Comparing the Responsibility of Some Pretreatment Types of Potato (*Solanum tuberosum*) to Reduce Acrylamide Formation

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Abstract

Acrylamide is a colorless, odorless, crystalline compound that is considered to be a genetic and reproductive toxin, and long-term consumption may cause damage in the nervous system. This research has studied the affect of pretreatment of potato slices with immersion solutions including (2% *citric acid*, 2% baker *yeast*, distal water, 100 units *glucose oxidase* and 2% *acetic acid*) to reduce *AA* content of fried potato slices. The results have showed some differences among the types of potato in their response to immersion solutions. Except *acetic acid*, Iraqi type had the minimum response ranging from 5.66 to 54.06% compared with Syrian type from 70.10 to 93.89% and Iranian from 7.97 to 69.95%. *Citric acid* was the effective immersion solution in decreasing *AA* which ranged from 54.06 % for Iraqi potato to 93.89% for Syrian type, while the *AA* reducing range of *yeast* solution was from 13.08 to 91.79% and of *glucose oxidase* from 5.66 to 82.97%. Distilled water increased *AA* formation in Iraqi type up to 44.91%, but decreased *AA* formation about 70.10% and 33.50% of Syrian and Iranian types respectively compared with control. This phenomenon might be attributed to the rate of equilibrium between exudation of soluble components and reconfiguration of them. *Acetic acid* oppositely behaved compared with most other immersion solutions since it highly increased the *AA* s that may be due to the longtime of immersion compared to most researches which used some minutes of steeping. However, this finding may be a key for more detailed researches

Keywords: potato, Acrylamide formation, Immersion solutions, pretreatment,

1- Introduction

Acrylamide (AA) is an industrial material which is used in many purposes such as adhesives, fibers, drugs, paper or textiles, tobacco consumption and other practices (Smith and Oehme, 1991). This material may have reached in contact with workers, causing a hazard to their heath (Tilson, 1981). Many agencies such as the International Agency for Research on Cancer, the U.S. National Toxicology Program's (NTP) and the U.S. Environmental Protection Agency (EPA) have reported acrylamide as "probably carcinogenic to humans (Group 2A)" (Lyon, 1994), "reasonably anticipated to be a human carcinogen" (Program, 2011). And "likely to be carcinogenic that have warned the unintentional contaminant *AA* in certain foods might be of public health concern since it has been shown to cause cancer in animals (Additives, 2005).

Acrylamide ($CH_2=CH–CONH_2$) is a colorless, odorless, crystalline compound with low molecular weight 71 g mol^{-1} (Friedman, 2003). Long-term *AA* consumption may cause damage in the nervous system. Acrylamide is also considered to be a genetic and reproductive toxin (Tilson, 1981).

It is also Intended to produce foods devoid of bacteria and other contaminants including harmful substances, although toxins produced during cooking are of great concern as they may have an impact on human health, and proper nutrition involves intake of carbohydrates, fats, proteins, fiber, vitamins and minerals at an appropriate level for an effective balance to body, however the consumption of fast food in this generation has led to various food behavioral changes with improper diet on daily basis (Dearfield et al., 1995). In addition, people are prone to non-communicable diseases, including obesity, diabetes and cancer (Baskar and Aiswarya, 2018). The extreme changes in processing food and methods of agriculture from organic to scientific methods have brought variations in the nutritional values of foods (Ouhtit et al., 2014).

Acrylamide is also used as a cement binder in the synthesis of polymers and gels. on the other hand, polyacrylamide polymers and copolymers are used in the paper and textile industries, as flocculants in the treatment of wastewater, as soil conditioners, in metal processing, and cosmetics (Friedman, 2003). After 2002, the concerns of *AA* exposure increased when it was found that some foodstuffs are produced at high temperatures above 120°C and low humidity (Biedermann et al., 2002).
Acrylamide is developed in a variety of high carbon categories, including fries, potato chips, baked or fried carbohydrates, bread and coffee (Friedman and Levin, 2008). The food items may undergo numerous thermal treatments during processing activities such as roasting, sprinkling, baking and pasteurization, with a major objective of stability and a suitable sensory profile (De Bock et al., 2013). These thermals can lead to some reactions including the Maillard reaction, lipid oxidation and caramelization in the food that can cause formation of various compounds that have sensible and unwanted effects (antimicrobials, antioxidant and anti-allergenic), (cytotoxicity, carcinogenicity and mutagenic reactions) (Rawel et al., 2006).
It was found that *citric acid, Acetic acid, yeast* solutions decrease formation of acrylamide in potato slices, while tap water had not any effect on it (Abou-Zaid, 2015, Maan et al., 2020, Albedwawi et al., 2021)
The aim of this research is to study the effect of solution immersion pretreatments of potato slices on activation or inhibition of acrylamide formation in fried potato slices.

2. Materials and Methods

Sampling
Three replicates of each different types of potatoes including Iraq, Iran and Syria were collected. The potato samples peeled and sliced as finger chips shape in 7 ml thickness and 5 cm long, then they were dried and smashed by mixture labeled and stored in plastic bag in laboratory for more analyses.

Potato slices preparation
All the steps of potato slices preparation were achieved according to with some modifications (Onishi et al., 2015, Doymaz, 2012). The potatoes peeled and sliced like French fries' shapes in 7 mm thickness with dimensions about 5*5 cm long and cross.

Potato slices steeping
Adequate amount of potato slice samples was immersed in the following solutions, 1% *Citric acid*, 1% *acetic acid*, 1% bakery *yeast* (Saccharomyces cerevisiae), 1 unit's ml^{-1} *Glucose oxidase*, and distill water for 1 h at room temperature (22±2°C). After steeping time, the potato slices were taking out from the solutions and they were put into distilled water for 5 minutes to discard the remains solutions. And then, the remained distill water was removed on the potato slices by filter paper so as to be prepared for frying.

Potato slices frying
Potato slices were fried in cooking oil by immersing the slices to about 2 mints at 220°C, the time and temperature were fixed during of frying, according to the procedure

reported by (Zhang et al., 2021). The remains of oil were removed by filter paper then dried very well at room temperature until complete drying. The dried slices were milled by electrical grinder (Gosonic GSB-708) and put in plastic package to store in freezer at -10 ± 2°C until the analysis by GC-Ms.

Sample analysis by (GC-MS)
According to (Zhu et al., 2008) method, samples were ground and homogenized in CNS-8161 multi-function food processor (Shunde Caina Electric Appliance Co. Ltd., Guangdong province, China) prior to sampling. For every sample, four aliquots (10 g of each sample) were weighted into 100 ml volumetric flasks, different concentrations of acrylamide (0, 0.5, 1.0, 2.0 mL) working solution (10 g/mL) was added to each flask respectively. Then distilled water was added to complete the volume. After mixing in a mixer for 30 min, the mixture was filtered. About 25 ml of the filtrate was transferred into the separator funnel and 25 mL n-hexane was added. After thorough hand mixing for 2 minutes, the aqueous phase was transferred into a 100 mL Erlenmeyer flask. The calcined potassium bromide (7.5 g) was dissolved into the separator aqueous phase with stirring, and the pH of the solution was adjusted between 1-3 by the addition of a few drops 0.4 mL of hydro bromic acid. Then 8 mL of saturated bromine-water solution was added to the flask whilst stirring. The flask was covered with aluminum foil and incubated in an ice bath for 1h, this is mainly to complete the reaction in the dark condition. After completing the reaction, the excess bromine was decomposed by adding a few drops of 0.4 mL of 1 M sodium thiosulfate solution until the yellow color disappeared. Then the mixture was extracted with 25 mL of ethyl acetate by shaking for 1 min. After phase separation, the organic phase was taken and dried over sodium sulfate. The solution was finally filtered through a 0.45 µm filter into an autosampler vial for GC analysis.

3-Results and Discussion

Effect of pretreatment of potato slices on *Acrylamide* formation
Table (1) shows results of the effect pretreatment of potato slices by using different types of immersion solutions to reduce *AA* content. The results showed there were a significant response of potato types to the different type of immersion solutions on the *AA* formation under ($p \leq 0.05$). However, the *AA* content differed among the potato samples compared to control which are ranged from 688 ppb for Iraqi potato to 2017 ppb for Iranian potato.

Comparing between the three selected types of potato samples in their content of protein which were 16.05, 13.37 and 16.68% for Iraqi, Iranian and Syrian potatoes respectively. The effect of their content on *AA* formation is not clear because the higher content of protein (Iraqi potato) produced a little amount of *AA* 688 ppb compared with the lower content of protein (Iranian potato) that produced the higher amount of *AA* 2107 ppb. On the other hand, this limitation of *AA* formation by protein content was in agreement with (Powers et al., 2017) And also, this range of *AA* was agreed with the results of Iraqi researcher (Al-Janabi, 2017) who found that the Iraqi potato chips contained between 339–1024 ppb of *AA*. While, (Yaseen et al., 2020) found that the fried potato chips at 180°C contained the higher amount of *AA* which was 2416 ppb compared to the fried at 100°C contained about 136 ppb for the same duration time of 4 min. Although, the percentage of protein and carbohydrate are the main factors that effect on the increasing of *AA* formation (Chuda et al., 2003). But, in the current studied, Iraqi potato which had the higher percentage of protein and carbohydrate did not increase the formation of *AA*. This may be attributed to the fact that the availability of free amino group and reducing sugar is more important. Long-term potato storage especially in low temperature and the relative humidity encourage enzymes activity such as proteases and *amylases* which will increase the availability of *amino acids* and reducing sugars (Amjad et al., 2019).

The results also, showed that the effect of immersion solution differed on potato. For example, distilled water caused the increase of *AA* in Iraqi potato sample whereas it was decreased *AA* content in Iranian and Syrian samples which may attribute to the balance between enzyme activity, especially amylase that can hydrolyze starch to reducing sugar and osmosis from potato slices to water which may affect positively or negatively on Maillard reaction. (Vlachojannis et al., 2010) reported that potato has a considerable of proteases activity which may increases Maillard reaction then assists for *AA* formation. (De Wilde et al., 2005) found that the formation of reducing sugar during storage, especially at low temperature, is a very important factor of increasing of *AA* formation. It is known that the storage at low temperature less than 8 to 5°C induces the activity of *β -amylase* which will hydrolyze starch to produce reducing sugar *Maltose* (Nielsen et al., 1997). However, except of *acetic acid* most of immersion solutions caused decreasing in *AA* formation in potato samples. Although, it was not found any article research related between the activity of potatoes amylases and the *AA* formation.

The results showed that immersion the potato strips in 2% *citric acid* solution had a significant effect on reducing *AA* formation during frying and it differed between potato samples. The percentage of reducing *AA* was highest in Syrian (93.89%) followed by Iranian (69.95%) and then Iraqi type (54.06%). The effect of immersion of potato slices in *citric acid* solution was widely studied (Torang and Alemzadeh, 2016). This result is slightly in agreement with (Yuan et al., 2014) who found that immersion potato slices in 1% *citric acid* caused reducing about 58% of *AA* formation.

However, immersion of the potato slices in 2% of *yeast* solution also causes reduction in the *AA* formation in fried potato slices. This is may be due to consumption of reducing sugar by the *yeast*. The results indicated that the effect of yeast solution was not similar in all potato samples. Syrian potato slices had the highest response to *yeast* solution which caused reducing of *AA* formation up to 91.79% compared to Iraqi and Iranian samples which their content of *AA* decreased by 13.08 and 7.97% respectively. These results slightly similar to (Baardseth et al., 2006) who found the fermentation of potato slices by *lactic acid* bacteria caused decreasing in the *AA* formation in fried potato slices in French fries by 48%, and by 71%.

The Immersion potato slices in distilled water also significantly differed between the potato samples. The *AA* formation of Iraqi slices increased in fried potato slices up to 44.91%. According to (De Wilde et al., 2005) who found that the higher activity of potato's amylases or cold storage increases reducing sugars which will increase acrylamide formation, that may be a cause of the acrylamide increasing in the Iraqi potato slices compared with Syrian and Iranian potato which their formation of *AA* decreased by 70.10 and 33.50% respectively. On the other hand, the osmosis pressure in all distilled water immersed potato samples must be similar because of same conditions of immersing but the accumulation of reducing sugar with fixed speed of osmosis was the effective factor caused recording different results between the potato samples (Nielsen et al., 1997). Therefore, it can be said that immersion in distilled water may not fit for the potato that stored at low temperature (less than 8°C) or at any conditions of storage (such as humidity and temperature) which permit to sprout of potato tubers (Zhang et al., 2014).

The results of immersion of the potato slices in *glucose oxidase* solution (100 U/mg protein) showed that this enzyme was effective in decreasing *AA* formation in the all samples of fried potato slices. However, not all samples had the same responsibility to the *glucose oxidase* activity. Iraqi potato showed the lower response which decreased the *AA* formation by 5.66% compared to Syrian and Iranian potatoes which were decreased by 82.97 and 36.68% respectively. These results were highlighted the findings of (Low et al., 1989) who noticed that treatment of potato paste with 0.01% *glucose oxidase* reduced its content of reducing sugar by 15% in raw potato and 25-35% in boiled potato. At the same time, these treatments decreased the browning color due to Maillard reaction. They also concluded that potato may contain some enzyme inhibitors which decrease the activity of *glucose oxidase* in raw potato (Vámos-Vigyázó and Haard, 1981). (SØE, 2004) found that hexoses oxidases, especially glucose oxidase have an effective effect on decreasing *AA* formation.

The final treatment of immersion solution is *acetic acid* which gave a reverse result compared with other solutions. The results showed that the immersion of potatoes slices increased of *AA* formation in all studied samples of fried potatoes slices. The results also showed significant differences between the studied samples of potatoes in their responsibility to *acetic acid* treatment. Iraqi potato had the highest response to *acetic acid* and its content of AA increased by 275.87% compared with Syrian and Iranian potatoes which were increased by 34 % and 26.24% respectively. Although, there were high differences between their percentage of the *AA* formation in fried potatoes samples in acetic treatment approximated to other resulted found by researchers which ranged between 2586 ppb for Iraqi to 2660 ppb for Iranian potatoes. While, these results are not in agreement with results reported by (Kita et al., 2004, Liu et al., 2020). They found that *acetic acid* work like *citric acid* in decreasing *AA* formation of fried potato slices. But, (Kato et al., 1969) found that at 2% *acetic acid* solution or neutral pH the range of pH between 3 to7). However, this phenomenon requires more research to find the mechanism of the *acetic acid* effect on *acrylamide* content of potato slices. The presence of significant differences between potato samples may emphasize that the chemical composition of the different types of potatoes is the main factor that can effect on *AA* percentage in fried potato slices when the other factors are fixed.

Table (1): Iraq and rate of *acrylamide*(ppb)

Treatment	**Iraq and rate of acrylamide(ppb)**	
	Mean	**SD**
Control	689.00	5.568
Citric Acid	310.00	6.557
Yeast	599.33	2.309
Distill Water	998.00	8.544
Glucose Enzyme	657.00	6.928
Acetic Acid	2590.00	4.583
LSD (P≤0.05)	**10.80529**	

Table No. (1) shows the value of *AA* in different Iraqi potatoes so that the value of *AA* is in the control phase and after conducting the experiment three times with an average value of (698±5.568) and the best result in this study was using *Citric Acid* so that the value of *AA* is reduced to the lowest value rate (310±6.556), which had the least effect in the increase the value of carcinogenic *AA*, As well as *Yeast* has a positive effect which was (599.33±2.309) but the value is greater than the use of *citric acid*. In the case of using *Glucose Enzyme*, its average value was (657±6.928) and it was higher than the use of yeast and Citric acid, but it is less than the control. In the use of distilled Water, the average value of *AA* significantly increased, which had a negative effect on the Iraqi potato product, which was (998±8.544). Using *Acetic Acid*, we see that the value of *acrylamide* increased unnaturally and dangerously, which has a negative effect on the increase in the rate of cancer, and its average value was (2590) with Std. Deviation (4.583).

Table (2): *Acrylamide* content of Iraqi potato samples.

Treatment	Replication			Mean	Difference with Control	Compare with LSD0.05	Conclusion
	1	2	3				
Control	688	695	684	689	-----------	----------	
Citric Acid	316	303	311	310	379	>	Significant
Yeast	598	598	602	599.33	89.67	>	Significant
Distill Water	997	990	1007	998	-309	<	Not-Sig
Glucose Enzyme	649	611	611	657	32	>	Significant
Acetic Acid	2589	2595	2586	2590	-1901	<	Not-Sig

The treatments (*Citric Acid*, *Yeast*, and *Glucose Enzyme*) in Iraqi Potato are showed Significantly different Yield Compared with Control at α = 0.05, While (Distill Water, *Acetic Acid*) did not show any significant different compared to control.

Table (3): Syrian and rate of *acrylamide* (ppb)

Treatment	Syrian and rate of acrylamide(ppb)	
	Mean	**SD**
Control	1970.333	27.024
Citric Acid	119.000	0.000
Yeast	178.333	17.559
Distill Water	583.000	0.000
Glucose Enzyme	332.666	17.009
Acetic Acid	2613.333	13.503
LSD (P≤0.05)	**28.19**	

Table (3) shows the value of *AA* with different values in Syrian potatoes control and the experiment, an average value of (1970.33±27.024) and it is higher compared to some treatments *Citric Acid, Yeast*, Distill Water, Glucose Enzyme. And the best result recorded with using *Citric Acid*, but value of *acrylamide* decreased to the lowest level of (119.00±.000), which had the minimum effect in increasing the value of the carcinogenic *AA*. as well as *Yeast* has a positive effect with an average of (178.33±17.559 ppb), but this value is greater than the value recorded with *citric acid* in reducing AA. In the case of using Glucose Enzyme the average value was (332.67±17.009) which was higher than the use of *yeast* and *citric* acid, and less than the control. In using the distilled water, the average value of *AA* increased, which was negatively affected the Syrian potato. The average value was (583±0.000), which was less than the control. Using *Acetic Acid*, that the result indicated that the value of *AA* increased irregularly and dangerously, which negatively affected the increase in the incidence of cancer, and its average value was (2613.0615±13.503).

Table (4): *Acrylamide* in Syrian potato sample.

Treatment	Replication			Mean	Difference with Control	Compare with $LSD_{0.05}$	Conclusion
	1	2	3				
Control	1950	1960	2001	1970.3	-------------	-------------	-------------
Citric Acid	119	119	119	119	1851.33	>	Significant
Yeast	160	180	195	178.33	1791.97	>	Significant
Distill Water	583	583	583	583	1387.33	>	Significant
Glucose Enzyme	332	350	316	332.66	1637.3	>	Significant
Acetic Acid	2613	2600	2627	2613.3	-643	<	Not-Sig

The treatments (*Citric Acid*, *Yeast*, *Glucose Enzyme* and Distill Water) in Syrian Potato are showed Significant difference yield Compared to Control at p ≤ 0.05, while *Acetic Acid* did not show any significant difference compared to control.

Table (5): Iran and rate of *acrylamide* (ppb)

Treatment	Iran and rate of acrylamide(ppb)	
	Mean	St. Deviation
Control	2107.000	0.000
Citric Acid	636.666	4.725
Yeast	1939.666	10.016
Distill Water	1407.666	11.547
Glucose Enzyme	1328.333	6.658
Acetic Acid	2657.333	12.220
LSD (P≤0.05)	**15.40631**	

Table (5) shows the value of *AA* in Iranian potato samples. The average *AA* content was (2107±0.000), this value is much higher compared to the Iraqi and Syrian potatoes. Using *Citric Acid* had the highest result as shown in the two previous experiments and then the value of *AA* decreases to the lowest level of (636.67±4.725), which had the least effect in the increase the value of carcinogenic *AA*. *Yeast* has a negative effect with an average of (1939.67±10.016), in the case of using *Glucose Enzyme*, the average value was (1328.33±6.658) which is higher than the use of *citric acid* but lower than the control and *yeast*. In the case of using of distilled water, the average value of *AA* had a negative effect on the Iranian potato product (1407.67±11.547). The use of *Acetic Acid* increased the value of Abnormally and dangerously, which has a negative effect in the incidence of cancer increase, the average value of which was (2657.33±12.220).

Table 6: *Acrylamide* in Iranian potato samples.

Treatment	Replication			Mean	Difference with Control	Compare with LSD0.05	Conclusion
	1	2	3				
Control	2107	2107	2107	2107	-------------	-------------	
Citric Acid	633	635	642	636.66	1470.34	>	Significant
Yeast	1939	1930	1950	1939.6	167.4	>	Significant
Distill Water	1401	1421	1401	1407.6	699.4	>	Significant
Glucose Enzyme	1334	1321	1330	1328.3	778.7	>	Significant
Acetic Acid	2660	2644	2668	2657.3	-550.3	<	Not-Sig

The treatments *Citric Acid, Yeast, Glucose Enzyme* and distilled water in Iranian Potato are showed significantly different in yield compared to control at (p ≤0.05). While, *Acetic Acid* did not show any significant differences compared to control.

Table (7): Pearson Correlation for *acrylamide* in (Iraq, Syrian, Iran) potato

Pearson Correlation	Iraq and rate of acrylamide	Syrian and rate of acrylamide	Iran and rate of acrylamide
Iraq and rate of acrylamide	1	0.787**	0.744**
Sig. (2-tailed)		0.00	0.00
Syrian and rate of acrylamide	0.787**	1	0.815**
Sig. (2-tailed)	0.00		0.00
Iran and rate of acrylamide	0.744**	0.815**	1
Sig. (2-tailed)	0.00	0.00	

** Correlation is significant at the 0.05 level (2-tailed).

Table (7) shows that there is a positive strong significant correlation between all the studied potato samples. The correlation was Iraq and rate of was (r^2= 0.744, p≤0.000) between Iraqi and Syrian *AA* and between Iraqi and Iranian, but the correlation was r^2= 0.0815, p≤0.000) between Syrian and Iranian *AA* and rate.

Conclusion

The results of this research show that the temperature degree and the period of thermal treatment were the effective factors in increasing the *AA* formation, but the type of flour and the processing methods may interrupt with this factor. The type of potato had significant effect on *AA* formation in fried potato slices, which may due to effect of agricultural operations especially the type of fertilizers, chemical or organic. The effect of steeping in distal water to reduce *AA* formation depended on the type of potato may due to the balance between the dialysis of soluble compounds and regeneration of it as a product of enzyme activity. Generally, most of immersion solutions had an effective role in decreasing the *AA* formation. *Acetic acid* solution behaved opposite of the current since it significantly works to increase *AA* formation may due to the long period of immersion

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