especially fungi, as alternative sources (Khalil et al. 2021). A number of L-asparaginase-producing fungi have been reported with potentials for acrylamide reduction (Munir et al. 2019). Most importantly, L-asparaginases from *Aspergillus niger* and *Aspergillus oryzae* have been licensed as commercial L-asparaginases to reduce or eliminate acrylamide formation in the food industry products (Wang et al. 2021). However, these enzymes are expensive and unstable to high temperatures, and the food industry may well benefit from more heat-stable L-asparaginases with better specific activities from other fungal sources by reason of food processing requirements (Chi et al. 2021).

Obtaining sufficiently high yield of L-asparaginase to meet food industry needs, as is frequently the case with other microbially-derived metabolites, is a huddle that must be crossed. Random mutagenesis remains a reliable method for firsthand strain improvement toward enhanced metabolite production. A number of physical agents including ultraviolet and gamma rays, and chemical agents including *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and ethyl-methane-sulfonate has been exploited in singles and in combination to mediate overexpression of value-added metabolite genes. Recently, Asitok et al. (2022a) reported successful application of alternate combinatorial random mutagenesis to improve *Stenotrophomonas acidaminiphila* for alkaline peptidase production. In current practice, a random mutagenic technique involving atmospheric and room temperature plasma (ARTP) exposure is being applied to a variety of microbial cells for improved production of microbial products. A dominant mechanism for ARTP effectiveness is its ability to reshuffle the DNA sequence of the exposed microorganism by introducing a large repertoire of breakages thus making way for countless possibilities of recombination with resultant generation of many altered species. A few of the strong points of the method include low plasma temperature, process safety and cost-effectiveness (Zhang et al. 2014).

Process optimization by response surface methodology (RSM) has been copiously documented but not so much by artificial intelligence technique. In a number of studies both techniques have been compared and artificial neural network (ANN) frequently emerges as superior, especially in terms of prediction ((Ekpenyong et al. 2021a). In typical industrial bioprocesses especially involving microorganisms where stochastic relationships of the non-linear kind dominate, it becomes significant to combine information gained from RSM and that from ANN, to get the best solutions for the process. Therefore, the two techniques have their strengths and weaknesses and are better combined especially where very significant improvements of the process are determined by interaction terms of the models (Amenaghawon et al. 2022).

This report describes the improvement of *Aspergillus sydowii* strain by ARTP mutagenesis to enhance L-asparaginase production followed by bioprocess optimization of parameters involved in L-asparaginase-mediated reduction of acrylamide in sweet potato chips using an RSM-ANN-GA hybrid technique. This, to the best of our knowledge, is the first report on optimization of influencing parameters involved in acrylamide reduction.

Materials and methods

Fungal isolation and molecular characterization

The producing mold was isolated from the epipellic sediment of the mesotidal river at Ikang, Cross River State, Nigeria along coordinates N 04° 50.127′; E 08° 32.976′, on sterile Czapek-Dox agar (CDA) enriched with fish entrails. The mold was selected for characterization by macro-and micro-morphology after demonstration of L-asparaginase production potential on asparagine-basal medium containing 2.5% NaCl at 55 °C. The filamentous fungus was subjected to molecular characterization by 18 S rRNA partial-gene sequencing using ITS1-F and ITS4 as universal primers. The selected mold was preserved at 4 °C until required.

Plasma mutagenesis

The protocol by Shu et al. (2020) was adopted to improve the strain by atmospheric and room temperature plasma (ARTP) mutagenesis. Mold spore suspension (3.5×10^6 spore-forming units per milliliter) was mutagenized by exposure to 120 W (radio frequency) at 10 L/min (flow-rate) from 2 mm (distance) for 240 s (exposure time). L-asparaginase overproducing strains were screened from sterile fermentation broth of survived cells by the assay method of Imada et al. (1973). Overproducing mutants were evaluated for stability for 1080 h at 72 h/generation.

Characterization of mutants by auxanography

Holliday (1956) developed a parallel plating method which was employed to determine the growth factor(s) for which L-asparaginase-overproducing mutants that were stable for 1080 h were auxotrophic. Filtered-stock solutions of growth factors, numbering 36 and comprising nucleotide bases, protein monomers, intermediates of metabolic pathways and vitamins, were incorporated into minimal medium (MM) using a 6-column 6-row arrangement. Similar concentrations (1 mM) of protein monomers, nucleotide bases, ornithine and hypoxanthine but variable microgram per milliliter concentrations (µg/mL) of vitamins and precursors were incorporated into twelve 8.5 cm Petri dishes containing minimal medium and incubated at 30 °C for 120 h (Mormann et al. 2006). Auxotrophic mutants were transferred to CDA as complete medium, incubated for 72 h at 30 °C and developed colonies preserved by refrigeration for further studies.

Bioreactor fermentation of L-asparaginase by selected *Aspergillus sydowii* ARTP mutant

The selected stable mutant was employed to produce L-asparaginase in submerged culture and compared with the wild strain. Fermentation medium composition was similar to that described in Ekpenyong et al. (2021b). A 5-L BioStat$^{(R)}$ CPlus bioreactor (Sartorius Stedim Biotech, Germany) with 70% (v/v) reaction volume was employed for fermentation in batch mode. The fermentation medium was sterilized 'in place' and inoculated with mold suspension (3% v/v) after cooling. Duration of fermentation was 48 h after which L-asparaginase activity and total protein were determined according to Imada et al. (1973) and Bradford (1976) respectively.

L-asparaginase purification and determination of molecular weight

Protein purification was achieved by a combination of three methods including ammonium sulfate precipitation, di-ethyl-amino-ethyl (DEAE)-cellulose and gel filtration and specific enzyme activity was calculated for each purification step. Molecular weight of protein was confirmed according to the procedure of Laemmli (1970) using 12% gel SDS-PAGE analysis with standard proteins of molecular weights (kDa) in the range 12.4–115.

Amino acid profiling of test L-asparaginases

The sequence of amino acids in wild-type and mutant L-asparaginases were determined using an analyzer (Agilent Technologies, Denmark). A comparison of test L-asparaginase sequences was made with L-asparaginase sequences obtained from Gene bank at NCBI for other species of *Aspergillus*.

Specificity of mutant L-asparaginase for substrates

The specificity of test L-asparaginases was evaluated with urea, D-glutamine, L-glutamine and L-asparagine as enzyme substrates in the assay mixture according to Imada et al. (1973).

Physicochemical properties of mutant L-asparaginase

Effect of pH was determined by pre-incubating 0.048 µM of mutant L-asparaginase in buffers at different pH ranging from 3 to 12 before measuring relative activity (%). Effect of NaCl was also determined by pre-exposure of 0.048 µM of mutant L-asparaginase to NaCl concentrations ranging from 2.5 to 25% before incubating with substrate and determining relative activity (%).

Temperature effect on the activity of L-asparaginase was studied over 25 to 95 °C by pre-exposure of the enzyme (5 µg/mL corresponding to 0.048 µM) at the respective temperatures in a water bath. The enzyme was allowed to re-fold on ice for 5 min before measuring residual enzyme activity in the presence of its substrate. Afterwards, thermal stability was determined using the thermal inactivation method at optimum temperature(s) for 240 min (at 30 min interval). The first-order thermal inactivation rate constant, K_d was calculated using Eq. (1).

$$LnA = LnA_0 - K_d t \quad (1)$$

where A is the final enzyme activity (U/mL), A_0 is the initial enzyme activity (U/mL), t is time (min). By plotting - ln A/A_0 against time t, K_d was determined as slope of the graph. Furthermore, with K_d determined, the activation energy of the L-asparaginase-catalyzed reaction was calculated using the Arrhenius expression in Eq. (2).

$$LnK_d = LnA - \frac{E_d}{RT} \quad (2)$$

where E_d is the activation energy for thermal denaturation (kJ/mol), A the Arrhenius frequency factor (Ln A is the integration constant, s^{-1}), R the universal gas constant (8.314×10^{-3} kJ/mol K) and T is the absolute temperature (K). The half-life of the enzyme, $t_{1/2}$ (defined as the time required to inactivate 50% of the enzyme) was then calculated from the expression in Eq. (3).

$$t_{1/2} = \frac{0.693}{K_d} \quad (3)$$

Michaelis-Menton kinetics of Val-Asp-S-180-L lasparaginase

The kinetic parameters, K_m (substrate concentration) and V_{max} (maximum velocity) of the enzyme were determined as described in Asitok et al. (2022b) using 0.02-20 mM of L-asparagine and extrapolating the parameters from the Lineweaver-Burke double reciprocal plot.

Design of experiment and neural network optimization of L-asparaginase-mediated acrylamide reduction

Eight variables that influence acrylamide reduction in sweet potato chips were identified in preliminary experiments that adopted one-variable-at-a-time approach. These included pH, temperature, NaCl concentration, asparagine content, asparaginase concentration, soaking duration, frying oil volume and duration of frying. The variables were subjected to 2^k factorial screening using Plackett-Burman design (PBD) in Minitab version 17 (Minnesota Pennsylvania, USA) to identify significant ones ((Ekpenyong et al. 2021b). Each variable was tested at + 1 (high) and − 1 (low) levels including 5 points at the center to determine model curvature as presented in the design matrix in Table S1 (Supplementary material). Analysis of variance (ANOVA) model was employed to determine significant variables at 95% confidence level and the method of least squares used to build a first-order regression model as given in Eq. (4).

$$y = b_0 + \sum b_i x_i + \sum b_{ij} \sum x_i x_j + \epsilon \quad (4)$$

where y is outcome variable, b_0 as coefficient for constant term; b_i as coefficient for linear term; b_{ij} as coefficient of interaction terms and e is error term.

The significant curvature of the PBD experiment informed skipping the steepest ascent experimentation step into response surface methodology (RSM) using the matrix of a central composite rotatable design (CCRD) in Matlab 2014 (Mathwaorks, USA). The second-order model built from analysis by RSM is given by the general expression in Eq. (5).

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \beta_{ij} x_i x_j + \epsilon \quad (5)$$

The CCRD levels were utilized to reduce acrylamide level in sweet potato chips. Briefly, sweet potato (*Ipomoea batatas*) was freshly peeled and cut into strips weighing 200 g and oven-dried at 85 °C for 10 min. The strips were fried in 200 mL of sunflower oil in a 15 × 10 cm pan to obtain chips. Acrylamide was extracted from the chips after pounding and soaking in ethanol (50 mL 90% v/v) to extract acrylamide. Residual ethanol was removed by vacuum evaporation and acrylamide concentration determined with Nessler's reagent (Imada et al. 1973) and recorded as baseline acrylamide before treatment. Next, sweet potato strips were prepared according to CCRD treatment conditions. After treatments and frying, strips were ground in a mortar and amount of acrylamide extracted and determined by Gas chromatography-Mass spectrometry (GC-MS) as described by Yassin et al. (2022). Acrylamide reduction was calculated as given by the expression in Eq. (6)

$$AR(\%) = \frac{Acryl_{wo} - Acryl_w}{Acryl_{wo}} \times 100 \quad (6)$$

where AR refers to acrylamide reduction, $acryl_w$ and $acryl_{wo}$ refer to acrylamide contents with and without treatment respectively.

Artificial neural network (ANN) modeling and optimization by genetic algorithm (GA)

The experimental CCRD results were modeled by a multilayer perceptron of a feed-forward artificial neural network (FF-ANN) after normalization using Eq. (7).

$$y = \frac{2x_i - (x_{max} + x_{min})}{x_{max} - x_{min}} \quad (7)$$

where $y = x_i$ normalized values and x_{max} = maximum value of x_i and x_{min} = minimum value of x_i.

First, the configuration of the network was optimized to include 2 hidden layers with 30 neurons and a symmetric sigmoid transfer function (*tansig*) to convey information from hidden layers to the output layer. The network was trained by Levenberg-Marquardt backpropagation algorithm (*trainlm*) to obtain a fitness level given by coefficient of determination, r^2 of 0.9591 and root mean squared error (RMSE) of 0.0538. Training of the network was terminated when the mean squared error (MSE) for the validation samples from which the RMSE was calculated no longer improved. Model performance was assessed by error metrics

including r^2, its adjusted version (adjusted r^2), mean squared error (MSE), root mean squared error (RMSE), mean absolute error (MAE) and average absolute deviation (AAD) as expressed in Eqs. (8)–(13).

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(x_{a,i} - x_{p,i}\right)^2}{\sum_{i=1}^{n}\left(x_{p,i} - x_{a,ave}\right)^2} \tag{8}$$

$$Adjusted\ R^2 = 1 - \left[\left(1 - R^2\right) \times \frac{n-1}{n-k-1}\right] \tag{9}$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(x_{p,i} - x_{a,i}\right)^2 \tag{10}$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(x_{p,i} - x_{a,i}\right)^2} \tag{11}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|\left(x_{a,i} - x_{p,i}\right)\right| \tag{12}$$

$$AAD = \frac{1}{n}\left(\sum_{i=1}^{n}\left(\frac{\left|\left(x_{a,i} - x_{p,i}\right)\right|}{x_{a,i}}\right)\right) \times 100 \tag{13}$$

Genetic algorithm optimization of ANN variable conditions

The best conditions from the ANN models were optimized using the GA optimization toolbox (*optimtool*) in Matlab 2014. The objective function was MSE which needed to be minimized. The GA optimization characteristics included a maximum population of 50, maximum generation of 150 and stall generation limit of 100 and adopted a stochastic selection. Variable conditions that gave the least MSE value were selected as optimized solutions to the problem and were validated in triplicate real-time acrylamide reduction experiments as earlier described.

Validation of ANN-GA optimized solution

L-asparaginase-mediated acrylamide reduction in sweet potato chips was repeated in real-time under the ANN-GA optimized conditions and results compared with model prediction. A more that 5% difference between the predicted and real-time values scored the optimized solutions as unworkable; otherwise they were accepted as true for future applications.

Global sensitivity analysis of variable contributions to model objective

Global sensitivity analysis (GSA) provides insight into the role of the input parameters in contributing to the total variance of the response viz. acrylamide reduction (Amenaghawon et al. 2022). To determine which of the five variables the acrylamide reduction process was most sensitive to, the first, second and total order sensitivity indices were estimated. The variance in the response attributed to the first order terms was measured by the first-order sensitivity indices. On the other hand, the variation in the response attributed to the combination of the first- and second-order terms was measured by the total-order sensitivity indices. A parameter was deemed sensitive if its total order sensitivity index was > 0.1.

Sensory evaluation of L-asparaginase-treated sweet potato chips

The study evaluated the overall acceptability of the processed potato chips using 10 trained Nigerian panelists comprising 5 men and 5 women accustomed to potato chip quality. Four sensory parameters were considered including appearance, flavor, texture and taste. Each parameter was scored on a scale ranging from 1 to 10 with '10' indicating extremely like and '1' indicating extremely dislike (Jung et al. 2013). Each panelist was made to evaluate commercially prepared sweet potato chips before beginning the evaluation. Results from all panelists were averaged and reported as average +/− standard deviation.

Results and discussion

The study mold

The study mold developed as patchy matted dull-brown growth on CDA as shown in Fig. 1a. Its cotton blue-in-lactophenol bright-field microscopic morphology is shown in Fig. 1b with biseriate phialides covering vesicles with long conidiophores characteristic of Aspergilli. L-asparaginase production was identified by dull pink coloration after 48 h of growth on asparagine-minimal agar medium as shown in Fig. 1c (Gulati et al. 1997). Its baseline L-asparaginase activity in liquid culture was recorded as 876.64 U/mL. The query sequence of 18 S rRNA partial-gene of the mold in Fig. 1d revealed 99.9% homology with *Aspergillus sydowii* strain AXM1 (Gene Bank accession: MK396475.1). The mold was deposited at www.wfcc.info/ccinfo/collection/by_id/652 and given the collection's code name *Aspergillus sydowii* strain UCCM 00124.

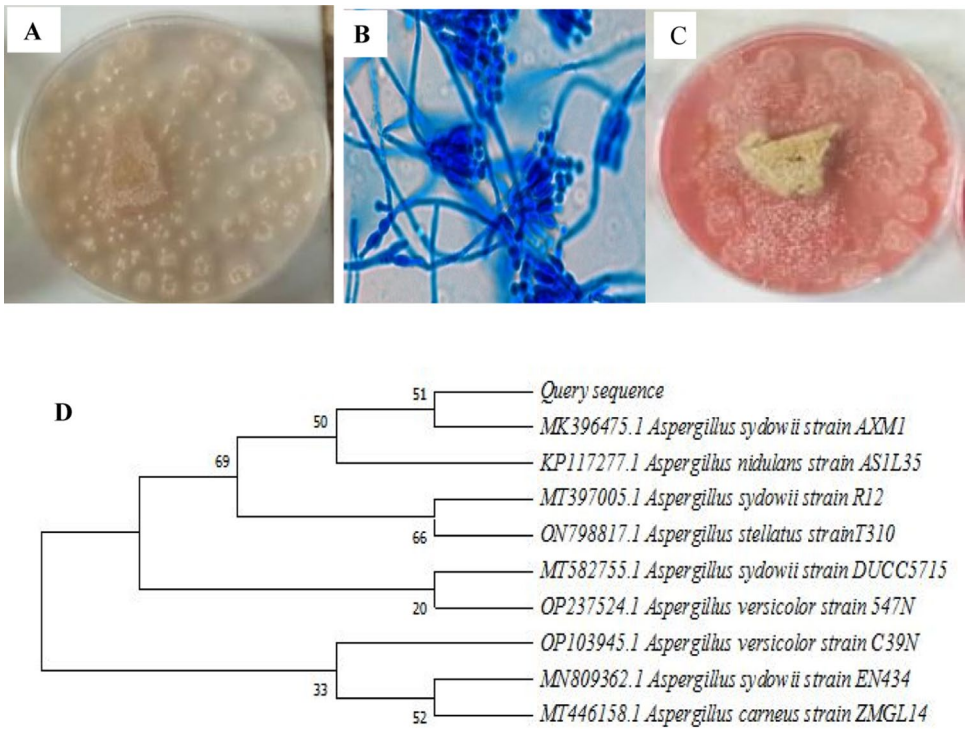

Fig. 1 Cultural (**A**), microscopic (**B**), L-asparaginase potential (**C**) and phylogenetic tree (**D**) of *Aspergillus sydowii* UCCM 00124

ARTP mutagenesis, stability and mutant characterization

Atmospheric and room temperature plasma (ARTP) mutagenesis conducted to improve strain yielded 154 mutants from different durations of exposure. Exposure for 150 s generated 4 mutants which were unstable and therefore discarded. One hundred and twenty-five mutants were generated by exposure for 180 s, with 46.67% showing overproduction (> 50% improvement) of L-asparaginase. However, only mutant-12 could stably produce an L-asparaginase activity of 10,173.28 U/mL for 15 generations, which when broken into 72 h/generation, gave a total of 1080 h. The mutant was characterized by auxanography and results showed that it could grow on the 6th (column) and 12th (row) plates indicating that the mutant was auxotrophic for a common growth factor between these plates (Holliday 1956). Since the common growth factor between the plates was valine, the mutant was described as valine-deficient and accordingly, designated Val$^-$Asp-S-180-L. Biosynthesis of valine occurs through the cooperation of three pathways namely pyruvate, aspartate and isoleucine-valine. Since leucine and alanine are also synthesized through these pathways, it follows that the *ilv*E gene was affected during the plasma mutagenesis. The *ilv*E gene encodes the enzyme transaminase B which synthesizes L-valine from α-ketoisovalerate. This intermediary substrate has been reported as a candidate therapeutic agent against prolonged renal problems (Krause et al. 2010).

L-asparaginase purification and determination of molecular weight

An L-asparaginase activity of 68,085.73 ± 5629.95 U/mL was obtained from the 48 h bioreactor fermentation by the mutant compared to 10,178.28 ± 1829.77 U/mL in shake flask, giving a 6.69-fold improvement (Table 1). In comparison, the wild strain could only muster 8,297.64 ± 1019.57 U/mL within the same time frame indicating a yield improvement of 9.47-fold in Val$^-$Asp-S-180-L from the wild strain. Total protein content was determined as 563.72 ± 82.38 mg for the mutant and 82.45 ± 10.06 mg for the wild strain indicating 6.84-fold improvement. Results of L-asparaginase purification (Table 1) show that purification factor (fold) increased with each successive purification step while enzyme recovery (yield) decreased with each purification step. Gel filtration purified the protein by 27.18-fold, with a final yield (recovery) of 14.37% and a specific activity of 3282.91 U/mg. Molecular weight of the purified protein determined using SDS-PAGE revealed a single band at 37.8 kDa (Fig. S1, Supplementary material).

The amino acid profile of the 37.8 kDa purified protein revealed a total of 208 amino acid residues as compared to 225 residues in the wild type (Fig. S2, Supplementary material) suggesting that the ARTP mutagenesis deleted about 17 codons that were not replaced resulting in shortened amino acid sequence of the Val$^-$Asp-S-180-L L-asparaginase. Since the mutant L-asparaginase without those 17 codons had a higher specific activity than the larger protein of the wild

Table 1 Effect of purification step on specific activity, yield and fold of Val-Asp-S-180-E L-asparaginase

Purification step	Total protein (mg/mL)	Total activity (U/mL)	Specific activity (U/mg)	Yield (%)	Fold purification
Sterile crude extract (wild strain)	82.45 ± 10.06	10,297.64 ± 1,819.57	124.90	100	–
Sterile crude extract (mutant)	563.72 ± 82.38	68,085.73 ± 5,629.95	120.78	100	–
$(NH_4)_2SO_4$ precipitation (wild strain)	22.46 ± 2.91	8,863.28 ± 593.35	394.68	86.07	3.16
$(NH_4)_2SO_4$ precipitation (mutant)	112.74 ± 13.18	49,135.26 ± 3928.17	435.83	72.17	3.61
DEAE-cellulose (wild strain)	5.71 ± 0.25	5,152.34 ± 185.36	901.78	50.03	7.22
DEAE-cellulose (mutant)	10.92 ± 1.07	25,524.34 ± 2637.21	1,421.64	37.49	11.77
Sephadex-200 (wild strain)	1.58 ± 0.02	3,735.22 ± 217.54	2,364.06	36.27	18.93
Sephadex-200 (mutant)	2.98 ± 0.01	9,783.08 ± 372.65	3,282.91	14.37	27.18

strain, then it follows that the 17 deleted codons would be somewhat responsible for regulation of protein activity (gene product regulation).

Substrate specificity and physicochemical properties of L-asparaginase

Urea, L-glutamine and L-glutamic acid all demonstrated low affinities toward Val-Asp-S-180-L L-asparaginase in the order of 0.56%, 8.56% and 4.21% respectively. This indicates that clinical applications could benefit from the low activities of glutaminase and urease of the study L-asparaginase. pH optimum for activity of Val-Asp-S-180-L L-asparaginase was in the acidic range (pH 3–7) as no significant difference ($p > 0.05$) was observed across this pH range (Fig. 2a), which is typical of fungal enzymes. However, at the alkaline pH of 9, an L-asparaginase activity of 79.79% was retained while 88% residual activity was recorded at pH 8.0. This indicates that the study L-asparaginase could be employed for catalytic applications over a pH range of 3 to 9.

Results of the effect of NaCl concentration on activity of the enzyme showed that optimum NaCl tolerance was 12.5% as significant loss (69.7%) in enzyme activity occurred when exposed to NaCl above this concentration (Fig. 2b). Influence of temperature is illustrated in Fig. 2c and Tukey's multiple comparison tests showed that optimum temperature for activity of the enzyme occurred between 45 and 75 °C. The study L-asparaginase was stable at 55 °C for 150 min with residual activity of 80% (Fig. 2d). The thermal deactivation rate constant, K_d and half-life, $t_{1/2}$ of the enzyme were determined at 45 °C (318 K), 55 °C (328 K), 65 °C (338 K) and 75 °C (348 K) using GraphPad Prism 8 software as 0.002222, 0.003819, 0.005618 and 0.009741 min^{-1}, respectively and were observed to follow first-order kinetics (El-Naggar et al. 2018). It was observed that half-life decreased with every 10 °C rise in temperature as 311.937, 181.476, 123.346 and 71.143 min, for 318, 328, 338 and 348 K, respectively. The activation energy, E_a, of the enzyme-catalyzed reaction was calculated as 53,801.5 J/mol.

Kinetic properties of Val-Asp-S-180-L L-asparaginase

The Michaelis-Menten constant K_m and maximum velocity, V_{max} of mutant L-asparaginase-catalyzed reaction were determined from Lineweaver-Burk plot (Fig. S3, Supplementary material). The enzyme displayed a K_m of 0.267×10^{-4} M, V_{max} as 666.67 µmol/min. The low K_m suggests its high substrate affinity while the V_{max} indicates its catalytic efficiency.

Optimization of L-asparaginase-mediated acrylamide reduction in sweet potato chips

Reducing sugars did not contribute significantly ($p > 0.05$) to acrylamide formation in sweet potato chips as earlier reported by Muttucumaru et al. (2017). This may derive from the vast difference in asparagine content of different potato varieties used in the studies. However, this important finding is corroborated by Teuschler et al. (2021) which found asparagine content as contributing the most to acrylamide formation. Results of the CCRD-RSM optimization are presented in Table 2; Fig. 2a as surface (top) and bottom contour (bottom) plots to illustrate two-way interaction of variables that led to ≥ 80% acrylamide reduction in sweet potato chips. Figure 2b shows that the interaction between temperature and soaking time (X_1X_5) made the highest contribution of 84% to acrylamide reduction followed by that between asparagine concentration and soaking time (X_2X_5) with 83.7% (Fig. 2d) and 82.4% reduction by NaCl concentration/soaking time interaction, X_4X_5 (Fig. 2f). Figure 2a (X_1X_2), 2c (X_2X_3), and 2e (X_3X_5) all barely led to 80% reduction in acrylamide formation in the chips.

Analysis of variance (ANOVA) for the second-order model is presented as Table S2 (supplementary material). The statistical CCRD-RSM model gave an r^2 of 0.9286, adjusted r^2 of 0.8794, a predicted r^2 of 0.7617, an adequate precision of 16.73, standard deviation of 3.34, percent covariance (C.V. %) of 4.59, lack-of-fit F-statistic (22, 29) of 1.92, $p = 0.1914 > 0.05$ and a mean squared error (mse) of

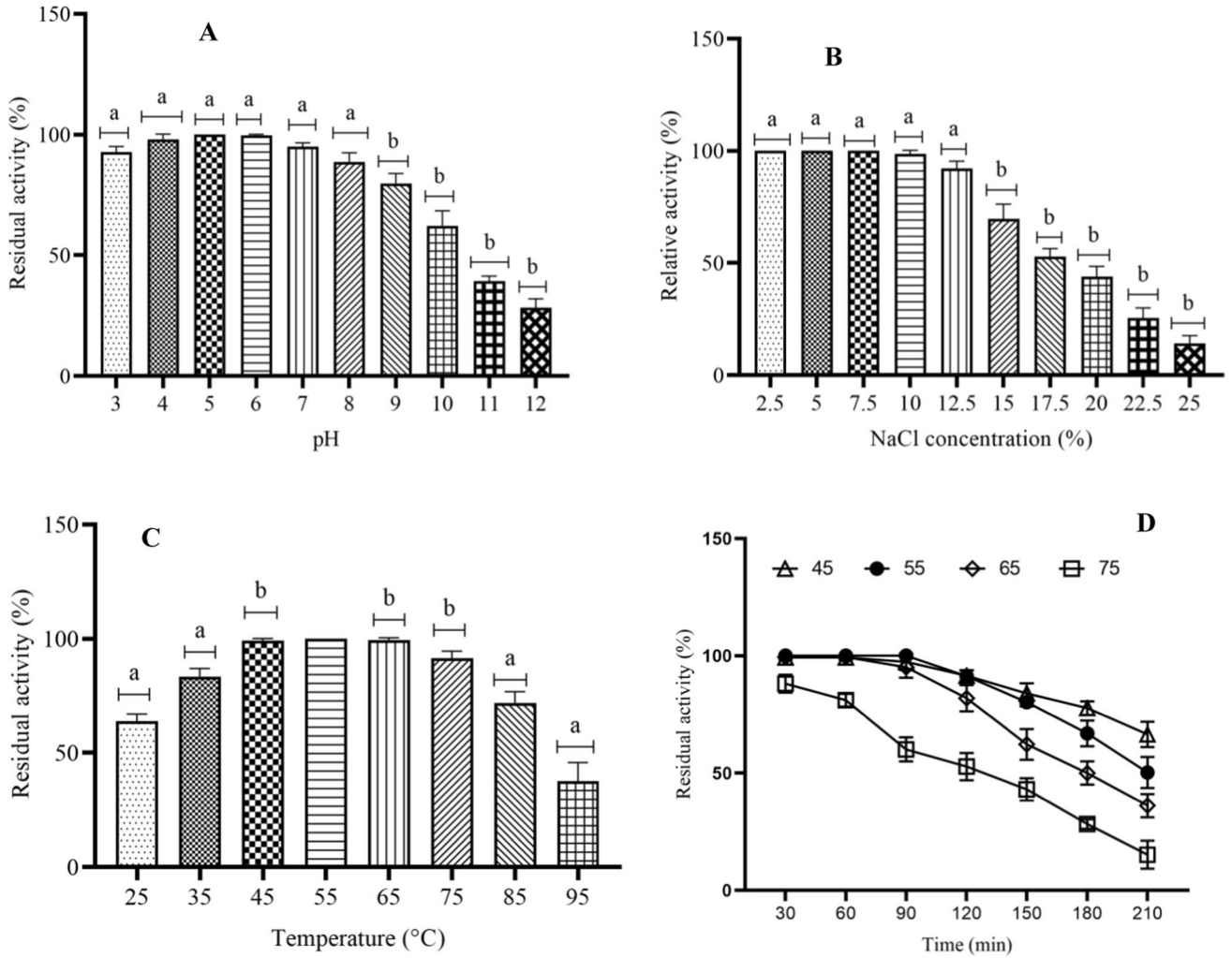

Fig. 2 Effect of physicochemical parameters on Val-Asp-S-180-L Lasparaginase

11.14. However, the ANN model by Levenberg-Marquardt back-propagation using 30 neurons in the hidden layers returned an acrylamide reduction potential of 99.75% at an r^2 of 0.9793, adjusted r^2 of 0.9592, MSE of 4.7631, RMSE of 2.1825, MAE of 0.9597 and AAD of 1.2116% (Table 1). This result was obtained under conditions set as 120 °C temperature, acrylamide content of 700 g/L, asparaginase concentration of 10 µg/mL, 5% NaCl and a soaking time of 12 h. However, genetic algorithm (GA), adopted to optimize ANN model conditions by minimization of MSE returned an acrylamide reduction of 98.53% under conditions set at 118.60 °C temperature, 726.37 g/L initial acrylamide content, 9.92 µg/mL L-asparaginase, 4.54% NaCl and a soaking time of 15.00 h with an MSE of 0.17203. These conditions, when validated in real time acrylamide reduction in sweet potato chips, resulted in 98.18% acrylamide reduction confirming that the ANN-GA hybrid model conditions set for the bioprocess were reliable and adequate. The significance of frying the chip at a temperature lower than 120 °C ensures that little or no acrylamide is formed in the process conferring overall safety to the product.

Global sensitivity analysis of input variables on acrylamide reduction process

The un-shaded bars in Fig. 4a represent the first order indices while the shaded bars represent the total indices. The variance in the response attributed to the first-order terms (single effect terms) was determined by first-order sensitivity indices. On the other hand, the variation in the response attributed to the combination of the first and second order terms is measured by the total order sensitivity indices. Zhang et al. (2016) had previously reported that a parameter is deemed sensitive if its total order sensitivity index is greater than 0.1. In this regard, Fig. 4a shows that all the input parameters were important. In ranking the parameters in order of importance, it is seen that X_2, which represents initial asparagine content was the most sensitive with a sensitivity index of

Table 2 CCRD actual and ANN predicted values for L-asparaginase-mediated acrylamide reduction in sweet potato chips

Run no.	Temp (°C)	Asparagine conc. (g/L)	ASNase conc. (µg/mL)	NaCl conc. (%)	Soaking time (h)	Actual acrylamide reduction (%)	Predicted acrylamide reduction (%)
1	140	700	5.0	10.0	6	62.25	62.35
2	120	900	10.0	10.0	6	60.50	60.50
3	130	800	7.5	7.5	9	82.38	85.61
4	130	800	7.5	7.5	9	84.39	85.61
5	130	800	7.5	7.5	9	85.52	85.61
6	120	700	10.0	10.0	12	77.53	77.53
7	120	900	10.0	10.0	12	78.53	78.53
8	120	900	5.0	5.0	6	73.27	73.27
9	120	700	5.0	10.0	12	72.15	72.16
10	130	800	7.5	7.5	9	88.35	85.61
11	130	800	2.5	7.5	9	64.37	64.37
12	130	800	12.5	7.5	9	75.25	75.25
13	120	700	10.0	5.0	6	73.84	73.84
14	140	700	5.0	5.0	12	69.77	69.78
15	130	800	7.5	2.5	9	78.55	78.55
16	120	700	5.0	5.0	6	62.94	62.94
17	140	700	5.0	10.0	12	75.36	75.36
18	130	800	7.5	7.5	9	82.19	85.61
19	120	900	5.0	5.0	12	77.16	77.16
20	120	900	5.0	10.0	6	62.88	62.88
21	120	700	5.0	5.0	12	82.53	82.53
22	140	700	5.0	5.0	6	63.27	63.28
23	140	900	5.0	5.0	12	60.28	60.28
24	120	900	10.0	5.0	6	67.42	67.41
25	130	800	7.5	7.5	9	83.27	85.61
26	140	700	10.0	5.0	12	81.56	86.18
27	140	900	10.0	10.0	6	57.93	57.94
28	110	800	7.5	7.5	9	75.37	75.37
29	140	900	5.0	5.0	6	61.49	61.49
30	130	600	7.5	7.5	9	71.43	71.43
31	120	700	5.0	10.0	6	60.06	60.06
32	130	800	7.5	7.5	9	85.28	85.61
33	130	800	7.5	12.5	9	67.34	67.35
34	130	800	7.5	7.5	3	68.45	61.73
35	120	900	5.0	10.0	12	73.28	74.12
36	130	800	7.5	7.5	15	90.66	90.66
37	150	800	7.5	7.5	9	61.35	61.35
38	120	700	10.0	5.0	12	92.73	99.75
39	120	700	10.0	10.0	6	64.17	58.71
40	130	1000	7.5	7.5	9	60.11	60.12
41	130	800	7.5	7.5	9	89.04	85.61
42	140	700	10.0	5.0	6	79.21	78.20
43	140	700	10.0	10.0	6	74.24	71.05
44	140	900	5.0	10.0	12	70.39	70.40
45	140	900	5.0	10.0	6	68.49	68.49
46	140	900	10.0	10.0	12	61.28	61.29
47	140	700	10.0	10.0	12	76.36	76.36
48	140	900	10.0	5.0	12	65.12	65.12

Table 2 (continued)

Run no.	Temp (°C)	Asparagine conc. (g/L)	ASNase conc. (μg/mL)	NaCl conc. (%)	Soaking time (h)	Actual acrylamide reduction (%)	Predicted acrylamide reduction (%)
49	140	900	10.0	5.0	6	58.94	58.94
50	120	900	10.0	5.0	12	80.42	80.41

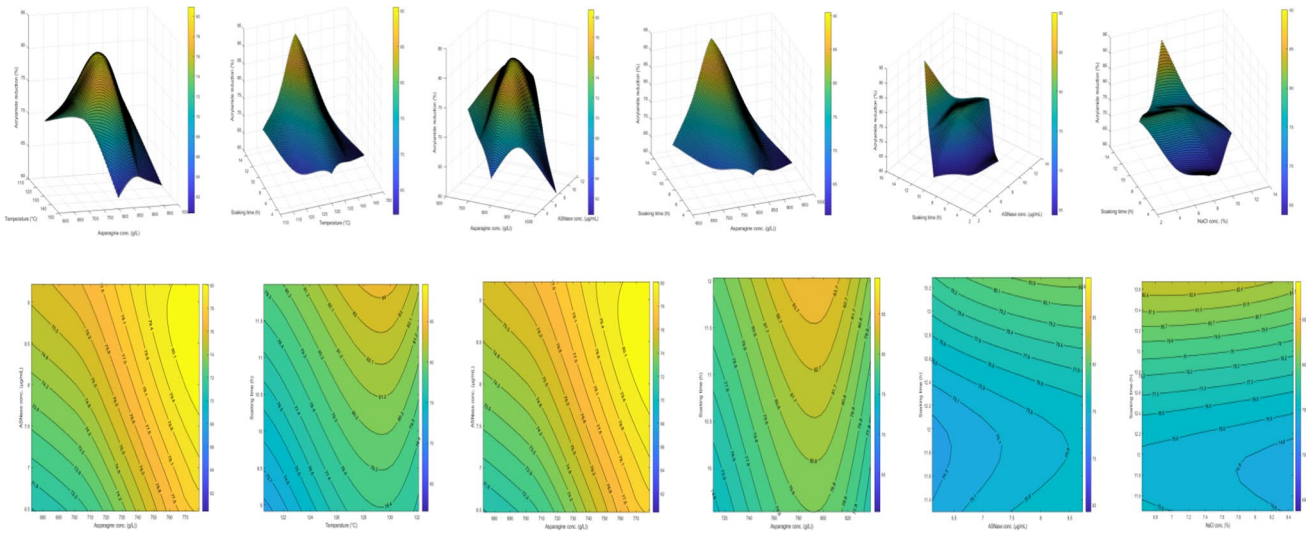

Fig. 3 Two-way interactions of significant factors toward ≥ 80% acrylamide reduction as surface (top) and contour (bottom) plots

0.35 accounting for 35% of the total variation in the response and suggesting that it contributed the most to the variance observed in the response (Teuschler et al. 2021). This was followed by X_1 representing temperature, X_5 representing soaking time and X_3 representing L-asparaginase concentration. The least sensitive parameter was blanching with NaCl (X_4) with a sensitivity index of 0.15 accounting for 15% of the total variation in the response. Thus, in designing a process such as this, a very sensitive parameter such as initial asparagine content of the raw food material should be given significant consideration.

The specific contributions to the variance of the response resulting from the interaction between each individual parameter were determined from the estimation of the second-order sensitivity indices as represented by the 3D histogram in Fig. 4b. In this instance, a 0.01 threshold was used to define the level of sensitivity. The 3D histogram presents the sensitivity indices in the vertical axis while the horizontal axes show the input parameters. Using a sensitivity threshold of 0.01, it was found that all the interactive terms were important apart from the terms representing the interaction between L-asparaginase concentration and soaking time (X_3X_5) and NaCl concentration and soaking time (X_4X_5), confirming the ANOVA results for two-way interactions in Fig. 3 already described. It should be recalled that initial asparagine content (X_2) already had a significant shared interaction with the other parameters as shown in Fig. 4a. In Fig. 4b, it can be seen that this same parameter had a significant one-on-one interaction with temperature (X_1X_2), L-asparaginase concentration (X_2X_3), NaCl concentration (X_2X_4) and soaking time (X_2X_5). The figure also shows that the interaction between asparagine content and L-asparaginase concentration (X_2X_3) was most sensitive as it contributed the highest (7.64%) to the variance observed in the response. The least contribution from the interaction between the input parameters came from NaCl concentration and soaking time (X_4X_5) with an overall contribution of 0.03%.

Sensory evaluation

Results of the sensory evaluations of L-asparaginase treated sweet potato chips and the untreated counterpart revealed no statistically significant difference ($p = 0.0721 > 0.05$) in taste and aroma. However, significant differences ($p = 0.0006 < 0.05$) existed in texture and appearance. Specifically, the panel of tasters adjudged the L-asparaginase-treated potato chips softer than the untreated chips and less brown too, which is a good thing as acrylamide content increases with browning. Further extension of treatment to

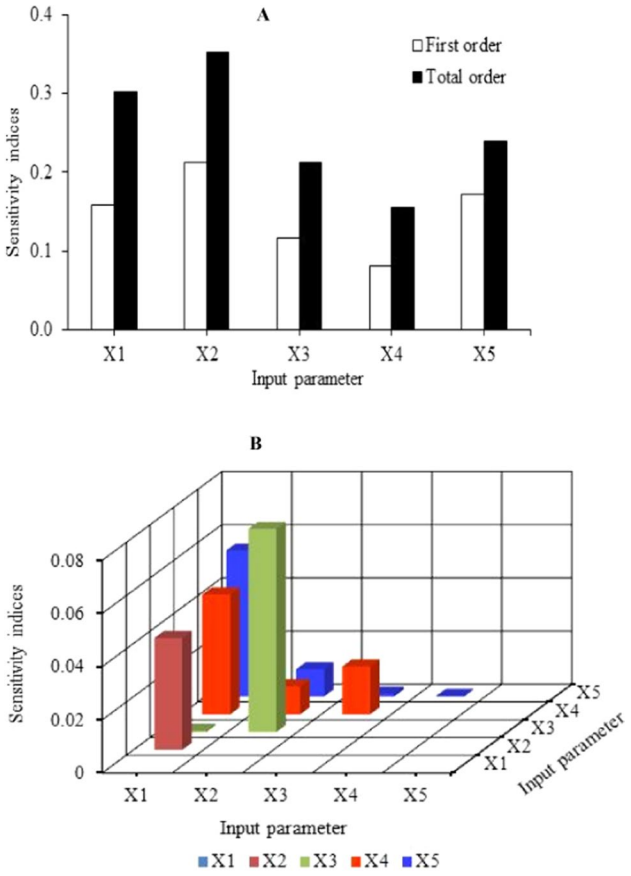

Fig. 4 Global sensitivity analysis of linear (**A**) and interaction (**B**) factor contributions to L-asparaginase-mediated acrylamide reduction optimization process

Irish potato and cocoyam (Fig.S5, Supplementary material) recorded similar sensory findings. These differences did not impact significantly on the general acceptability of the final product. The complete loss of activity of the enzyme during the 118 °C frying completely eliminated all trace of adverse reaction arising from L-asparaginase consumption thus underlying the general safety of the product.

In conclusion, a combination of strain improvement by ARTP mutagenesis and batch mode bioreactor fermentation enabled the production of a large quantity of low molecular weight (~ 37 kDa) L-asparaginase by Val^{-}Asp-S-180-L. The enzyme demonstrated an acidic pH optimum with 65 °C as temperature optimum for activity coupled with phenomenal Michaelis-Menten kinetics. The enzyme achieved a 98.18% acrylamide reduction in sweet potato chips with most sensitive optimized parameter being initial asparagine content. The technology allows frying at a temperature below 120 °C and did not significantly influence general acceptability of the final product during sensory evaluation.

Acknowledgements Authors are grateful to personnel at Sheda Facility, Abuja, Central laboratory, University of Ibadan, Nigeria and Food Science and Technology laboratory, University of Calabar, Nigeria and University of Calabar Collection of Microorganisms, for technical assistance.

Authors' contributions ME conceived the idea; EA, DU, EI conducted the experiments while ME and AA supervised the work; ME, AAm, HK analyzed data; ME, AA, AAm prepared original draft; HK and SA edited the final manuscript. All authors read approved the final manuscript.

Funding The submitted work did not receive funding from any government or non-government sources.

Data availability Data generated in this study are provided in the main manuscript and Supplementary material. Additional data may be provided upon reasonable request by the corresponding author.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval Not applicable.

Consent to participate The participant at the sensory evaluation of L-asparaginase treated sweet potato fries freely and willingly consented to participate but opted to remain anonymous.

Consent for publication Not applicable.

References

Amenaghawon AN, Orukpe PI, Nwanbi-Victor J, Okedi MO, Aburime EI (2022) Enhanced lipase production from a ternary substrate mix of agricultural residues: a case of optimization of microbial inducers and global sensitivity analysis. Bioresour Technol Rep. https://doi.org/10.1016/j.biteb.2022.101000

Asitok AD, Ekpenyong MG, Takon IA, Antai SP, Ogarekpe NM, Antigha RE, Edet, PE, Ben UC, Akpan AE, Antai AS, Essien JP (2022a) Overproduction of a thermo-stable halo-alkaline protease on agro-waste-based optimized medium through alternate combinatorial random mutagenesis of *Stenotrophomonas acidaminiphila*. Biotechnol Rep 35:e00746. https://doi.org/10.1016/j.btre.2022.e00746.

Asitok AD, Ekpenyong MG, Ogarekpe NM, Antigha RE, Takon IA, Rao AP, Iheanacho JN, Antai SP (2022b) Intracellular-to-extracellular localization switch of acidic lipase in *Enterobacter cloacae* through multi-objective medium optimization: aqueous two-phase purification and activity kinetics. World J Microbiol Biotechnol 38:235. https://doi.org/10.1007/s11274-022-03429-8.

Bradford M (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Chi H, Chen M, Jiao L, Lu Z, Bie X, Zhao H, Lu F (2021) Characterization of a novel L-asparaginase from *Mycobacterium gordonae*

with acrylamide mitigation potential. Foods 10:2819. https://doi.org/10.3390/foods10112819.
Ekpenyong MG, Asitok AD, Antai SP, Ekpo BO, Antigha RE, Ogarekpe NM (2021a) Statistical and artificial neural network approaches to modeling and optimization of fermentation conditions for production of a surface/bioactive glyco-lipo-peptide. Int J Pept Res Ther 27:475–495, https://doi.org/10.1007/s10989-020-10094-8.
Ekpenyong MG, Asitok AD, Antigha RE, Ogarekpe NM, Ekong US, Asuquo MI, Essien JP, Antai, SP (2021b) Bioprocess optimization of nutritional parameters for enhanced anti-leukemic L-asparaginase production by *Aspergillus candidus* UCCM 00117: a sequential statistical approach. Int J Pept Res Ther 27:1501–1527. https://doi.org/10.1007/s10989-021-10188-x.
El-Naggar NE, Deraz SF, El-Ewasy SM, Suddek GM (2018) Purification, characterization and immunogenicity assessment of glutaminase free L-asparaginase from *Streptomyces brollosae* NEAE-115. BMC Pharmacol Toxicol 19:51–65. https://doi.org/10.1186/s40360-018-0242-1.
Gulati R, Saxena RK, Gupta R (1997) A rapid plate assay for screening L-asparaginase producing micro-organisms. Lett Appl Microbiol 24:23–26. https://doi.org/10.1046/j.1472-765x.1997.00331.x.
Holliday R (1956) A new method for the identification of biochemical mutants of microorganisms. Nature 178:987. https://doi.org/10.1038/178987a0.
Imada A, Igarasi S, Nakahama K, Isono M (1973) Asparaginase and glutaminase activities of microorganisms. J Gen Microbiol 76:85–99. https://doi.org/10.1099/00221287-76-1-85.
Jung H-J, Ko E-J, Kwak H-S (2013) Comparison of physicochemical and sensory properties between cholesterol-removed Gouda cheese and gouda cheese during ripening. Asian Australas J Anim Sci 26:1773–1780. https://doi.org/10.5713/ajas.2013.13255.
Khalil NM, Rodríguez-Couto S, El-Ghany MNA (2021) Characterization of *Penicillium crustosum* L-asparaginase and its acrylamide alleviation efficiency in roasted coffee beans at non-cytotoxic levels. Arch Microbiol 203:2625–2637. https://doi.org/10.1007/s00203-021-02198-6.
Krause FS, Blombach B, Eikmanns BJ (2010) Metabolic engineering of *Corynebacterium glutamicum* for 2-ketoisovalerate production. Appl Environ Microbiol 76:8053–8061. https://doi.org/10.1128/AEM.01710-10.
Laemmli UK (1970) Cleavage of structural protein during the assembly of the head 385 of bacteriophage T4. Nature 227:680–685. https://doi.org/10.1038/227680a0.
Lingnert H, Grivas S, Jägerstad M, Skog K, Törnqvist M, Åman P (2002) Acrylamide in food: mechanisms of formation and influencing factors during heating of foods. Scandinavian J Nutr, 46:159–172. https://doi.org/10.1080/110264802762225273.
Liyanage DWK, Yevtushenko DP, Konschuh M, Bizimungu B, Lu Z-X (2021) Processing strategies to decrease acrylamide formation, reducing sugars and free asparagine content in potato chips from three commercial cultivars. Food Control 119:107452. https://doi.org/10.1016/j.foodcont.2020.107452.
Mormann S, Lömker A, Rückert C, Gaigalat L, Tauch A, Pühler A, Kalinowski J (2006) Random mutagenesis in *Corynebacterium glutamicum* ATCC 13032 using an IS6100-based transposon vector identified the last unknown gene in the histidine biosynthesis pathway. BMC Genomics 7:205–224. https://doi.org/10.1186/1471-2164-7-205.
Munir N, Zia MA, Sharif S, Tahir IM, Jahangeer M, Javed I, Riaz M, Sarwar MU, Akram M, Shah SMA (2019) L-asparaginase potential in acrylamide mitigation from foodstuff: a mini-review. Progress in Nutrition 21:498–506. https://doi.org/10.23752/pn.v21i3.6771.
Muttucumaru N, Powers SJ, Elmore JS, Dodson A, Briddon A, Mottram DS, Halford NG (2017) Acrylamide-forming potential of potatoes grown at different locations, and the ratio of free asparagine to reducing sugars at which free asparagine becomes a limiting factor for acrylamide formation. Food Chem 220:76–86. https://doi.org/10.1016/j.foodchem.2016.09.199.
Negoita M, Mihai AL, Hornet GA (2022) Influence of water, NaCl and citric acid soaking pre-treatments on acrylamide content in french fries prepared in domestic conditions. Foods 11:1204. https://doi.org/10.3390/foods11091204.
Nematollahi A, Meybody NM, Khaneghah AM (2021) An overview of the combination of emerging technologies with conventional methods to reduce acrylamide in different food products: perspectives and future challenges. Food Control 127:108144. https://doi.org/10.1016/j.foodcont.2021.108144.
Shu L, Si X, Yang X, Ma W, Sun J, Zhang J, Xue X, Wang D, Gao Q (2020) Enhancement of acid protease activity of *aspergillus oryzae* using atmospheric and room temperature plasma. Front Microbiol 11:1418. https://doi.org/10.3389/fmicb.2020.01418.
Teuschler L, Gasperl A, Remele K, Pfeifhofer HW, Müller M (2021) Asparagine content is a key factor for acrylamide formation in sweet potato (*Ipomoea batatas* L.) fries. J Agric Food Dev 7:16–24. https://doi.org/10.30635/2415-0142.2021.07.04.
Wang Y, Xu W, Wu H, Zhang W, Guang C, Mu W (2021) Microbial production, molecular modification, and practical application of L-asparaginase: a review. Int J Biol Macromol 186:975–983. https://doi.org/10.1016/j.ijbiomac.2021.07.107.
Wen C, Shi X, Wang Z, Gao W, Jiang L, Xiao Q, Liu X, Deng F (2016) Effects of metal ions on formation of acrylamide and 5-hydroxymethylfurfural in asparagine-glucose model system. Int J Food Sci Technol 51:279–285. https://doi.org/10.1111/ijfs.12966.
Yassin MA, Shindia A, Labib M, Soud M, El-Sayed ASA (2022) Thermostable chitosan-L-asparaginase conjugate from *Aspergillus fumigatus* is a novel structurally stable composite for abolishing acrylamide formation in french fried potatoes. LWT-Food Sci Technol 162:113494. https://doi.org/10.1016/j.lwt.2022.113494.
Yeo MTY, Bi X, Henry CJ (2021) Acrylamide contents of local snacks in Singapore. Front Nutr 8:764284. https://doi.org/10.3389/fnut.2021.764284.
Zhang X, Zhang X-F, Li H-P, Wang L-Y, Zhang C, Xing X-H, Bao C-Y (2014) Atmospheric and room temperature plasma (ARTP) as a new powerful mutagenesis tool. Appl Microbiol Biotechnol 98:5387–5396. https://doi.org/10.1007/s00253-014-5755-y.
Zhang W, Cho C, Piao C, Choi H (2016) Sobol's sensitivity analysis for a fuel cell stack assembly model with the aid of structure-selection techniques. J Power Sour 301:1–10. https://doi.org/10.1016/j.jpowsour.2015.08.076