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Acrylamide Reduction in Potato Crisps using: Asparaginase from *Candida utilis*, Commercial Asparaginase, Salt Immersion, and pH Treatment

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ABSTRACT

This paper investigates the reduction of acrylamide formation in potato crisps as a result of asparaginase treatment, using calcium chloride and sodium chloride solutions with different concentrations, immersion in different pH solutions, and different frying conditions. The main aim is to compare the reduction of acrylamide in potato crisps using two kinds of asparaginase enzyme; the first enzyme is commercial but the second is an enzyme made from *Candida utilis* specifically for food treatment. Before frying, samples were treated in one of following ways: Washing in distilled water (control I); Blanching in hot water; Immersion in commercial asparaginase (or asparaginase of *Candida utilis*) solution; Both blanching in hot water and immersion in commercial asparaginase (or asparaginase of *Candida utilis*) solution; Blanching in hot water plus immersion in medium temperature water (control II).While commercial asparaginase reduces acrylamide formation by 39%, asparaginase obtained from *Candida utilis* makes a higher reduction of 58% in potato crisps. However, both enzymes in combination with blanching inhibit much higher amount of acrylamide formation. Treatments with calcium chloride, sodium chloride, and citric acid have considerable effects on the content of acrylamide in fried potato. The maximum reduction of acrylamide is 95% caused by commercial treatment plus blanching.

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1. INTRODUCTION

In 2002, a scientific group at Swedish National Food Administration in cooperation with Stockholm University researchers revealed data on problems caused as a result of high concentrations of acrylamide in a wide variety of starchy foodstuffs which are mostly fried, roasted or baked [1, 2]. International Agency for Research on Cancer (IARC) has introduced acrylamide as a "probable human carcinogen" (Group 2A) [3]. Acrylamide in foods is formed in Maillard reaction pathway, which is a synthesis of asparagine, as an amino group, and a reducing sugar such as glucose and fructose [4, 5]. This reaction affects the development of flavor and color [6]. This chemical reaction mainly occurs in high temperatures (>120°C) [2] and low moisture conditions [7]. Meanwhile, the relationship between these reactants and final product (acrylamide) is surprisingly complicated [8].

All kinds of high temperate processed potato, coffee and cereal based food have been introduced by the European Food Safety Agency (EFSA) as the chief sources of exposure to acrylamide in diet [8]. Potato has high levels of acrylamide precursors; hence, at the presence of high temperatures acrylamide is generated [9]. This toxic substance can have concentrations of even more than 4000 µg/kg in potato products [10] which are one of the most popular foodstuffs specially among the youth [11, 12] and contribute to 56% of total exposure to acrylamide in the Western adults diet [13]. Consequently, researchers frequently have tried to find methods precluding or reducing acrylamide formation [8].

The content of acrylamide in cooked foods depends on cultivar, fertilization, storage, processing temperature and time, blanching, and the amount of precursors in potatoes [14-16]. As reducing sugar content in potato is

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relatively low, compared to its asparagine concentration, it is considered as limiting factor in acrylamide formation [8]. However, in another study [17] it is suggested that both asparagine and reducing sugar contribute to acrylamide generation evenly. Yet other study [16] showed that a direct correlation between sugar level and acrylamide in high sugar concentration exists, while in low sugar content acrylamide formation has a direct relation with asparagine amount. On the contrary, another study [18] suggested that reducing sugar content is the only determining factor.

A possible way to reduce acrylamide formation is the control of time and temperature. Lower frying time and temperature have been shown that lead to the more reduction [8, 19]. In addition, the pretreatment by using blanching has been suggested in several studies as a way of both asparagine and reducing reduction [20-22]. Moreover, in the low pH, it has been shown that the amount of acrylamide is decreased [23]. Using citric acid and other organic acids, which reduce the pH in pre-treatment stage, were introduced as a method to decrease acrylamide level [23, 24].

Despite unquestionable progresses achieved in reducing the acrylamide formation in foods, there is not comprehensive knowledge of the advantages of using additives like enzymes, salts and antioxidant agents [1, 25]. Recently, it has been shown that the addition of flavonoids can significantly reduce the amount of acrylamide reduction [26]. Also authors of [27] show that adding vitamin B_3 and B_6 to potato slices decline acrylamide formation. Different types of salts have been examined in some studies. Vanadyl sulphate, in one study [28], made up to 92.5% reduction in acrylamide formation. Also, calcium-containing additives and NaCl diminished the amount of oil absorbed by food and as a result, reduced the heat transfer and acrylamide generation [29]. NaCl has been introduced as a considerable factor for reducing acrylamide formation in glucose-asparagine model systems [30]. In addition, to suggested methods, in a study [31] the role of yeast fermentation treatments in reduction of reducing sugar and as an outcome, reduction of acrylamide production was investigated and shown that it can be a practical way to reduce this toxic substance. One alternative is the use of enzymes. Asparaginase hydrolyses asparagine and produces aspartic acid that has no rule in acrylamide formation [32]. Furthermore, the use of this enzyme has no detrimental effect on the sensorial properties of cooked products [33]. It has been shown that asparaginase can significantly reduce the amount of acrylamide formation, especially when the time of treatment increases [34].

Aspergillus oryzae generates commercial asparaginase based on cloning technology. The optimum pH for this enzyme has been determined at 6-7. The best enzymatic activity is gained at the pH between 5 and 8 which is actually suitable for producing of potato crisps [35]. *Aspergillus niger*, in addition to asparaginase, can produce xylanase [36] and Lipase [37]. Dried potato powder which is treated by asparaginase showed considerably lower acrylamide content 90-97% [38].

Previously, asparaginase obtained from *Aspergillus oryzae* and *Aspergillus niger* were used to treat in baking industry in order to reduction of acrylamide formation [39]. Recently, native l-asparaginase from *Aspergillus oryzae* CCT 3940 has been shown to have a significant impact on acrylamide reduction in French fries [40]. However, enzyme obtained from the two works optimally at lower temperature and has no suitable thermal stability at frying temperature higher than 120˚C. As an outcome, a stable and active enzyme in high temperatures is required [41]. L-asparaginase enzymes of yeasts are thermostable [42] and utilizable for food treatments. It has been shown [43, 44] that eukaryotic microorganisms as yeasts and filamentous fungi genera can produce asparaginase with fewer side effects than prokaryotes. While another study [45] has focused on this yeast, *Candida utilis* has a potential of producing a huge amount of asparaginase [41].

The main aim of this study is a comparison between the effect of commercial asparaginase and asparaginase obtained from *C. utilis* ATCC9950, which is produced by [41], on reduction of acrylamide formation in potato crisps. Hence, the impact of blanching in combination with the two asparaginase enzymes on reducing of acrylamide formation during frying is studied. In addition, this study aims to examine the effects of factors, which have been studied in other papers, such as frying conditions (time and temperature), salts (of NaCl and $CaCl_2$), and pH, in the same conditions to make comprehensive comparable results.

2. MATERIALS AND METHODS

2. 1. Materials Potatoes tubers (Agria variety, 82.42 g/100 g of wet weight, 1.36 mg/1 g of asparagine, 89.48 mg/1 g of reducing sugar, length>12 cm) and vegetable oil (sunflower oil, Bahar, Iran) were the raw materials. Potatoes used in this study were grown in Iran and stored at 8°C and 95% of relative humidity. Then, they were washed and peeled manually. Potatoes were cut in size of $4\times0.5\times0.7$ cm^3 by using of a ruler and a knife from the pith of the parenchymatous region.

2. 2. Pre-treatments To eliminate starch material sticking to the surface of slices, raw potato slices were rinsed instantly after cutting for 1 min in distilled water prior to frying. Other pre-treatments were performed before frying:

2. 2. 1. Enzyme Treatments and Blanching C1: Rinsed slices in distilled water without any treatment (control I).

B: Blanched slices in hot distilled water at 85°C for 3 min in a ratio of potato to water (g/g) of approximately 0.5.

E-C: Immersed slices in a commercial asparaginase 300 U/ml(*Escherichia coli*-derived L-asparaginase (Elspar, Lundbeck, Deerfield, IL, USA) as a preservative-free lyophilized powder in vial of 10000 units /vial for injection) solution at 50°C for 30 min in a ratio of potato to enzyme solution (g/g) of approximately 0.5.

E-L: Immersed slices in a solution of asparaginase made of *C. utilis* 246 U/ml (produced in department of chemical and petroleum engineering in Sharif University of Technology, Tehran, Iran) at 50°C for 30 min in a ratio of potato to enzyme solution (g/g) of approximately 0.5.

B_E-C: Blanched slices in hot distilled water at 85°C for 3 min (ratio of potato to water (g/g) of approximately 0.5) and then, immersed in a commercial asparaginase solution at 50°C for 30 min (ratio of potato to enzyme solution (g/g) of approximately 0.5).

B_E-L: Blanched slices in hot distilled water at 85°C for 3 min (ratio of potato to water (g/g) of approximately 0.5) and then, immersed in an asparaginase solution of *C. utilis* at 50°C for 30 min (ratio of potato to enzyme solution (g/g) of approximately 0.5).

C2: Blanched slices in hot distilled water at 85°C for 3 min (ratio of potato to water (g/g) of approximately 0.5) and then, immersed in distilled water at 50°C for 30 min (ratio of potato to water (g/g) of approximately 0.5) (Control II).

Eventually, all potato samples were deep-fried at 165°C for 4 min.

2. 2. 2. Treatments by Calcium Chloride and Sodium Chloride The rinsed potato slices were immersed in three different concentrations of calcium chloride (36% w/v) (supplied by Brenntag (Belgium)) and sodium chloride (>99% w/w) separately (0.001, 0.01, and 0.1 mol/l) at 25°C for 30 min (ratio of potato to water solution (g/g) of approximately 0.5). After that, all samples were deep-fried at 165°C for 4 min.

2.2.3. pH Treatment Potato slices were soaked in different solutions of citric acid (>99.8% w/w) (supplied by Brenntag (Belgium)) at 25°C for 30 min (ratio of potato to acid solution (g/g) of approximately 0.5). The pH was adjusted to 4.0, 5.0, 6.0 and 7.0 with 1 N citric acid and 1 N NaOH. After that, all samples were deep-fried at 165°C for 4 min.

2. 3. Frying Conditions The rinsed slices were deep-fried at three temperatures (150, 165, and 180°C) with different frying times (1, 2, 3, and 4 min). Frying temperature was maintained constant because the ratio of potato to oil (g/g) was kept very low. The experiments were made in duplicate.

2. 4. Sample Preparation A standard acrylamide ((>99%) obtained from Aldrich (Milwaukee, WI, USA)) stock solution (1.0 mg/ml) was prepared by dissolving 1.0 mg of acrylamide in 1.0 ml of Milli-Q (Millipore Corp., Bedford, MA, USA) water. This solution was diluted with Milli-Q water to obtain a series of calibration standards (50, 100, 200, 300, 400, 500, 1000, and 2000 mg/l). All standard solutions were stored at 4°C.

The potato samples were ground homogeneously and passed from 10 No mesh. 1.00 g of each sample was put into separate 50-ml centrifuge tubes. 10 ml of water was then added to each centrifuge tube. The samples were extracted in a tube shaker for 3 min at 25°C. Carrez I and II solutions were prepared by dissolving 15 g potassium hexacyanoferrate and 30 g zinc sulphate (both were obtained from Merck (Darmstadt, Germany)) in 100 ml water, respectively [46]. Solid part of each sample was separated by centrifugation at 9000 rpm for 10 min. 50 µl Carrez I and 50 µl Carrez II were added to the centrifuge tubes to precipitate proteins and again were centrifuged at 9000 rpm for 10 min. Afterwards, clear supernatant was transferred into another tube and filtered through a 0.4 µm filter paper. After that 2.5 ml of each sample was separated and evaporated under a stream of nitrogen to almost dryness. The remnant was re-dissolved in 1 ml of deionized water. Prior to HPLC-UV analyses, all sample extracts were again filtered through 0.2 µm syringe filters.

2. 5. Acrylamide Analysis HPLC-UV analysis was operated on HP 1010 series. HPLC device furnished with a vacuum degasser, a duplex pump, and a diode array detector (Hewlett Packard, Wilmington, DE, USA). 20 µl of the calibration standards and sample extracts were injected via a 20 µl sample loop onto a 250 mm × 4.6 mm, 5 µm Hypersil ODS-C18 column (Thermo Scientific, Waltham, MA, USA) at 22°C. The wavelength was 202 nm. MilliQ water as the mobile phase at a flow rate of 1 ml/min was used.

3. RESULTS AND DISCUSSION

3. 1. The Effect of Pre-treatments on Acrylamide Formation in Potato Crisps

3. 1. 1. Influence of Enzyme Treatment and Blanching As shown in Figure 1, the non-treatment potatoes sample (C1) contained 3207 µg/kg of acrylamide. Blanching (B) made approximately 41% less acrylamide content in potato slices. Similar to explanation of previous authors [35], blanching not only leaches out reducing sugars but also declines asparagine

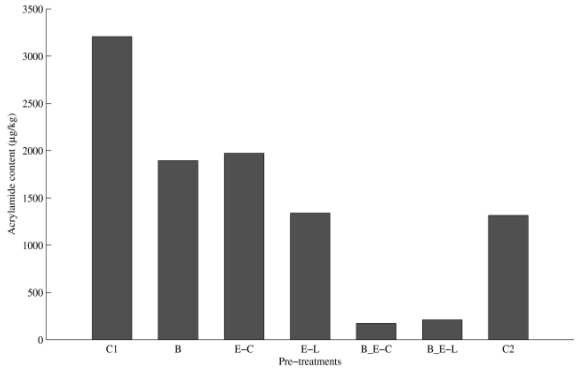

Figure 1. Acrylamide concentration of potato crisps treated with asparaginase. C1. Non-treatment potato slices (control I); B. Blanched potato slices at 85°C for 3 min; E-C. Immersed potato slices in commercial asparaginase solution at 50°C for 30 min; E-L. Immersed potato slices in a solution of asparaginase from *C. utilis* at 50°C for 30 min; B_E-C Blanched potato slices at 85°C for 3 min in combination with immersion in commercial asparaginase solution at 50°C for 30 min; B_E-L Blanched potato slices at 85°C for 3 min in combination with immersion in a solution of asparaginase from *C. utilis* at 50°C for 30 min; C2. Blanched potato slices at 85°C for 3 min in combination with immersion in pure water at 50°C for 30 min (control II). All samples fried at 165°C for 4 min in sunflower oil.

(acrylamide precursors). Consequently, as both reducing sugars and asparagine decrease, the final amount of acrylamide is lower than normal situation. In addition, blanching prepares potato crisps in order to sensory quality, such as crispiness, taste and colour [47]. When potato slices were soaked in the commercial asparaginase solution (E-C), the acrylamide formation decreased in almost 39%. This decrease could be attributed chiefly to a reduction of amino acid (asparagine) in the external layers of the potato slices reached by the asparaginase. This enzyme hydrolyses asparagine to produce aspartic acid and ammonia. Similarly, repetition of this stage by using asparaginase made of *C. utilis* (E-L) had almost similar results and diminished acrylamide formation by about 58%. This decrease in the acrylamide content is due to reduction of asparagine which also has been explained in other studies [40, 48]. These results showed that blanching and the enzyme immersion are almost equivalent in reducing acrylamide. Besides, when blanched slices are soaked in commercial enzyme solution (B_E-C), the acrylamide formation diminished significantly (95%) from 3207 µg/kg to 173 µg/kg and in this case the reduction resulted by asparaginase of *C. utilis* (B_E-L) was 93%. Surprisingly, a combined treatment of blanching and distilled water (C2) accounted for 59% of acrylamide reduction. The blanching facilitates both enzyme diffusion and asparagine to move toward the surface. Thermal treatment such as blanching during potato processing makes a microstructure change in the potato strips like the starch swells and cell wall degradation, that could make the move of asparagine toward the enzyme easier in the around solution [35, 49]. Blanching in conjunction with asparaginase treatment led to a complete removal of glucose and asparagine in potato slices with considerably lower acrylamide formation.

3. 1. 2. The Effect of Treatment by Calcium Chloride and Sodium Chloride As Figure 2 shows, NaCl and $CaCl_2$ reduced the acrylamide content in potato crisps. The addition of $CaCl_2$ to the concentrations of 0.001, 0.01, and 0.1 M caused a decline in acrylamide content by 28%, 53%, and 69%, respectively, in comparison with control I. These experiments were repeated by NaCl at the same concentrations and showed reductions of 11%, 25%, and 43%, respectively. The maximum reduction of acrylamide content occurred in the system containing 0.1 M $CaCl_2$. Considerable differences were shown between the reactions containing salts and the control reaction in which salts were not added. When the potato slices were soaked in 0.1 M NaCl and 0.1 M $CaCl_2$ water solutions, the acrylamide reductions were 43% and 69%, respectively. Hence, according to our study, $CaCl_2$ was the stronger deterrent of the acrylamide formation than NaCl. As reported by other studies [47], additives such as NaCl and $CaCl_2$ reduce oil content and consequently a lower heat transfer from the oil to the potato slices reduces acrylamide formation [50]. Therefore, a mitigated oil uptake seems an acceptable mechanism for acrylamide reduction in salts treatments. Besides, these additives can probably alter the potato tissue's structural properties, which result in a different oil uptake and changed heat transfer and acrylamide content in the potato crisps [47]. Some authors have investigated the possible lowering effect of metal cations like Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+} on acrylamide generation [51]. Also, it has been acceptable that metal cations may interact with amino acid (asparagine) precluding Schiff base formation and eventually inhibiting the acrylamide formation [28].

3. 1. 3. Reductions Obtained by pH Treatment Figure 3 investigates the impact of pH as an effective factor on formation of acrylamide. An study showed that the pH has a critical role in inhibiting acrylamide formation in foodstuffs [28]. This study further examined whether there is any correlation between acrylamide formation and the pH. The adjustment of pH had been done by adding 1 N citric acid to decrease or 1 N NaOH to increase. While the concentrations of acrylamide generated at pH 4, 5, and 6 after frying at 165°C for 4 min in comparison to control I decreased by 86%, 65%, and 54%, respectively, acrylamide content in potatoes fried at the same condition but at a solution with pH of 7 increased by 8%.

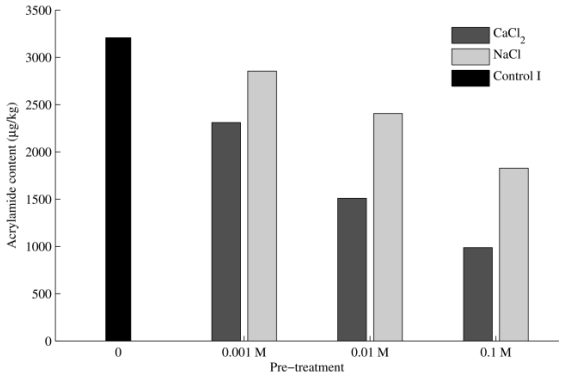

Figure 2. The effects of $CaCl_2$ and NaCl on acrylamide formation in potato crisps. Samples were immersed in 0.001, 0.01 and 0.1M of each salt separately for 30 min, and fried at 165°C for 4 min in sunflower oil.

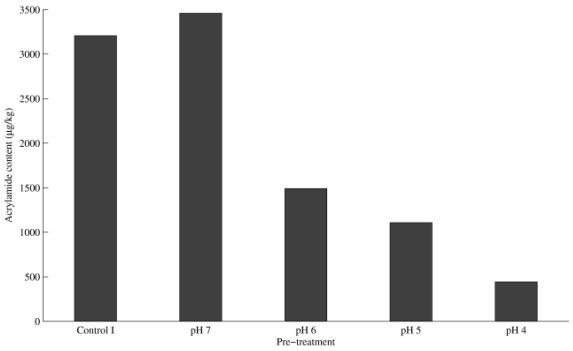

Figure 3. The effects of different pH on acrylamide formation in potato crisps. Samples were immersed in solutions with pH of 4, 5, 6, and 7 which were adjusted by 1 N citric acid and 1 N NaOH, and fried at 165°C for 4 min in sunflower oil.

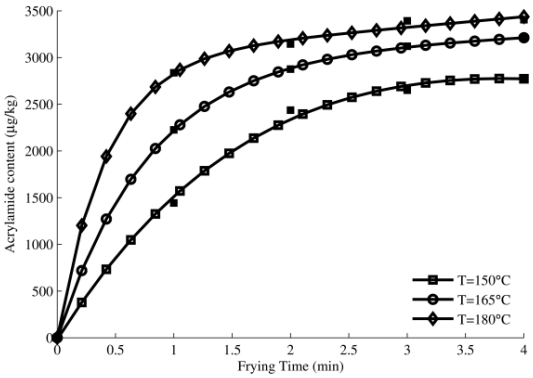

Figure 4. The effect of frying time on acrylamide formation in potato crisps. Samples were fried for 1, 2, 3, or 4 min at 3 constant temperatures.

Previously a reduction of oil absorption was reported for the potato slices treated with acids [52], that just similar to salts investigated in previous section, the lower amount of oil absorption results in the lower heat transfer that ends to a decrease in acrylamide formation.

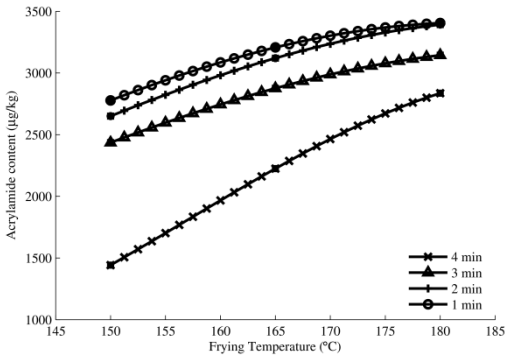

Figure 5. The effect of frying temperature on acrylamide formation in potato crisps. Samples were fried at 150, 165, or 180 for 4 constant times.

It has been also reported that maximum acrylamide content occurred at the pH of approximately 8.0 [53]. Besides, citric acid was confirmed as a factor of reducing pH and consequently reducing acrylamide formation [23].

Diminishing the pH could block the nucleophilic asparagine to add a carbonyl compound, precluding the Maillard reaction and as a result, acrylamide formation [28].

3. 2. The Content of Acrylamide Formed in Potato Crisps at Different Frying Conditions

Figure 4 indicates the effect of frying time on the formation of acrylamide in fried potatoes. Acrylamide formation increased dramatically with frying time for all three temperatures. In constant, temperature of 150°C, acrylamide formed in first and fourth minute by 1442 and 2777 µg/kg, respectively. However, these numbers rose to 2836 and 3405 µg/kg when potato slices fried at 180°C. The rate of formation of acrylamide grew up with time and the greatest amount of acrylamide formed when potato slices fried at 180°C for 4 min. The amount of acrylamide content had no significant change after the first 2 min of frying and more than 88% of final concentration in each temperature formed in this period. In addition, at the higher temperatures the final concentration of acrylamide formed in the lower frying time. These results shows a contrast information given by another study [54]. As shown in Figure 5, lowering the frying temperature from 180 °C to 165 °C and to 150 °C, decreased acrylamide content after 4 min by 6% and 18%, respectively. The gaps between the levels of acrylamide formed during frying at different times had been narrowed at the highest temperature (180°C) in comparison with the temperature of 150°C. It is most evident in the frying times of 3 and 4 min where this difference decreased from 127 µg/kg to only 13 µg/kg. The greatest slope of increasing acrylamide by increasing temperature was at t=1 min. However, this slope continuously decreased as time grew up. These

results can be proved by those given in other paper [55]. Increase in acrylamide formation by rising the frying time also reported by other study [56].

4. CONCLUSIONS

The challenge of this research was to examine strategies to minimize acrylamide content. Asparaginase enzyme is produced from several microbial sources mainly with the goal of therapeutic treatments. In the present study, we used a specific kind of asparaginase produced with the aim of food treatment. We hope that as international knowledge of acrylamide as a carcinogenic substance is growing; the use of enzyme from *C. utilis* becomes widespread in the food industry.

Blanching enhanced the effect of both commercial and asparaginase made from *C. utilis* in diminishing asparagine concentration in potato slices and led to decrease of acrylamide concentration in potato crisps. When asparaginase acts lonely on potato, its impact is significantly lower. Several additives, namely organic acid, NaCl or $CaCl_2$, reduced the final acrylamide formation. Our results showed that pre-treatment with $CaCl_2$ prior to frying decreased the amount of acrylamide in potato crisps. However, between these additives citric acid treatment made the largest reduction of acrylamide formation.

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Acrylamide Reduction in Potato Crisps using: Asparaginase from *Candida utilis*, Commercial Asparaginase, Salt Immersion, and pH Treatment

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چکیده

این مقاله کاهش تشکیل آکریل آمید در چیپس سیب زمینی در نتیجه اعمال آسپاراژیناز، استفاده از محلول های کلسیم کلرید و سدیم کلرید با غلظت های مختلف، غوطه ورسازی در محلول ها با pH های مختلف و شرایط سرخ کردن متفاوت را بررسی می کند. هدف اصلی مقایسه کاهش آکریل آمید در چیپس سیب زمینی در نتیجه استفاده از دو نوع آنزیم آسپاراژیناز است؛ آنزیم اول تجاری است اما آنزیم دوم به طور خاص برای استفاده در صنعت غذا از ***Candida utilis*** تولید شده است. پیش از سرخ کردن، نمونه ها را به یکی از روش های زیر آماده کردیم: شستشو در آب مقطر (کنترل ۱)؛ بلنچینگ در آب داغ؛ غوطه ور کردن در محلول آسپاراژیناز تجاری (یا آسپاراژیناز به دست آمده از ***Candida utilis***)؛ بانچینگ در آب داغ و غوطه ور کردن در محلول آسپاراژیناز تجاری (یا آسپاراژیناز به دست آمده از ***Candida utilis***)؛ بلنچینگ در آب داغ و غوطه ور کردن در آب با دمای متوسط (کنترل ۲). در حالی که آسپاراژیناز تجاری تشکیل آکریل آمید را تا۳۹٪ کاهش می دهد ، آسپاراژیناز به دست آمده از ***Candida utilis***سبب کاهشی بالاتر تا ۵۸٪ در چیپس سیب زمینی شده است. با این حال، هر دو آنزیم در ترکیب با بلانچینگ مقدار بسیار بالاتری از تشکیل آکریل آمید را مانع شده اند. همچنین استفاده از کلسیم کلرید، سدیم کلرید و اسید سیتریک اثرات قابل توجهی بر روی محتوای آکریل آمید در سیب زمینی سرخ شده دارد. حداکثر کاهش آکریل آمید به دست آمده ۹۵٪ بوده که حاصل اعمال آنزیم تجاری توام با بلانچینگ است.

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