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Impact of Pre-Treatments on the Acrylamide Formation and Organoleptic Evolution of Fried Potato Chips

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ABSTRACT

The main objective of this investigation was to study the effect of different pre-frying treatments on reduction of acrylamide formation of fried potato Moreover; the impact of different phenol compounds and leaves on acrylamide formation was evaluated. In addition, the effects of these treatments on the sensorial quality of fried potato chips were studied. Results showed that blanching process caused significant decreases in acrylamide content of fried potato. The highest decrease was observed for those samples blanched in MgCl2 (0.1 M), L-cysteine (0.05 M) and 0.01 M of citric acid solutions, 97.97, 97.17 and 93.43%, respectively. Soaking of potato slices in water or different solutions significantly reduced the formation of acrylamide. The decreases in acrylamide content ranged from 61.61 to 97.47%. Soaking in crude, semi-purified asparaginase solutions, blanching in hot water plus immersing in the enzyme solutions and soaking in phenolic acid solutions caused significant reduction in the formation of acrylamide of potato chips. Addition of fresh leaves into frying oil significantly influenced acrylamide formation. Oregano, rosemary, bamboo, guava and olive leaves caused the greatest reductions. Potato slices blanched in distilled water at 65°C, NaCl, Mg Cl2 and 0.1 M glutamine had significantly the highest scores of overall acceptability.

Keywords: Acrylamide, Potato, Blanching, Soaking, Protein, Antioxidants

1. INTRODUCTION

Potato (*Solanum tuberosum*) is one of the world's major agricultural crops and it is consumed daily by millions of people from diverse cultural backgrounds. Potatoes are always cooked before consumption traditionally by frying and other cooking methods (Pedreschi *et al*., 2006). Deep fat frying is extensively used in food processing both industrially and at home and fried potato products are one of its largest applications (Pedreschi *et al*., 2007). Acrylamide is a chemical compound that is formed from food components during heat treatment (frying, baking, roasting and extrusion) as a result of the Mailard's reaction between asparagine and reducing sugars (Pedreschi *et al*., 2004). Acrylamide shows a variety of adverse effects in animals and humans. It is known to be neurotoxic (causing peripheral neuropathy) in humans and a reproductive toxic agent in rodents (Tritscher, 2004). Acrylamide is positive in a number of tests for genotoxicity, inducing chromosomal aberrations, micronuclei, sister chromatid exchange, polyploidy, aneuploidy and other mitotic disturbances in mammalian cells in the absence of metabolic activation (Tritscher, 2004).

Acrylamide formation in foods is influenced by several factors, including processing temperature, time, content and species of reducing sugars and amino acids, pH, moisture content and frying oils, indicating that

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acrylamide in foods can be decreased by changing processing technology (Ciesarova *et al*., 2006). Ferulic acid, hydrogen peroxide, ferulic acid combined with H_2O_2 or Fe^{2+}, tea catechin,$NaHCO_3$ and $NaHSO_3$ were used to test the eliminating capacity for acrylamide under different temperatures and it was found that combination of ferulic acid with H_2O_2 or Fe^{2+} showed highest efficiency for eliminating acrylamide (Ou *et al*., 2004). Also, (Levine and Smith, 2005) found that $NaHCO_3$, NH_4HCO_3, cysteine, sodium bisulfite and ascorbate could eliminate acrylamide; and citric acid, ferulic acid and NaCl decreased the amount of acrylamide produced. In addition, pre-treatment with the enzyme L-asparaginase is sufficient to reduce acrylamide content, since L-asparagine is considered to be one of the main precursors for the acrylamide formation in foods (Friedman, 2003).

The main objective of this investigation was to study the effect of different pre-frying treatments on reduction of acrylamide formation of fried potato chips including soaking in water and different solutions (organic acids, salts and amino acids); blanching in hot distilled water with different solutions (citric acid, salts and amino acids) as well as blanching followed by soaking. Moreover, the impact of different phenolic compounds and leaves on acrylamide formation was evaluated. In addition, the effects of these treatments on the sensorial quality of fried potato chips were studied.

2. MATERIALS AND METHODS

2.1. Materials

Potatoes (*Solanum tuberosum L*.) of the variety Hermas were obtained from Chipsy for Food Industries Co. Giza, Egypt. Sunflower oil was obtained from Arma for Food Industries, 10th of Ramadan, Egypt. *Pseudomonas aeruginosa* 50071 bacteria obtained from Microbiological Resource Center, Ain-Shams University, Cairo, Egypt. All reagents and chemicals that were used in this study were of analytical grade.

2.2. Methods

2.2.1. Induction of Crude Enzyme from Pseudomonas Aeruginosa 50071 Bacteria

A pure culture of *Pseudomonas aeruginosa* 50071 was maintained on tryptic soy agar slants (30 g of Tryptic Soy Broth (TSB) per liter, 1.5% agar) and was subcultured regularly. To prepare an inoculum, a loopful of cells from an agar slant was first transferred into a test tube containing 10 mL of TSB and incubated at 37°C for 18 h (Chayabutra and Ju, 2000). About 0.5 mL of freshly grown single colonies of the isolates were picked up, with an inoculation loop and stirred into 50 mL tryptone soy broth in 250 mL Erlenmeyer flasks. The culture flasks were incubated at 37°C for 4 days. The cell free supernatant was obtained after a centrifugation at 8000 rpm for 20 min and used as crude enzyme after determination of enzyme.

2.3. Purification of the Crude Enzyme

The method of (El-Bessoumy *et al*., 2004) was used for the enzyme purification. The Production media was centrifuged at 10,000 rpm for 12 min to obtain the supernatant. The supernatant was saturated with 80% ammonium sulfate. The mixture was left for 12 h at 4°C, followed by centrifugation at 8,000 rpm for 20 min at 4°C. The precipitate was dissolved in a 0.01M phosphate buffer pH 8.5 and dialyzed overnight against the same buffer at 4°C to obtain the Semi-purified enzyme, then the specific activity of enzyme was measured.

2.4. Preparation of Potato Chips

2.4.1. Potato Chips (Slices)

Potato tubers were washed under running water, hand-peeled and cut into round slices (5 cm diameter, 2 mm thickness).

2.5. Blanching Treatments

Blanching was done by immersing the potato slices in distilled water (65 and 85°C), citric acid solutions (0.01and 0.05M), $CaCl_2$, $MgCl_2$ and NaCl (0.1M) solutions as well as amino acids solutions (glycine, L-arginine, L-glutamine, 0.1 M) and L-cysteine (0.1; 0.05M). Samples were removed after 5 min. The product: solution ratio was 1:5 (w/w). After blanching, potato slices were cooled in ice water for 10 min. The blanched slices were drained and fried at 190±5°C.

2.6. Soaking Treatments

Potato slices were soaked in running tap water (15 min), distilled water (60 min), citric acid (0.01and 0.05M); acetic acid(0.15, 0.3 and 0.45M) solutions, salt solutions, 0.1 M [$CaCl_2$, $MgCl_2$ and NaCl], amino acids solutions (glycine, L-arginine and L-glutamine, 0.1 M), L-cysteine (0.1; 0.05M) solution, 2% protein (chickpea and egg albumin) solutions and phenolic (ferulic acid, protocatechoic acid, caffeic acid, catechin and gallic acid) solutions, (1:10, w/v) for 60 min at room temperature (˜25°C). The soaked slices were drained and fried at 190±5°C.

2.7. Addition of Different Fresh Leaves with into Frying Sunflower Oil

Guava, rosemary, oregano, olive, cranberry and green tea leaves were added into sunflower oil during frying process at level (5%).

2.8. Blanching Followed by Soaking Treatments

Potato slices were blanched in distilled water at 85°C for 5 min, the blanched potato were drained and divided into two portions. A portion of the drained blanched potato was soaked in crude enzyme solution (specific activity, 46.57 units) at 37°C for 60 min. A second portion of the blanched potato was soaked in semi-purified enzyme solution (specific activity, 232.84 units) at 37°C for 60 min. All treated potatoes were drained and fried at 190±5°C for 6 min.

2.9. Frying Conditions

Sunflower oil (500 g) was placed in a stainless steel pan of electric fryer (20 cm depth×20 cm length×10 cm) and heated at 190°C ± 5°C. Then, 100 g of potato slices of each treatment were fired for 6 min.

2.10. Determination of Total Polyphenols

2.10.1. Preparation of Leaves Juice

Fresh leaves of guava, rosemary, oregano, olive, cranberry and green tea were cleaned, cut into pieces and then pressed by means of the hydraulic laboratory press model C S/N 37000-156 Freds from Carver (WI, USA). The resultant crude juices were centrifuged at 4000 rpm for 30 min at 4°C; the supernatant was concentrated at 45°C using a rotary evaporator (Laborota 4000-efficient, Heildolph, Germany). The obtained juice was kept in light-protected containers at-18°C until further use.

Total polyphenols were determined according to the method of (Jayaprakasha *et al*., 2003). An aliquot of leaves juice (0.1 mL) was dissolved in a 10 mL mixture of acetone and water (6:4 v/v). Sample (0.2 mL) was mixed with 1.0 mL of ten-fold diluted Folin-Ciocalteu reagent and 0.8 mL of 75 g L^{-1} sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 725 nm. Phenolic contents were calculated on the basis of the standard curve for Gallic Acid (GAL). The results were expressed as mg gallic acid/100 g.

1.11. Determination of Free Radical Scavenging Activity (DPPH)

The antioxidant activity of fresh leaves juice was evaluated by using the 2, 2'Diphenylpicrylhydrazyl (DPPH) assay (Cuendet *et al*., 1997; Burits and Bucar, 2000). Fifty microliters of the juice were added to 5 mL of 0.004% (w/v) DPPH in methanol (100%v/v). After, 30 min incubation period at room temperature the absorbance at 517 nm was compared to DPPH in methanol without sample (blank). The percent inhibition of free radical formation (I%) was calculated as follow:

$$I\% = (A\ blank_{at517} - A\ sample_{at517} / A\ blank_{at517}) \times 100$$

2.12. Determination of Acrylamide in Potato Samples

The homogenized sample (5g) was extracted with 50 mL of methanol (5%) and shaked by hand for 30 min. After centrifugation for 30 min at 9,000 rpm, the supernatant solution was transferred to a 200-mL separating funnel and the aqueous layer was collected and used for analysis. The aqueous layer was centrifuged at 13 000 rpm for 5 min. The pooled supernatant was filtered through a 0.2µ PVDF syringe filter. The C18 SPE column (strong cation exchange, 0.5-1.5 g sorbent, 0.6 mequiv. g^{-1}) was conditioned with 5 mL methanol and the extract (2 mL) was loaded with 5 mL of water onto the column. The extracts were eluted with 2 mL of water. The eluent was collected for HPLC analysis.

The samples were analyzed using HPLC (Hewlett Packard 1050) with a C18 column (250×4.6 nm, 5µ purity C18). The injected volume was 20 µL. The separation was carried out using 80% methanol/water (v/v) with flow rate 1 mL min^{-1} and the effluent of the column was continuously monitored with the UV detector at 254 nm (Geng *et al*., 2010).

2.13. Organoleptic Evolution of Potato Chips

The sensory evaluation of fried potato chips were conducted two times and the mean score values was reported in the text. The potato chip samples were rated on a 10-point scale (1,2: bad,3,4: poor,5,6: fair,7,8: good and 9,10: excellent).

The potato chips from each treatment, placed randomly in codified plates with 3-digit codes, were served to each panelist. Judges were placed in different places to avoid communication during the evaluation and asked to score chips for taste, texture, appearance, color, odor and overall acceptability (Carpenter *et al*., 2000).

2.14. Statistical Analysis

Data were statistically analyzed in completely randomized design in factorial arrangement according to the procedures outlined by (Gomez and Gomez, 1984) and

the treatments means were compared by Least Significant Differences (LSD) and Duncan multiple range using SPSS program package. Data are presented in text and tables as means of three determinations.

3. RESULTS AND DISCUSSION

3.1. Effect of Different Blanching Treatments on the Formation of Acrylamide in Potato Chips

Table 1 shows the effect of different blanching treatments on the formation of acrylamide of potato chips. These result show that the formation of acrylamide was higher in control than in blanched samples. Blanching process caused significant ($p \leq 0.05$) decreases in acrylamide content. These reductions ranged from 73.58 to 97.97%. Blanching reduces the formation of acrylamide of French fries probably due to the leaching out of reducing sugars, previous to frying, inhibiting in this way non-enzymatic browning reactions and leading to lighter and less red French fries (Pedreschi and Moyano, 2005). Lightness of French fries decreased as the acrylamide formation increased since the pieces get darker as a result of Maillard reactions. The highest decrease was observed for those samples blanched in $MgCl_2$ (0.1 M), L-cysteine (0.05 M) and 0.01M of citric acid solutions, 97.97, 97.17 and 93.43%, respectively. Recently, evidence was found that cations such as Ca^{2+} or Mg^{2+} would change the reaction path from the Maillard reaction toward dehydration of glucose (Gokmen and Senyuva, 2007). Some authors diminished acrylamide formation in fried snacks products by adding amino acids, such as lysine, glycine and cysteine (Kim *et al*., 2005). The addition of glycine or glutamine during blanching of potato chips reduced the amount of acrylamide by almost 30% compared to control (Claeys *et al*., 2005). Acrylamide formation can be reduced significantly as well by introducing other amino acids, such as cysteine, lysine, or glycine, which would compete with asparagine for the carbonyl compounds in the Maillard reaction and/or enhance acrylamide elimination (Claeys *et al*., 2005). The nucleophilic sulphur atom of L-cysteine and the amino groups of the other amino acids might readily give rise to Michael type addition reactions with acrylamide (Fennell *et al*., 2005; Stadler *et al*., 2004). Average acrylamide contents for potato chips immersed in $MgCl_2$ (0.1 M), L-cysteine (0.05M) and 0.01M of citric acid solutions were 40, 56 and 130 μg 100^{-1} g of fried potato chips, respectively. For these treatments, acrylamide content was lower than the maximum permissible level of WHO (2005) (0.3-2 μg/kg/day for the general population, body weight 70kg). Blanching of potato chips in hot water (65 and 85°C) or solutions containing 0.05M citric acid, 0.1 M $CaCl_2$ or NaCl, 0.1M glycine, L-glutamine or L-cysteine reduced significantly acrylamide formation of potato chips by 90.40, 88.88, 90.40, 73.58, 76.91, 85.20 and 76.96% respectively **Table 1**. However, acrylamide contents of those samples were higher than the maximum permissible level of WHO (2005). The reduction of acrylamide formation in potato chips blanched in citric acid solutions may be attributed to both pH lowering and leaching out of free asparagine and the reducing sugars from the surface layer of potato slices to the solutions (Jung *et al*., 2003). Additionally, (Gokmen and Senyuva, 2007) showed that dipping of potato stips into calcium chloride solution inhibited the formation of acrylamide by up to 95%during frying. The reduction of acrylamide formation in potato chips immersed in sodium chloride and calcium chloride solutions may be due to its complexation with amines and some intermediates of the Maillard reaction products, especially acrylic acid, a prevalently recognized precursor for forming acrylamide (Stadler *et al*., 2004). Also Na+ or Ca2+ was indicated to interact with asparagine to prevent the formation of acrylamide (Park *et al*., 2005; Lindsay and Jang, 2005; Gokmen and Senyuva, 2007). Yet, in the previous study, NaCl did not lower the final acrylamide content in the potato model system (Mestdagh *et al*., 2007). On the other hand, the addition of NaCl, CaCl2 or citric acid might also change the oil uptake (Bunger *et al*., 2003; Rimac-Brncic *et al*., 2004; Pedreschi *et al*., 2007). This could therefore be an additional factor, possibly influencing the formation of acrylamide in fried foodstuffs.

3.2. Effect of Different Soaking Treatments

Soaking of potato slices in water or different solutions caused significant reduction in the formation of acrylamide. The decreases in acrylamide content ranged from 61.61 to 97.47%. Some authors reported that the reduction of the sugar content by soaking could decrease acrylamide concentration by about 60%in potato chips (Haase *et al*., 2003; Pedreschi *et al*., 2004). Potato slices treated with running water for 15 min and distilled water for 60 min showed a reduction of acrylamide formation of 61.61 and 63.63%, respectively. Soaking process leads to a higher leaching of one important acrylamide precursor such as glucose that finally results in lower acrylamide formation (Pedreschi *et al*., 2004). These results are coincident with those of (Jung *et al*., 2003) who reported that dipping potato strips in distilled water for 1 h induced almost 25% reduction of acrylamide formation in French fries after frying at 190°C.

Table 1. Effect of different blanching treatments on the formation of acrylamide (µg/Kg) in potato chips fried at 190°C±5°C for 6 min compared with maximum permissible level WHO (2005)*

Blanching treatments (for 5 min at 65°C)	Acrylamide content	Reduction (%)	Status
Control (without blanching)	1980^{a}±104	-	+
Blanching in distilled water			
At 65°C	164^{ef}±16	91.70	+
At 85°C	405^{c}±24	79.54	+
Citric acid solution			
0.01 M	130^{efg}±52	93.43	-
0.05 M	190^{de}±30	90.40	+
Salt solution (0.1 M)			
$CaCl_2$	220^{de}±43	88.88	+
$MgCl_2$	40^{ef}±2	97.97	-
NaCl	190^{de}±34	90.40	+
Amino acid solution (0.1 M)			
Glycine	523^{b}±63	73.58	+
L-Glutamine	457^{b}±70	76.91	+
L- Arginine	293^{d}±49	85.20	+
L-cysteine	456^{bc}±86	76.96	+
L-cysteine (0.05M)	56^{ef}±8.71	97.17	-
LSD = 90.21			

(+,-) Daily intake (µg), based on consumption of 100 gm of fried potato strips per day, was higher or lower than maximum permissible level of WHO limit (0.3-2 µg/kg/day for the general population, body weight 70 kg), respectively. * Maximum permissible level of WHO (2005) at range of 21-140 µg/70kg/day for the general population. Data are expressed as mean ± SD. Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p<0.05$)

Table 2. Influence of different soaking treatments on the formation of acrylamide (µg/Kg) in potato chips fried at 190°C±5°C for 6 min compared with maximum permissible level WHO (2005)*

Soaking treatments at room temperature for 60 min	Acrylamide content	Reduction (%)	Status
Control (without soaking)	1980^{a}±104	-	+
Soaking in water			
Running water (15 min)	760^{b}±127	61.61	+
Distilled water (60 min)	720^{bc}±36	63.63	+
Organic acid			
Citric acid (0.01M)	50^{h}±5	97.47	-
Citric acid (0.05M)	60^{gh}±15	96.96	-
Acetic acid (0.15M)	290^{de}±43	85.35	+
Acetic acid (0.3M)	190^{efg}±26	90.40	+
Acetic acid (0.45M)	290^{de}±55	85.35	+
Salt solution (0.1 M)			
$CaCl_2$	130^{fgh}±36	93.43	+
$MgCl_2$	218^{ef}±20	88.98	+
NaCl	170^{efgh}±36	91.41	+
Amino acid solution (0.1M)			
Glycine	298^{de}±34	84.94	+
L-Glutamine	634^{c}±84	67.97	+
L-Arginine	422^{d}±37	78.68	+
L-cysteine	621^{d}±44	68.63	+
L-cysteine (0.05M)	302^{de}±1	84.74	+
Protein solution (2%)			
Chickpea	510^{b}±101	74.24	+
**Egg albumin	160^{c}±10	91.91	+
LSD = 93.41			

(+,-) Daily intake (µg), based on consumption of 100 gm of fried potato strips per day, was higher or lower than maximum permissible level of WHO limit (0.3-2 µg/kg/day for the general population, body weight 70 kg), respectively. * Maximum permissible level of WHO (2005) at range of 21-140 µg/70kg/day for the general population. Data are expressed as mean ± SD. Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p<0.05$). **2 % dry weight or 16.4% fresh weight

Table 2 shows the effect of soaking in 0.01 and 0.05 M of Citric acid or 0.15, 0.3 and 0.45 of acetic acid on the formation of acrylamide of potato chips. Potato slices soaked in 0.01 and 0.05 M of Citric acid and 0.3 M of acetic acid had significantly the lowest values of acrylamide 50, 60 and 190 μg Kg^{-1} of fried potato. Organic acids, such as citric, acetic and L lactic acid reduced the final acrylamide content, but merely due to a reduced pH (Mestdagh *et al*., 2007). Lowering the pH using organic acids of the food system to reduce acrylamide generation may attribute to protonating the α-amino group of asparagine, which subsequently cannot engage in nucleophilic addition reactions with carbonyl sources (Jung *et al*., 2003). Lowering the pH of the cut potatoes (e.g., with citric acid 0.5%-1.0% <20 min) has been shown to lower the levels of acrylamide formed (Jung *et al*., 2003). Potato slices soaked in 0.1M of $CaCl_2$, $MgCl_2$ and NaCl solutions for 60 min showed significant reduction in the formation of acrylamide by 93.43, 88.98 and 91.41%, respectively. CaCl2 showed more efficient in inhibiting acrylamide formation than $MgCl_2$ and NaCl. The acrylamide inhibiting mechanism for calcium may be due to its complexation with amines and some intermediates of the Maillard reaction products as reported before (Delgado-Andrade *et al*., 2004; O'Brien and Morrissey, 1997), especially acrylic acid, a prevalently recognized precursor for forming acrylamide (Stadler *et al*., 2004; Yaylayan *et al*., 2003). Soaking of potato slices in 0.1M of glycine, L-glutamine, L-arginine and L-cysteine for 60 min induced significant reductions of acrylamide levels in fried potato chips. However, those potatoes soaked in 0.1 M of glycine and 0.05 of L-cysteine had the highest reduction 84.94 and 84.74%, respectively. A related study showed that the addition of cysteine or lysine to an asparagine/glucose model system heated between 140 and 200°C significantly lowered acrylamide formation (Claeys *et al*., 2005).

Glycine and lysine can exert their beneficial effects by competing with asparagine for the carbonyl group of the sugar moiety and/or form adducts with acrylamide after it is formed. In principle, the SH group of cysteine (or other thiols) can exert its beneficial effects in two ways: it can form an adduct with acrylamide as well as undergo heat-induced H_2S elimination to form dehydroalanine [CH_2 = CH (NH_2) COOH]. The NH_2 group of asparagine can then competitively participate in addition reactions with the double bond of the dehydroalanine, as it does with acrylamide. Dehydroalanine can, in principle, also be formed by elimination of H_2O from serine (Friedman, 1978). Evidently, free amino acids in foods can reduce as well as enhance acrylamide levels during processing and storage. **Table 2** shows that soaking potato slices in protein solutions containing 2% Chickpea or egg albumin resulted in significantly reduced acrylamide levels in fried potato by 74.24 and 91.91% respectively. The protein content of the fried samples was inversely related to acrylamide levels. The protective effect of the chickpea proteins may be due to their known thermo stability. The heat-stable proteins appear to function as a thermal barrier of the potato slices and/or to combine with some of the acrylamide as it is formed in the food matrix during frying (Friedman and Levin, 2008; Fiselier *et al*., 2004) coated potato croquettes with a mixture of egg and breadcrumbs. Even though the product was browner than regular croquettes, the acrylamide content was reduced from 280 to 50 ppb.

Effect of soaking in crude (specific activity, 46.57 units) and semi-purified (specific activity, 232.84 units) asparaginase solutions and blanching plus immersing in the enzyme solutions.

Table 3 shows that enzyme treatments significantly lowered acrylamide formation by 93.93 to 97.97%. Acrylamide content was lower than the maximum permissible level of WHO (2005). The application of asparaginase represents another strategy for acrylamide reduction. As in the presence of acids, asparagine is hydrolysed to aspartic acid, thus inhibiting acrylamide generation in the Maillard reaction. When the enzyme preparation was added to wheat cracker production, acrylamide levels were decreased by at least 70% without any changes in the colour or flavour of the products (Vass *et al*., 2004).

3.3. Effect of Soaking in Phenolic Solutions

Table 4 shows the effect of soaking in ferulic, protocatechoic, caffeic, gallic acids and catechin solutions on the formation of acrylamide of potato chips. Soaking in phenolic acid solutions for 60 min resulted in significant reductions in the formation of acrylamide of potato chips. These reductions ranged from 31.81 to 98.03%. Gallic acid had the highest efficiency for reducing acrylamide in fried potato, 98.03. Acrylamide content was lower than the maximum permissible level of WHO (2005). Reducing agents, ferulic acid and catechin were also used to reduce acrylamide formation by inhibiting transformation of acrolein to acrylic acid proposed by (Mottram and Wedzicha, 2002). Since this mechanism was discounted by several researches (Stadler *et al*., 2004; Vattem and Shetty, 2003; Yaylayan *et al*., 2003), these two agents may have other inhibiting mechanism, for example, they form quinone at higher temperature and react with asparagine and the formed amines (Hurrel and Fiont, 1984) and therefore may decrease acrylamide production.

Table 3. Effect of soaking in crude and semi-purified asparaginase solutions and blanching plus immersing in crude and semi-purified asparaginase on the formation of acrylamide of potato chips, fried at190±5°C for 6 min compared with maximum permissible level WHO (2005)*

Treatment	Acrylamide content	Reduction (%)	Status
Control (without any treatment)	1980^{a}±104	-	+
Soaking at 37°C for 60 mi			
Semi-purified enzyme			
(specific activity, 232.84 units)	120^{b}±2.0	93.93	-
Crude enzyme (specific activity, 46.57 units)	40^{b}±9.0	97.97	-
Blanching in hot distilled water at 85°C			
for 5 min and followed by soaking in			
Semi-purified enzyme, specific activity,			
232.84 units) (at 37°C for 60 min)	70^{b}±9.2	96.46	-
Crude enzyme, specific activity,			
46.57 units) (at 37°C for 60 min)	40^{b}±5.8	97.97	-
LSD = 93.0			

(+,-Daily intake (µg), based on consumption of 100 gm of fried potato strips per day, was higher or lower than maximum permissible level of WHO limit (0.3-2 µg/kg/day for the general population, body weight 70 kg), respectively.* Maximum permissible level of WHO (2005) at range of 21-140 µg/70kg/day for the general population. Data are expressed as mean±SD. Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p<0.05$)

Table 4. Acrylamide contents (µg/Kg) in potato chips pre-soaked (potato: solution, 1: 2) for 60 min on different phenolic solutions (70 mg 60 mL^{-1}, in traces of tween 80) and fried on sunflower oil at190°C±5°C for 6 min comparing with maximum permissible level WHO (2005)*

Treatments	Acrylamide content	Reduction (%)	Status
Control	1980^{b}±104	-	+
Ferulic acid	461^{c}±34	76.71	+
Protocatechoic acid	1350^{a}±95	31.81	+
Caffeic acid	528^{c}±143	73.33	+
Catechin	180^{d}±26	90.90	+
Gallic acid	39^{e}±6	98.03	-
LSD = 149.75			

(+,-) Daily intake (µg), based on consumption of 100 gm of fried potato strips per day, was higher or lower than maximum permissible level of WHO limit (0.3-2 µg/kg/day for the general population, body weight 70 kg), respectively.* Maximum permissible level of WHO (2005) at range of 21-140 µg/70kg/day for the general population. Data are expressed as mean±SD. Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p<0.05$)

3.4. Total Phenol Content and Free Radical Scavenging Activity of Different Fresh Leaves

Phenolic Compounds (PCs) may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content. **Table 5** shows total phenolic content of leaves. They varied significantly ($p<0.05$) from 577.8 to 1490.1 mg Gallic Acid Equivalents (GAE) per 100 g DW. Granberry, guava and green tea leaves had the highest ($p<0.05$) levels of total phenolics 1490.1, 1092 and 1020 mg Gallic Acid Equivalents (GAE) per 100 g DW, respectively.

Table 5. Total phenolic content and free radical scavenging activity of different fresh leaves

Leaves	Total phenolic	Free radical scavenging (%)
Guava leaves	1092.6^{b}±1.52	88.7^{b}±1.20
Rosemary leaves	899.5^{d}±1.00	83.2^{c}±1.03
Oregano leaves	577.8^{f}±0.577	79.2^{d}±1.40
Olive leaves	751.1^{e}±0.577	73.2^{e}±1.09
Cranberry leaves	1490.1^{a}±0.587	42.5^{f}±1.01
Green tea leaves	1020.4^{c}±1.00	95.7^{a}±1.20
LSD =	1.59	1.64

Data are expressed as mean ± SD. Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p<0.05$). Total polyphenols are expressed as mg gallic acid/100g

However rosemary, oregano and olive leaves contained moderate amounts of total phenolics 899.5, 577.8 and 751.1 mg Gallic Acid Equivalents (GAE), respectively. The total phenolics content of granberry leaves was about 1.3,1.4,1.65, 1.98 and 2.57-fold greater than that of the guava, green tea, respectively. Although the granberry leaves contained the highest level of phenolics, the lowest DPPH radical scavenging activity was detected in granberry leaves. Radical Scavenging Activity (RSA) depends on the composition of phytochemicals, including antioxidatively active compounds in plants (Baltrusaityte *et al*., 2007). Green tea, guava leaves had the higher DPPH radical-scavenging activity 95.7 and 88.7%, ‘‘respectively’’, followed by rosemary and oregano leaves 83.2 and 79.2%, “respectively”. The radical-scavenging activities of these extracts can be attributed to the presence of some compounds that

have antioxidant activity. Baardseth *et al*. (2010) stated that the higher content of total phenolic compounds, the stronger antioxidant activity. Although the granberry leaves contained the highest level of phenolics, the lowest DPPH radical scavenging activity was detected in granberry leaves.

3.5. Effect of Different Fresh Leaves into Frying Oil on Acrylamide Formation

Table 6 shows the effect of addition of different fresh leaves into frying sunflower oil (5 g 100^{-1} g) on the formation of acrylamide in potato chips fried at190±5°C for 6 min. Addition of fresh leaves into frying oil significantly influenced acrylamide formation. Oregano, rosemary, bamboo, guava and olive leaves caused the greatest reductions, of 92.8, 85.7, 84.6, 84.0 and 75.7%, respectively, however the lowest reductions were showed for Cranberry and Green tea leaves 59.6 and 56.5%, respectively. (Hedegaard *et al*., 2008) reported that addition of aqueous rosemary extract, rosemary oil, dried rosemary leaves to wheat dough reduced the content of acrylamide in wheat buns by 62%, 67 and 57%, respectively. Significant acrylamide reduction was reported by (Zhang and Zhang, 2007) in fried bread sticks upon addition of Antioxidants of Bamboo Leaves (AOB) and antioxidant from Green Tea (EGT). Levine and Smith (2005) reported a slight lowering effect upon addition of ascorbic or ferulic acid to wheat/water model systems.

3.6. Sensory Evaluation

Table 7 shows the effect of blanching in water or different solutions on sensorial attributes of potato chips. Potato slices blanched in distilled water at 65°C, NaCl, MgCl2 and glutamine (0.1 M) had significantly the highest scores of taste, texture, appearance, color, odor and overall acceptability. However those blanched in distilled water at 85°C, citric acid (0.05), L-cysteine (0.1M) had significantly the lowest values. Acidification may moreover result in a sour product taste (Kita *et al*., 2004; Franke *et al*., 2005). This effect however depends upon the applied soaking or blanching treatment and the type and concentration of the acid used. Addition of (sulphur containing) amino acids may also generate unpleasant off-flavours upon heating, which should be taken into account as well (Claeys *et al*., 2005). Effect of soaking in water or various solutions were shown in **Table 8**. These results show that soaking in citric acid (0.3 and 0.45 M) $CaCl_2$and L-cysteine (0.1 and 0.05M) solutions had significantly the lowest values of taste, odor and overall acceptability. It was suggested that acetic acid would be a better acidulant for the pre-treatment of potato crisps compared to citric acid, due to the less appearing sourness (Kita *et al*., 2004). Addition of (sulphur containing) amino acids may also generate unpleasant off-flavours upon heating, which should be taken into account as well (Claeys *et al*., 2005). Calcium chloride might improve product texture, but on the other hand can cause a bitter aftertaste (Varela *et al*., 2007). Consequently, these pre-treatments may also cause unwanted sensorial defects. While those potato slices treated with running water, distilled water, NaCl, 0.1 M glycine, 0.1 glutamine, 0.1 arginine, chickpea and egg albumin solutions had significantly ($p \leq 0.05$) the higher values of overall acceptability with a score above 7. The result presented in **Table 9** showed that the acceptability of fried potatoes was not affected by the addition of fresh leaves into frying oil, which ensures good taste, texture, appearance, color, odor and overall acceptability of fried potato.

Table 6. Impact of adding different fresh leaves with frying sunflower oil (5 g 100 g^{-1}) on the formation of acrylamide (µg $100g^{-1}$) in potato chips fried at190°C±5°C for 6 min compared with maximum permissible level WHO (2005)*

Treatments	Acrylamide content	Reduction (%)	Status
Control	1980^{a}±104	-	+
Bamboo	305^{d}±15.6	84.6	+
Guava leaves	317^{d}±14.7	84.0	+
Rosemary leaves	280^{d}±52.9	85.7	+
Oregano leaves	140^{e}±20.0	92.8	-
Olive leaves	480^{c}±72.1	75.7	+
Olive leaves (juice) equivalent 5 g fresh leaves	810^{b}±26.4	59.0	+
Cranberry leaves	800^{b}±100	59.6	+
Green tea leaves	860^{b}±35.3	56.5	+
LSD = 101.90			

(+,- Daily intake (µg), based on consumption of 100 gm of fried potato strips per day, was higher or lower than maximum permissible level of WHO limit (0.3-2 µg/kg/day for the general population, body weight 70 kg), respectively.* Maximum permissible level of WHO (2005) at range of 21-140 µg/70kg/day for the general population. Data are expressed as mean±SD. Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p<0.05$)

Table 7. Effect of blanching treatments on sensorial attributes of potato chips

Blanching treatments (for 5 min at 65°C)	Taste	Texture	Appearance	Color	Odor	Over all acceptability
Control	$8.0^{a}\pm0.0$	$7.8^{abc}\pm0.4$	$7.8^{ab}\pm0.4$	$8.0^{a}\pm0.5$	$8.0^{a\pm}0.7$	$7.6^{ab}\pm0.3$
Commercial product	$8.8^{a}\pm0.5$	$8.4^{ab}\pm0.5$	$8.6^{a}\pm0.5$	$8.3^{bc}\pm0.5$	$8.2^{ab}\pm0.4$	$8.5^{ab}\pm0.5$
Distilled water at 65°C	$7.8^{ab}\pm0.4$	$8.5^{a}\pm0.5$	$8.2^{a}\pm0.4$	$8.2^{a}\pm0.4$	$8.0^{a\pm}0.3$	$7.8^{ab}\pm0.2$
Distilled water at 85°C	$6.2^{d}\pm0.4$	$7.4^{bc}\pm1.0$	$7.6^{abc}\pm0.5$	$7.4^{a}\pm0.8$	$7.8^{a}\pm0.8$	$7.4^{abc}\pm0.8$
Citric acid 0.01 M	$7.8^{ab}\pm0.4$	$8.2^{ab}\pm0.4$	$7.8^{ab}\pm0.4$	$7.8^{a}\pm0.4$	$7.2^{a}\pm0.8$	$7.7^{ab}\pm1.0$
Citric acid 0.05 M	$5.0^{e}\pm0.0$	$6.2^{e}\pm0.5$	$7.0^{cd}\pm0.0$	$7.4^{a}\pm0.5$	$7.4^{a}\pm0.5$	$6.7^{bc}\pm0.3$
$CaCl_2$(0.1 M)	$7.0^{c0.0}\pm0.0$	$6.4^{de}\pm0.4$	$7.6^{abc}\pm0.5$	$7.8^{a}\pm0.4$	$7.0^{a}\pm1.0$	$7.2^{abc}\pm0.4$
$MgCl_2$(0.1 M)	$8.2^{a}\pm0.0$	$7.2^{bcd}\pm004$	$8.0^{ab}\pm0.0$	$8.0^{a}\pm0.7$	$8.2^{a}\pm0.4$	$8.2^{ab}\pm0.4$
NaCl(0.1 M)	$7.2^{bc}\pm0.4$	$8.2^{ab}\pm0.0$	$7.2^{bcd}\pm0.4$	$7.4^{a}\pm0.5$	$7.2^{a}\pm0.8$	$7.6^{ab}\pm0.9$
Glycine(0.1 M)	$7.8^{ab}\pm0.4$	$7.0^{cde}\pm0.4$	$8.0^{ab}\pm0.0$	$7.8^{a}\pm0.4$	$7.8^{a}\pm0.4$	$7.7^{ab}\pm0.5$
L-Glutamine(0.1 M)	$8.4^{a}\pm0.5$	$7.2^{bcd}\pm0.0$	$8.4^{a}\pm0.5$	$8.0^{a}\pm0.0$	$8.2^{a}\pm0.4$	$8.3^{a}\pm0.8$
L-Arginine(0.1 M)	$5.2^{e}\pm0.4$	$8.0^{ab}\pm0.4$	$6.6^{d}\pm0.5$	$7.6^{a}\pm0.5$	$6.0^{b}\pm0.7$	$7.0^{abc}\pm1.0$
L-cysteine(0.1 M)	$5.6^{e}\pm0.5$	$5.2^{f}\pm0.5$	$6.0^{e}\pm0.4$	$5.4^{b}\pm0.4$	$5.6^{b}\pm0.8$	$5.6^{d}\pm0.5$
L-cysteine (0.05M)	5.6e±0.5	$5.4^{f}\pm0.5$	$5.8^{e}\pm0.0$	$6.4^{b}\pm0.5$	$5.4^{b}\pm0.5$	$6.2^{cd}\pm0.4$
LSD=	0.54	0.66	0.53	0.68	0.86	0.89

Data are expressed as mean±SD. Values followed by the same letter are not significantly different ($p<0.05$). The potato chip samples were rated on a 10-point scale (1,2: bad,3,4: poor, 5,6: fair,7,8: good and 9,10: excellent)

Table 8. Effect of different soaking treatments on sensorial attributes of potato chips

Soaking treatments at room temperature for 60 min	Taste	Texture	Appearance	Color	Odor	Over all acceptability
Control	$1.2\pm^{abc}7.8$	$0.8\pm^{abc}7.4$	$0.4\pm^{abc}7.2$	$0.5\pm^{cd}7.4$	$0.5\pm^{bcd}7.4$	$0.3\pm^{bcd}7.7$
Commercial product	$0.5\pm^{a}8.8$	$0.5\pm^{a}8.5$	$0.5\pm^{a}8.6$	$0.5\pm^{bc}8.3$	$0.4\pm^{ab}8.2$	$0.5\pm^{ab}8.5$
Running water (15 min)	$0.4\pm^{abc}7.6$	$0.4\pm^{cde}6.8$	$0.0\pm^{ab}8.0$	$0.0\pm^{bc}8.0$	$0.4\pm^{abcd}7.8$	$0.2\pm^{bcd}7.8$
Distilled water (60 min)	$0.8\pm^{bcde}6.4$	$0.8\pm^{abc}7.8$	$0.5\pm^{abc}7.4$	$0.5\pm^{bcd}7.0$	$0.4\pm^{cde}7.2$	$0.3\pm^{bcd}7.5$
Citric acid (0.01M)	$0.8\pm^{bcde}6.4$	$0.5\pm^{abc}7.6$	$0.8\pm^{ba}7.6$	$0.4\pm^{bcd}7.8$	$0.4\pm^{abcd}7.8$	$0.3\pm^{bcd}7.5$
Citric acid (0.05M)	$0.8\pm^{bcde}6.8$	$0.8\pm^{cde}6.6$	$0.4\pm^{ab}7.8$	$0.4\pm^{bcd}7.8$	$0.5\pm^{bcd}7.6$	$0.1\pm^{bcd}7.5$
Acetic acid (0.15M)	$1.3\pm^{cde}6$	$0.8\pm^{cde}6.6$	$0.5\pm^{ab}7.6$	$0.5\pm^{bcd}7.6$	$0.4\pm^{cde}7.2$	$0.3\pm^{d}7.2$
Acetic acid (0.3M)	$0.7\pm^{bcde}6.2$	$0.0\pm^{cde}6.8$	$0.8\pm^{abc}7.4$	$0.4\pm^{bcd}7.8$	$0.4\pm^{cde}7.2$	$0.3\pm^{cd}7.4$
Acetic acid (0.45M)	$1\pm^{bcde}6.4$	$0.0\pm^{abcd}7.0$	$0.5\pm^{abc}7.4$	$0.4\pm^{bcd}7.8$	$0.5\pm^{bcd}7.4$	$0.4\pm^{bcd}7.4$
$CaCl_2$ (0.1 M)	$1.3\pm^{bcde}6.6$	$0.8\pm^{de}6.2$	$0.0\pm^{bc}7.0$	$0.0\pm^{d}7.0$	$0.0\pm^{de}7.0$	$0.5\pm^{de}7.0$
$MgCl_2$ (0.1 M)	$0.8\pm^{abcd}7$	$0.5\pm^{abc}7.6$	$0.8\pm^{ab}7.6$	$0.4\pm^{bcd}7.8$	$0.4\pm^{abcd}7.8$	$0.4\pm^{de}7.0$
NaCl (0.1 M)	$1.0\pm^{abcd}7.2$	$0.8\pm^{abc}7.8$	$0.8\pm^{ab}7.8$	$0.0\pm^{bc}8.0$	$0.0\pm^{abc}8.0$	$0.5\pm^{bcd}7.8$
Glycine (0.1 M)	$0.4\pm^{abcd}7.4$	$0.4\pm^{ab}8.2$	$0.4\pm^{ab}8.6$	$0.5\pm^{b}8.4$	$0.0\pm^{abc}8.0$	$0.4\pm^{abc}8.3$
Glutamine (0.1 M)	$0.3\pm^{ab}8.2$	$0.4\pm^{ab}8.2$	$0.8\pm^{a}8.6$	$0.0\pm^{a}9.0$	$0.5\pm^{a}8.6$	$0.2\pm^{a}8.8$
Arginine (0.1 M)	$0.8\pm^{e}5.0$	$0.5\pm^{e}5.6$	$0.0\pm^{bc}7.0$	$0.4\pm^{f}5.2$	$0.8\pm^{e}6.4$	$1.5\pm^{d}7.1$
L-cysteine (0.1 M)	$0.2\pm^{de}5.6$	$0.5\pm^{e}5.6$	$0.8\pm^{d}5.2$	$0.5\pm^{f}5.4$	$0.0\pm^{g}5.0$	$0.5\pm^{f}5.6$
L-cysteine (0.05M)	$0.5\pm^{bcde}6.6$	$0.4\pm^{cde}6.6$	$0.6\pm^{c}6.2$	$0.5\pm^{e}6.4$	$0.2\pm^{f}5.8$	$0.4\pm^{ef}6.2$
Chickpea (2%)	$0.8\pm^{bcde}6.9$	$0.4\pm^{e}5.6$	$0.1\pm^{bc}7.1$	$0.6\pm^{b}8.0$	$0.4\pm^{abcd}7.8$	$0.6\pm^{d}7.1$
Egg albumin (2%)	$0.9\pm^{bcde}6.8$	$0.5\pm^{e}5.6$	$0.2\pm^{bc}7.2$	$0.7\pm^{b}8.1$	$0.5\pm^{de}7.0$	$0.7\pm^{d}7.0$
LSD =	1.18	0.85	0.81	0.55	0.57	0.64

Data are expressed as mean ± SD. Values followed by the same letter are not significantly different ($p<0.05$). The potato chip samples were rated on a 10-point scale (1,2: bad,3,4: poor,5,6: fair,7,8: good and 9,10: excellent)

Table 9. Effect of addition of fresh leaves into sunflower frying oil on sensorial attributes of potato chips

Soaking treatments at room temperature for 60 min	Taste	Texture	Appearance	Color	Odor	Over all acceptability
Control	$8.0^{a}\pm0.2$	$7.8^{a}\pm0.4$	$7.2^{ab}\pm0.4$	$7.6^{a}\pm0.5$	$7.4^{ab}\pm0.5$	$7.5^{a}\pm0.5$
Commercial product	$0.5\pm^{a}8.8$	$0.5\pm^{a}8.5$	$0.5\pm^{a}8.6$	$0.5\pm^{bc}8.3$	$0.4\pm^{ab}8.2$	$0.5\pm^{ab}8.5$
Guava leaves	$7.4^{a}\pm0.4$	$7.6^{a}\pm0.5$	$7.6^{a}\pm0.5$	$7.8^{a}\pm0.4$	$7.4^{ab}\pm0.5$	$7.4^{a}\pm0.5$
Rosemary leaves	$7.4^{a}\pm0.4$	$7.4^{a}\pm0.5$	$6.6^{b}\pm0.8$	$7.2^{a}\pm0.4$	$8.0^{ab}\pm1.0$	$7.6^{a}\pm1.0$
Oregano leaves	$7.4^{a}\pm0.4$	$7.8^{a}\pm0.4$	$7.8^{a}\pm0.4$	$7.6^{a}\pm0.5$	$8.0^{ab}\pm1.0$	$8.2^{a}\pm0.8$
Olive leaves	$6.2^{b}\pm0.8$	$6.6^{a}\pm0.5$	$7.6^{a}\pm0.5$	$7.8^{a}\pm0.4$	$7.0^{bc}\pm0.1$	$7.0^{a}\pm0.7$
Olive leaves juice	$6.2^{b}\pm0.5$	$6.8^{a}\pm1.0$	$7.0^{ab}\pm0.0$	$7.0^{a}\pm1.0$	$6.2^{c}\pm0.4$	$6.6^{a}\pm1.1$
Green tea leaves	$8.0^{a}\pm0.5$	$7.6^{a}\pm0.5$	$7.8^{a}\pm0.4$	$8.0^{a}\pm0.1$	$8.4^{a}\pm0.5$	$8.2^{a}\pm0.8$
LSD=	0.65	0.94	0.69	0.72	0.86	1.10

Data are expressed as mean ± SD. Values followed by the same letter are not significantly different ($p<0.05$). The potato chip samples were rated on a 10-point scale (1,2: bad,3,4: poor,5,6: fair,7,8: good and 9,10: excellent)

4. CONCLUSION

Results of current investigation showed that blanching of potato chips in hot water (65 and 85°C) or solutions containing 0.05M citric acid, 0.1 M $CaCl_2$ or NaCl, 0.1M glycine, L-glutamine or L-cysteine reduced significantly acrylamide formation of potato chips by 90.40, 88.88, 90.40, 73.58, 76.91, 85.20 and 76.96% respectively. Soaking of potato slices in water or different solutions caused significant decrease in the formation of acrylamide. Potato slices soaked in 0.01 and 0.05 M of Citric acid and 0.3 M of acetic acid had significantly the lowest values of acrylamide 50, 60 and 190 μg Kg^{-1} of fried potato. Soaking in phenolic acid solutions for 60 min resulted in significant reductions in the formation of acrylamide of potato chips. These reductions ranged from 31.81 to 98.03%. Soaking in crude, semi-purified asparaginase solutions, blanching in hot water plus immersing in the enzyme solutions and soaking in phenolic acid solutions caused significant reduction in the formation of acrylamide of potato chips. Addition of fresh leaves into frying oil significantly influenced acrylamide formation. Oregano, rosemary, bamboo, guava and olive leaves caused the greatest reductions. Potato slices blanched in distilled water at 65°C, NaCl, $MgCl_2$ and glutamine (0.1 M) had significantly the highest scores of taste, texture, appearance, color, odor and overall acceptability. However those blanched in distilled water at 85°C, citric acid (0.05), L-cysteine (0.1M) had significantly the lowest values.

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