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Asparaginase: A promising aspirant for mitigation of acrylamide in foods

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Abstract
Acrylamide leading to cause neurotoxicity having gentotoxic properties and potential carcinogen is naturally formed in processed food(s) (>120°C) as a by-product of maillard reaction arises worldwide concern for the safety measures of various food products. Mitigation procedures that ameliorate maillard reaction may have negative effect on flavor and color. Hence, presently research is mainly focused on minimizing or completely eliminating the acrylamide formation in processed food(s) in which use of aspraginase pretreatment epitomize a promising technique for obviation of acrylamide by direct conversion of precursor asparagine to aspartic acid and ammonia, while also maintaining sensory quality of the foods. Thus, present review catalogues the research assessment of acrylamide and their mitigation strategies with emphasis on preventive techniques based on the use of asparaginase in food products.

Keywords: acrylamide, asparaginase, maillard reaction, food procesing

Introduction

The foods are processed in heat in general way to enhancing the quality, promoting the safety and improved the sensory characteristic properties of foodstuff. However, heating of foods may also lead to toxicant formation like acrylamide (2-propenamide) [1]. It is considered as the important heat induced naturally occurring process contaminant in potato chips, french fries, cereal and bakery products via a process called maillard reaction, which is a non-enzymatic browning reaction responsible for the golden color, desirable aroma and tasty flavor of foods) [2]. According to the International agency for research on cancer (1994), acrylamide was classified as a Group 2A carcinogen and has also been categorized as Category 2 carcinogen and mutagen [3, 4, 5]. Acrylamide causes paralysis of the cerebrospinal system, it is further oxidized into glycidamide (GA), which is more reactive and having higher potential of causing toxicity [6]. With regard to toxic properties of acrylamide European Union (EU) confirmed that exposure of acrylamide to human should be kept as low as possible [5]. The preventive strategies are aimed to minimize acrylamide formation during the heating process; the removal interventions are aimed to remove or decompose the already formed molecules in the finished product [7]. For the mitigation of dietary acrylamide, the food industry faces the challenges of changing processes and product parameters without compromising the taste, texture and appearance of their products [8]. Some relevant technological strategies of mitigation are summarized in (Table 1) which has been implemented by the industry. They can be applied in one or more steps of the food process, as pretreatments, formulation, and process and post process interventions [9]. Note worthily, Asparaginase pretreatment is one of the significant technologies to overcome such nutritional or health drawbacks considered over the past years [10]. As shown in the Figure.1, naturally present free aminoacids asparagine and reducing sugars (mainly glucose and fructose) in food combine to produce color, aromas and flavor via maillard reaction is responsible for the browning of food at high temperature leads to produce acrylamide where as in treatment with asparaginase the key precursor hydrolyze into aspartic acid and mitigate acrylamide formation with maintaining the sensory characteristics of food products [11]. Towards this end, the present review focused on the summarization of promising mitigation techniques but will focus on the use of enzymes, in particular asparaginase, to reduce levels of acrylamide precursor in food products.

Table 1: Strategies of prevention and removal of acrylamide in foods (Adapted from Anese *et al*., 2009)

Process step	Type of intercession	Mechanism of action	Food	Reference
Mitigation Strategy:				
Pretreatment	Dipping in additive solutions	Decrease of formation rate precursor consumption Decrease of formation rate	Potato derivatives	[48]
	Thermal treatment (blanching)	Leaching of precursors	Potato derivatives	[49, 50]
	Yeast or lactic acid bacteria fermentation	Precursor consumption Decrease of formation rate	Potato- and cereal-based products	[51, 52, 53, 54, 9, 43,]
	Use of asparaginase	Precursor (asparagine) Decomposition	Potato and cereal-wheat based products	[38, 40, 20, 7, 31, 2]

Formulation	Use of inhibiting or competing Ingredients	Decrease of precursor concentration Decrease of formation rate degradation reactions	Potato- and cereal-based products	[53, 39, 47]
Processing	Thermal input reduction	Decrease of formation rate	Potato- and cereal-based products coffee, chicory, jarred foods	[54, 55, 56,]
Post processing	Physical removal	Evaporation or sublimation	Potato- and cereal-based products, coffee, chicory, jarred foods	[1]

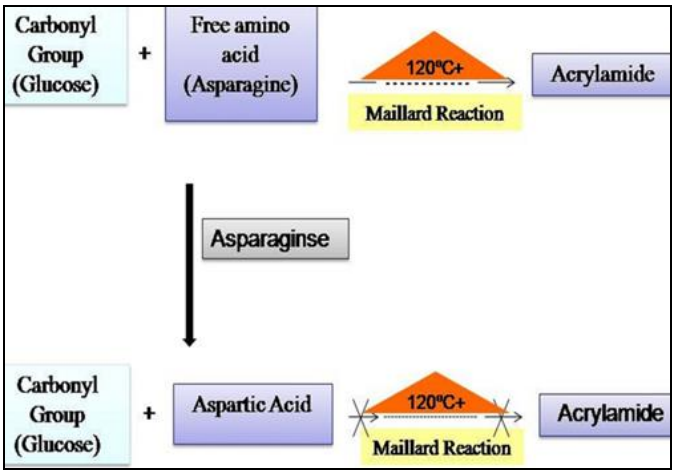

Fig 1: Mode of action of aspraginase in acrylamide formation during food processing

Occurrence of acrylamide

Acrylamide is present in a wide range of foods in plant based foods stuffs such as potato, cereal and bakery products and found very low levels in animal based food products like meat and fish, It is not found in foods that are not fried or baked such as boiling or microwaving [12]. The exposure to this process contaminant is a public health concern and a priority for the National Food Safety Authorities. Foods that are contributing most dietary intake of acrylamide are different in countries within individual food types. In addition, variations in raw materials or processing conditions can contribute to differences in the levels observed [13]. According to the EFSA, the most important food groups contributing to acrylamide exposure are fried potato products, coffee and cereal based food Biscuits & crackers [14]. Instead, EFSA's experts estimated the dose range within which acrylamide is likely to cause a small but measurable tumour incidence (called "neoplastic" effects) or other potential adverse effects (neurological, pre- and post-natal development and male reproduction)[15]. The lower limit of this range is called the Benchmark dose lower confidence limit (BMDL10). For tumors, experts selected a BMDL10 of 0.17mg/kg bw/day. For other effects, neurological changes were seen as the most relevant with a BMDL10 of 0.43 mg/kg bw/day. By comparing the BMDL10 to human dietary exposure to acrylamide, scientists can indicate a "level of health concern" known as the margin of exposure [16]. Total diet repot from Food and drug administration 2016 reported that highest concentrations of acrylamide (>100 µg/kg) were found in: the sweet and savory biscuits; potatoes based products and other snacks (not potato based) and Lowest amounts of acrylamide (≤10 µg/kg) were measured in takeaway fish based meals; coffee, cocoa and branded food drinks (as consumed); canned or jarred tomatoes; white unsliced bread; tree nuts; canned or jarred beans; meat or yeast extracts; other cereal products; spreads and dressings; mushrooms; turnips and Swedes; other canned or frozen fruit; and canned peaches, pears and pineapples [5, 17].

Acrylamide formation in food(s)

Acrylamide is not a substance that is added to food but it is naturally formed in food during heat processing. Heating of food is an important phenomena for maintaining the quality and safety of food stuff but formation of acrylamide takes starts in high temperatures at above 120°C during frying, roasting or baking and in low moisture conditions and the formation increases drastically towards the end of the frying process at temperatures higher than 170-180°C [18]. The main route for the formation of acrylamide in fried potato products is due to the presence of large concentrations of its precursor asparagine in the tuber and to the processing conditions applied [19]. The formation of acrylamide in foods has two ways major and minor for the formation, major route by free asparagines with carbohydrates resulting in the corresponding N-glycosyl conjugation and the formation of a decarboxylated schiff base in the dehydration at high temperature. After its decarboxylation, the Schiff base may lead to decomposition directly to acrylamide and an imine or followed by hydrolysis to aminopropionamide and carbonyl compounds. With this respect, it should be noted that aminopropionamide may also yield acrylamide after the elimination of an ammonia group [20]. The minor route with amino acids such as glutamine, cysteine and aspartic acid has also been found to produce low amounts of acrylamide [21]. However, this mechanism of formation might be irrelevant for acrylamide formation in foods as stated because studies in potato- and cereal-based foods have demonstrated the importance of asparagine by effectively reducing AA through the use of the substrate-selective enzyme asparaginase [22].

Risk assessment of acrylamide

In accordance with the Guidelines for Carcinogen Risk Assessment [23], acrylamide is characterized as "likely to be carcinogenic to humans." The chronic oral exposure of F344 rats to acrylamide in drinking water induced statistically significant increased incidences of thyroid follicular cell tumors (adenomas and carcinomas combined in both sexes), scrotal sac mesotheliomas (males), and mammary gland fibroadenomas (females) in bioassays [24]. After dietary consumption, Acrylamide is rapidly absorbed from the gastrointestinal tract and widely distributed to the tissue. In the liver it is metabolized to an epoxide glycidamide by the liver metabolizing system CYP2E1 to induce a variety of genotoxic effects in mammalian cells. It form covalent adducts with DNA in mice and rats and induces gene mutations and chromosomal aberrations in somatic cells and germ cells [14]. Dietary acrylamide may increase the risk of endometrial and possibly ovarian cancers, especially among

high acrylamide consumers, multiple myeloma and follicular lymphoma in men [25], and oesophageal cancer, with a stronger association among obese individuals [26]. In women was it is not associated with bladder, prostate, renal cancers [27] brain cancers [28], lung cancer [29], or ovarian cancer [30].

Strategies of acrylamide reduction by asparaginase

Asparaginase (L-asparagine amidohydrolases EC 3.5.1.1) has the physiological function of hydrolyzing amide group of the side chain in asparagine to produce aspartic acid and ammonia [7].It is an enzyme widely distributed in animal tissues, plants and in microorganisms, but not in mankind. Apart from its usage in acrylamide reduction, asparaginases have been a hallmark in multidrug chemotherapeutic regimens and broadly used for the treatment of lymphoid systems malignancies, childhood acute lymphoblastic leukemia, melanosarcoma, Hodgkin's lymphoma and lymphosarcoma [31], Tumor cells take asparagine from blood circulation or body fluid as it cannot synthesize asparagines. As a chemothera-peutic agent, asparaginase injected into bloodsteam hydrolyses free asparagine into aspartic acid and ammonia enzyme may indirectly starve tumor cells and lead to cell death [21]. Moreover, asparaginases have been used as a diagnostic biosensor for asparagine due to the large amount of ammonia produced by the enzymatic reaction and its direct correlation to the level of asparagine in a patient's blood, [32]. The exceptional ability of the enzyme asparaginase attracted considerable attention worldwide. Although asparaginases are broadly distributed among various living organisms; microorganisms are more efficient and inexpensive. Microbial enzymes are preferred over plant or animal sources due to their economic production, consistency, ease of process modification, optimization and purification. They are relatively more stable than corresponding enzymes derived from plants or animals [33]. Asparaginase production using microbial systems has attracted considerable attention owing to the cost-effective and eco-friendly nature. A wide range of microorganisms such as filamentous fungi, yeasts, and bacteria have proved to be beneficial sources of this enzyme [32] However, the production of the enzyme is complex with low yield. Production of this enzyme depends on various parameters like concentration of carbon and nitrogen sources, pH of culture medium, temperature, fermentation time and oxygen transfer rate. It has been observed that these parameters vary for different organisms [34]. Most asparaginases are quite specific for asparagine. Optimal activity is usually achieved at pH 5–7 and 37 °C. However, as glutamine has similar structure to asparagine, some enzymes also have a low activity towards glutamine [35]. A small group of enzymes, called glutaminase-asparaginases, have activities for both asparagine and glutamine but prefer glutamine as a substrate [36, 37] reported that in Crystallographic study that both types of asparaginase, common asparaginase and glutaminase-asparaginase, have the same basic structure and catalytic mechanism but differ in working conditions (pH and temperature) (Researchers believe that glutaminase activity caused by glutaminase-asparaginase will exert serious adverse effects on human health, such as liver dysfunction, pancreatitis and leucopoenia [31]. Therefore, this specific type of asparaginase should be strictly avoided in the food industry.

As shown in Table. 2, over the last decade there have been numerous studies monitoring the reduction of acrylamide formation by means of asparaginase treatment. The first study was carried out by Zyzak *et al*. in 2003, immediately after FDA 2002, announcement of acrylamide in food. However, Zyzak's research was focused on the formation mechanism rather than the mitigation efficiency. He used commercial asparaginase (A2925 from *Erwinia chrysanthemia*) and reported hydrolization of the asparagine, in order to verify that asparagine is indeed the precursor of acrylamide by added 50 U asparaginase to 60 g of mashed potato slurry (15 g potato, 45 g water) and achieved an 88% asparagine reduction that led to 99% acrylamide reduction in a microwave mashed potato snack, heated at full power until brown [38]. The following year, the first paper on the use of asparaginase as an acrylamide mitigation method was published. Asparaginase (from *E.coli*, 4 U/kg) added to gingerbread hydrolysed approximately 75% of the free asparagine, leading to a 55% acrylamide reduction in the final product. The acrylamide-reduced product was identical to a control product in both color and taste [39]. Both asparagine and sugars are not only important and desirable nutrients, naturally present in many foods, they are also important for plant growth and development. In most foods, they cannot be considered in isolation, since they are part of the highly complex chemical composition and metabolism of food plants. Though this enzyme application formed only a small part of the research, it stressed the advantage of the enzymatic method on mitigating acrylamide while maintaining the organoleptic properties of the products [40] set up a model system to examine the importance of all the related factors, such as temperature, dosage and application time. Applying asparaginase to dried potato powder led to a 90% acrylamide reduction in cooked product. However, instead of considering the effect of the cut and shape of the potato products, this research focused more on potato varieties. Although more than enzyme research discussed agronomic factors. Asparaginase with low glutaminase activity was successfully extracted from *Bacillus licheniformis* and was used to reduce acrylamide in fried potato strips by up to 80% [31]. In 2013, Hendriksen *et al*. were carried out on a much wider range of foods, including gingerbread, crispbread, semi-sweet biscuits, French fries and crisps. The optimum conditions of temperature and pH were 60°C and 7.0 as found to be best for enzyme [40]. In this study, other factors were also taken into consideration, depending on the food matrix. Frying is used in food processing both at industrial and home levels where blanching is an important unit operation in the prediction of French fries production. This reduces acrylamide formation mainly by leaching the precursors (reducing sugars) prior to frying blanching conditions i.e temperature and time can be varied in order to maintain the final product specifications constant., Acrylamide formation of 65% and 96% for French fries and potato chips reduced by blanching at 70°C for 10 to 15 min, respectively [42].

An investigation of acrylamide formation in fried potatoes in relation to blanching (75 °C, 10 min) and asparaginase treatments (10000 U/L, 40 °C, 20 min) before final frying (175 °C, 3 min) showed that the enzyme reduced the acrylamide content of 2075 μg kg−1 by 30%. This study

suggesting that the blanching heat treatment prior to frying might cause swelling of the potato starch that improves the diffusion of asparagines towards the asparaginase solution surrounding the strips and that the physical structure of the food affects efficacy of the enzyme [43, 6]. The authors assumed that the microstructure of the potato tissues was changed in the blanching process, causing the asparagine in the cell to have a more effective interaction with the enzyme outside the cell [43]. Asparaginase 40 U from food-grade *Bacillus subtilis* was applied to potato chips as compared to a control sample of asparaginase led to 80% reduction in acrylamide. One unit of the enzyme was defined as the amount that catalysed the formation of 1 µmol ammonia per min. They suggested that BAsnase, as the enzyme was christened, could be used to spray potatoes prior to cooking at home[44]. The author recently reported a Thermostable asparaginase from *Thermococcus zilligii* evaluated in French fries using this enzyme retained 70 % of its original activity after 2 h of incubation at 85 °C. When potato samples were treated with 10 U/mL of l-asparaginase at 80 °C for only 4 min, the acrylamide content in final French fries was reduced by 80.5 % compared with the untreated control [46].

Table 2: Mitigation of acrylamide using asparaginase from various sources (adapted from Xu *et al*., 2016)

Mitigation Strategy: Removal					
Food(s)	**Source of enzyme**	**Quantity of enzyme**	**Processing stipulation**	**Reduction of acrylamide**	**References**
Potato	*Escherichia coli*	Not declared	Not avowed	99%	[38]
Gingerbread	*E .coli*	4U/kg	Various time/temperature combinations	55%	[39]
Potato	*E. coli*	0.2-1U/g	180°C, 20 min	50-90%	[40]
French fries	*A. oryzae*	10000ASNU/L	175°C,3min	67%	[20]
Semi-sweet biscuits, ginger biscuits, crispbread, french fries, potato crisps	*A. oryzae*	Various dosages	Various time/temperature combination	Semi- sweet biscuits: 65-84% ginger biscuits:34-90% crispbread:84-92% french fries:59% potato crisps:60%	[41]
Fried dough model system	*A. oryzae*	100,500,1000 U	180 or 200°C; 4,6/8min	90%	[57]
Bread	*Pisum sativum L*	Not avowed	220°C,22-25 min	Wheat bran bread :57% Whole-grain bread: 68%	[58]
Potato chips	*A. oryzae*	10000 ASNU/L	170°C, 5min	90%	[43]
Potato	*Bacillus licheniformis*	30 IU/mL	175 °C, 15 min	80%	[31]
Potato lebkuchen, tortilla chips, potato snack, French fries, coffee	*A.oryzae*	30 IU/mL Various dosages	175°C 15 min Lebkuchen:200 °C,14 min tortilla chips:190 °C,60s French fries:175 °C,3min other not specified	80% Lebkuchen:95% tortilla chips:90% potato snack:40% French fries:57% Coffee:55-74%	[44]
Cookies	*A.oryzae*	500U/kg	205°C,11 or 15 min	23-75%	[59]
Wheat-oat bread	*Aspergillus niger*	500U	220,230 and 250°C;10,30 and 40 min	90%	[60]
Sweet bread	*Cladosporium sp.*	50-300U	220°C;25min	Crust 97% Crumb:73%	[61]
Potato crisp	*Bacillus subtilis*	0-40U	170°C;90s	80%	[44]
French fries	*Thermococcus zilligii*	0-20 U/mL	175°C:5min	80%	[46]
Potato chips	*Bacillus subtillis*	500µl	170°C,6min	90-95%	[62]
Potato chips Sweet Bread	*Fusarium culmorum*	300U	170-180°C,90s	85%-738%	[63]

Commercially available asparaginase for mitigation

Enzyme asparaginase is available in the market with the trade Name PreventASeTM from DSM (Heerlen, The Netherlands) and Acrylaway® from Novozymes A/S (Bagsvaerd, Denmark). PreventASeTM was obtained after analyzing the gene sequence of *Aspergillus niger* and produced recombinant in the *Aspergillus niger* host. It has an acidic profile (optimum pH 4–5, temperature 50 °C) [6]. Acrylaway® obtained from *Aspergillus oryzae* and has an almost neutral profile (optimum pH 7, temperature 37°C). Although different dosages of asparginase will be used in different types of food so, there is no unified standard for the maximum dosage of the enzyme [45]. It has also been proved to be significant food additive by the Joint FAO/WHO Expert committee. Asn has received "generally recognized as safe" status from the US government [46] and it is currently used in several countries, including United States, Australia, New Zealand, China, Russia, Mexico and several European countries. Acrylaway® and Prevent

ASeTM have shown high specificity and therefore minimum activity towards glutamine and other aminoacids [6] Although asparaginase has advantages over other mitigation methods but there are also issues with the industrial application of asparaginase in a continuous process, to achieve good results in a relatively short time more research is needed to better incorporate asparaginase usage into industrial-scale food production.

Conclusions

Present review concludes that asparaginase treatment has become significant and safe approach for acrylamide mitigation in the food industry and having potential to overcome with the adverse effects on sensory characteristics of processed foods. There is still a need to achieve sufficient enzyme–substrate contact coming in areas for future research. Further studies and approvals will enable the introduction of new asparaginase with potential benefits food processing commercially to avoid acrylamide formation in foods.

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