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Research Article

"ROLE OF ASPARAGINASE ENZYME IN FOOD PROCESSING INDUSTRY TO REDUCE THE ACRYLAMIDE LEVEL-A REVIEW"

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ABSTRACT

Enzymes are proteins produced by living organisms isolated for large-scale commercial production and with wider application in the food industry. Asparaginase is a hydrolytic enzyme that breakdown of non-essential amminoacid L-asparagine to L-aspartic acid with the release of ammonia. The naturally occurring enzyme expressed and produced by humans, plants and microorganisms. L-asparaginase has gained attention in recent years due to its significant applications in food industry to prevent the acrylamide formation. The enzyme asparaginase is added to certain foods in order to reduce the quantity of the amino acid asparagine that is naturally present in foods. Under specific cooking conditions, asparaginecan react with certain carbohydrates in the food to form acrylamide, a potential neurotoxin and human carcinogen. By reducing the amount of asparagine in the food prior to cooking or processing, the amount of acrylamide that can be formed will also be reduced. In recent years, to reduce exposure of foodborne acrylamide has strongly encouraged in the food industry to develop and implement acrylamide reduction strategies. Asparaginase is a powerful tool for the food industry and it is likely that its use will increase in future. This review explores the use of asparaginase enzyme and the reduction of acrylamide formation in food processing.

INTRODUCTION

Microbial Enzymes

Microorganisms are being the most important source of commercial enzymes today can usually be found to produce related enzymes that catalyse the desired reaction[1]. Enzymes have always been important to food technology and food industry, because of their ability to act as catalysts to improved food products. The main values of enzymes are their own substrate specificity[2,3]. L-asparaginase (L-asparagine aminohydrolase) is an enzyme belongs to an amidase group that catalysis the hydrolysis of asparagine to aspartic acid and ammonia[4]. L-asparaginase is the first enzyme with anti-tumor activity which has been thoroughly researched by many researchers throughout the world and has been a clinically acceptable for the effective treatment of acute lymphoblastic leukemia as well as lymphosarcoma[5].L- Asparaginase is a hydrolytic enzyme that breakdowns of non-essential amino acid L-asparagine to L-aspartic acid with the release of ammonia. Asparagine is found in plant sources like asparagus, potatoes, legumes, nuts, seeds, soy, whole grains and the animal sources like dairy, whey, beef, poultry, eggs, fish, seafood[6]. Asparaginase produced by various microorganisms like *Aspergillus niger*, Aspergillus oryzae, *Bacillus subtilis*, *Erwinia herbicola*, *Erwinia carotovora, Escherichia coli*, *Fusarium oxysporum*, *Penicillium granulatum*, *Vibrio* etc.,[7].L-asparaginase has gained attention in recent years due to its significant application, as its use in food industry to prevent the acrylamide formation when foods are processed in high temperatures[8]. Different types of asparaginase can be used for different industrial and pharmaceutical purposes[9]. The most common use of asparaginase have been used as food processing aid in order to reduce the formation of acrylamide, which is a suspected carcinogen in starchy food products such as snacks and biscuits and is marketed under the brand names Acrylaway® and PreventASe[10]. Acrylamide is a compound formed naturally during the preparation of foods by major

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pathway involves reaction between asparagine and reducing sugar at temperatures in excess of 120ºCin low moisture[11]. Acrylamide is mainly formed in food as part of the Maillard reaction, also responsible for the desirable flavour, aroma and colour compounds in baked, roasted, as well as fried foods[12].

L-Asparaginase

L-Asparaginase (L-asparagine amidohydrolases EC 3.5.1.1) is an enzyme widely distributed in plants, animals and microorganisms thatare used as a medication and in food manufacturing[13]. As a medicine it is used to treat acute lymphoblastic leukemia, acute myeloid leukemia and Non-Hodgkin's lymphoma[14]. The discovery and development of asparaginase as an anti-cancer drug began in 1953, when scientists first observed that lymphomas in rat and mice regressed after treatment with guinea pig serum[15]. Asparaginase produced by *Erwinia chrysanthemi* is known as crisantaspase, the trade name Erwinase. In *E. coli* branded formulation Medac, Ciderolase, and Oncaspar[16]. Acrylamide is often formed in the cooking of starchy foods. The food processing aid asparaginases can effectively reduce the level of acrylamide up to 90% in a range of starchy foods without changing the taste and appearance[17].The optimal activity is usually at pH 5-7 and 37°C. However, as asparagine has similar structure to glutamine, some enzymes are low active towards glutamine[18]. Crystallographic study has shown that common asparaginase and glutaminase-asparaginase have same basic structure and catalytic mechanism but differ in working condition like pH and temperature[19].The glutaminase-asparaginase shows serious adverse effects on human health, so it should be strictly avoided in food industry[20].L-asparaginase promises to be a potential way to reduce the amount of free L-asparagine in the starting materials of food production, thus reducing the risk of generating acrylamide, a potentially carcinogenic and neurotoxic compound. Recombinant L-asparaginase of *Aspergillus niger* and *Aspergillus oryzae* used in processing of starchy food products. It converts the amino acid asparagine to aspartic acid then reduces acrylamide formation during processing of high starch food products. A new microbial source for asparaginase sourced from a genetically modified strain of *Bacillus subtilis* for use in food production to reduce the risk of acrylamide formation[21].

Asparaginase Enzyme and Source of Organism

The systematic name of the enzyme is L-asparaginase amidohydrolase and has the Enzyme Commission (EC) number of 3.5.1.1. The commercial name of the Novozyme asparaginase preparation is Acrylaway® L source organism *Aspergillus oryzae* and has a typical activity of 3500 ASNU/g[22]. One ASNU is the amount of enzyme that produces one µmole of ammonia per minute under specific reaction conditions. The products of this reaction are aspartic acid and ammonia is normal constituents of food[23]. The source of microorganism is a genetically modified selected strain of *Aspergillus oryzae* which contains extra copies of the asparaginase gene. This enzyme used to reduce the formation of acrylamide in food, as a result indicated reduction between 68-83% depending on incubation time. *Aspergillus niger* is a filamentous fungus that commonly occurs in the environment and is generally regarded as non-pathogenic[24]. Food Standards Australia New Zealand (FSANZ) has assessed an application made by Novozymes Australia Pty Ltd to permit the use of a new microbial source for asparaginase sourced from a genetically modified strain of *Bacillus subtilis* containing the gene for thermo-tolerant asparaginase from *Pyrococcus furiosus* for use in food production to reduce the risk of acrylamide formation[25]. Asparaginase with low glutaminase activity was successfully extracted from *Bacillus licheniformis* and it reduced acrylamide formation in fried potatoes by up to 80%[26]. Asparaginase has been extracted from *Rhizomucor miehei* and designated as RmAsnase, was optimally active in pH 7.0 and stable at 45°C for 30 minutes. RmAsnase was cloned and expressed in *Escherichia coli* and proved highly specific towards asparagine[27].

Production of Asparaginase

Asparaginase is mass-produced by pure culture fermentation of a genetically modified strain of *Aspergillus niger* that contains multiple copies of the asparaginase gene[28]. The asparaginase production strain constructed by transformation of the *Aspergillus niger* host strain DS 51563 with DNA fragments derived from two plasmids, one containing the asparaginase gene from *Aspergillus niger* and other acetamidase gene from *Aspergillus nidulans*[29]. The acetamidase gene used as a selectable marker to identify transformants and subsequently removed from the production strain. The strain complies with the criteria suggested by the organization for economic co-operation and development for r-DNA good industrial large scale practice microorganisms'cellular cultures

Asparaginase in Food Processing

Asparaginase is used as a food processing aid to reduce the formation of acrylamide, a suspected carcinogen, in starchy food products. Acrylamide is a chemical compound, formed from L-asparagine and reducing sugar in carbohydrate containing foods that are heated above 120ºC such foods includes bread and other baked goods, fried or baked potato products and reaction flavours[30]. During heating the amino acid asparagine, naturally present in starchy foods, is converted into acrylamide in a process called the Maillard reaction[31]. The reaction is responsible for giving baked or fried food their brown color, crust and toasted flavour. By adding asparaginase before baking or frying the food, asparagine is converted into another common amino acid, aspartic acid, and ammonium. As a result, asparagine cannot take part in the Maillard reaction, and therefore the formation of acrylamide is significantly reduced[32].

Acrylamide

Acrylamide is a known carcinogen substance in experimental animals which occurs in carbohydrate-rich foods as a result of cooking methods at high temperatures[33]. As acrylamide has not been detected in unheated or boiled foods, it was considered to be formed during heating at high temperatures. This fact attributed at the higher temperatures reached in maillard non-enzymatic browning reactions required for desirable color, flavour, and aroma production, especially in the fried potatoes[34]. Acrylamide was formed by heating above 120°C certain starch-based foods, such as potato chips, French fries, bread, and processed cereals[35]. In April 2002, Swedish researchers shocked the food safety world when they presented preliminary findings of acrylamide in some fried and baked

foods, most notably potato chips and French fries[36]. Acrylamide is mainly formed unintentionally in the maillard reaction when the free amino acid asparagine reacts with the reducing sugars, especially glucose and fructose that are present in food. Major food items contaminated by acrylamide include potato chips, crisps, bakeries, fried vegetables and coffee[37]. Foods prepared by boiling do not typically produce acrylamide[38]. Maillard reaction has been suggested as the major pathway for acrylamide formation in foodstuffs, and asparagine is mainly responsible for acrylamide formation in heated foods after condension with reducing sugars or a carbonyle source[39]. Glycoconjugates, such as N-glycosides and related compounds formed in the early stage of maillard reaction, have been proposed as key intermediates leading to acrylamide[40].Liquid chromatography and tandem mass spectrometry-LC-MS-MS analytical methodology for simultaneous analysis of acrylamide and their precursors, such as asparagines and glucose, was implemented with a detection limit for acrylamide of 20µg/kg, for French fries analysis.

Asparaginase in acrylamide mitigation

In acrylamide mitigation, the first study was focused on the acrylamide formation rather than the mitigation efficiency by using commercial asparaginase from Aldrich (A2925 from *Erwinia chrysanthemi*), achieved an 88% asparagine reduction[41].Thefollowing year, the first paper on the use of asparaginase from *E. coli*, added to gingerbread hydrolysed approximately 75% of the free asparagines, leading to a 55% acrylamide reduction[42]. The commercial asparaginase (Acrylaway®) produced from *Aspergillus oryzae* based on cloning technology and a reduction of 67% in acrylamide was achieved in French fries. Dough based application was carried out on a much wider range of foods, including gingerbread, crispbread, semi-sweet biscuits, French fries and crisps with a reduction in final acrylamide contents of 34%-92% when asparaginase was employed. The acrylamide content of BAsnase-treated fried potato chips decreased to below 20% of that of BAsnase-untreated fried potato chips[43]. The impact L-asparaginase on the acrylamide content reduction after high heat treatment in a model system as well as in potato based material was investigated[44]. Addition of partial purified L-asparaginase enzyme from *Penicillium cyclopium* followed by incubation of the mixture at 37°C for 30 min led to 92% reduction of acrylamide content[45]. One another study, the purified L-asparaginase enzyme from *Trichoderma viride* to used potato slice to deep fried in oil at 150°C for 6 min to reduce the acarylamide level in fried potatoes[46].

Application of Asparaginase in food Products

Application of Asparaginase in food products used for the two commercially available enzymes Acrylaway® by Novozymes and PreventASe by DSM, two different methods to determine the activity of the enzymes and the first commercially available "acrylamide-free" product, biscuits treated with Prevent ASe, was announced to launch in Germany for Christmas, 2008[47]. Both methods are based on measuring the ammonia that is generated from the asparagines hydrolysis. However, in the method used to measure the activity of Acrylaway®, ammonia subsequently reacts with α-ketoglutarate to form L-glutamic acid. Asparaginase activity is expressed in ASPU activity units. One ASPU is defined as the amount of asparaginase that liberates one micromole of ammonia from L-asparagine per minute under standard conditions pH 5; 37°C[48].The amount of asparaginase added to food preparations is indicated by the amount of enzyme activity required for that particular food application[49]. In food application PreventASe was used as the asparaginase enzyme source[50]. A solution of 500 ASNU in 10ml of water was added and spread over the surface of a wheat bread loaf prior to baking. The asparaginase enzyme was effective during a proofing step of 15 min at 32°C. This treatment led to a 46% reduction in the acrylamide content of the baked bread crust[51]. Asparaginase Acrylaway® used in a laboratory-scale experiment indicated that a low dosage 2000-6000 ASNU of asparaginase could achieve 55-74% acrylamide reduction in coffee beans.

CONCLUSION

There are number of scientific evidences that proved Asparaginase enzyme used in food processing to reduce the more acrylamide content, is achieved data given by researchers. Anti-cancer enzyme asparaginase is a powerful tool for acrylamide mitigation in the food industry. With the success of commercial enzyme used to reduce the acrylamide level in the food products.The benefits of using asparaginase for acrylamide mitigation are related to food safety and brand protection. Acrylaway® is currently being used in industrial scale to mitigate acrylamide formation in food. This enzyme application will increase all over the country in future.

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