



EFFECT OF ADDITION OF VARIOUS PROPORTIONS OF ROSEMARY POWDER, CITRIC ACID AND TABLE SALT IN REDUCING THE RATIOS OF ACRYLAMIDE IN POTATO FRIES

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Abstract

The study was conducted to demonstrate the effect of adding different percentages of rosemary powder, citric acid and food salt individually or combined with different ratios in reducing the percentage of acrylamide in foods rich in carbohydrate when exposed to high temperatures during manufacturing. Acrylamide is a toxic substance believed to cause various neurological and genetic health problems. Potatoes were treated before frying by adding different percentages of rosemary powder, citric acid, food salt and a combination of the three additives. The results of the treatments showed different percentages of acrylamide in (C1) treatment potato slices with rosemary (0.1, 0.2, and 0.3%) which amounted to (31, 50 and 7 mg/kg) respectively. Potato slices treated with citric acid (C2) (0.5, 1.5, 2 %) amounted to (43.66, 35.28 and 38.55 mg / kg) respectively while the ratio of acrylamide in potato slices treated with table salt (C3) with proportions of (1.5, 2, 2.5%) amounted to (60, 44 and 33 mg / kg). the ratio of acrylamide in the synergistic action of the (C4) treatment consisting of potato slices + rosemary + citric acid + table salt with addition rates of (0.2, 1.5, 2) and (0.3 2, 2.5) % amounted to 2 mg / kg for both treatments compared to the control treatment which amounted to 186 mg / kg. A sensory evaluation for the fried potato treated with additives was carried out where the (C4) treatment showed a clear superiority over the rest of the treatments in the taste and smell characteristics. This was equal to the control treatment (C) and was associated with it in terms of texture. However, it was of an unacceptable color common with C2 treatment due to low percentage of acrylamide, which causes the golden color of fries and to avoid their health effects, food colors can be added to acquire the desired color.

Key words : Acrylamide, potato fries, Rosemary powder, citric acid, table salt.

Introduction

Acrylamide is a white crystalline chemical compound whose molecular weight is 71.08 with a melting point of 84.5, which has a polarity that makes its degradation in water high. Acrylamide chemical formula is $\text{CH}_2 = \text{CH}-\text{CO}-\text{NH}_2$, naturally formed in a large group of foods, Toasted bread, breakfast cereals, potato chips, biscuits, chocolate, coffee, etc. (Krishnakumar and Visvanathan, 2014; Eriksson and Karlsson, 2005). Acrylamide is formed during Millard reaction from the interaction of free amino acids, especially asparagine with reduced sugar such as glucose or fructose at high temperatures up to more than 120°C in food products baked, roasted or fried or through

the interaction between ammonia and acrolein in high fat foods (Borda and Alexe, 2011). The discovery of high-temperature starchy foods by the (Swedish National Food Administration) and (Stockholm University) in 2002 a major shock for many researchers, drawing researchers' attention to the study of levels of acrylamide in different types of foods. Studies of several factors affecting the formation of acrylamide, some of them related to the composition of food such as types of amino acids and sugars, the value of pH and water activity, and other factors related to manufacturing processes such as temperature, time, humidity and pressure (Lingnert *et al.*, 2002).

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Acrylamide was first synthesized in Germany in 1893 by Mario, but it did not receive much attention until the middle of the last century, after the production of polyacrylamide in various applications in wastewater treatment, drinking water purification, crude oil refining, paper processing, soil processing and packaging industry products and cosmetics. It was then observed that there was a risk of exposure to a monoacrylamide molecule not associated with polyacrylamide, which could be absorbed by the skin, by breathing or enter into the mouth by contaminated hands which may cause a serious risk to the workers in factories and laboratories that use them, especially when exposed for long periods (Zyzak *et al.*, 2003). Furthermore, in a study conducted on the presence of acrylamide in cigarette at maximum concentrations ranging from 1-2 micrograms per cigarette, before being detected in food at a long time, Showed that smokers who did not work on preparing this substance showed higher levels of compounds that resulted from the association of acrylamide with hemoglobin than twice that of those who worked in the preparation of non-smoking acrylamide gel, however, it did not take such concern as in food (Husamo, 2010). The Swedish National Food Administration and the University of Stockholm have reported high levels of acrylamides of up to 1000 µg / kg in high-temperature starchy foods, which were directly supported by the US Food and Drug Administration (FDA), which showed that chips and fingers of fried potatoes contain high levels ranging from 17 to 2762 µg/kg. The International Agency for Research on Cancer (IRAC) has identified acrylamide as a potential compound in the development of cancer in humans. This is based on the biological experiments that showed the carcinogenic effects of many tissues in rats and mice, especially kidney and lung cancer. It is classified in the substances that affect neurotransmitter, especially peripheral nerves, has a negative effect on the growth and development of embryos, and has mutagenic toxicity on genes (Friedman, 2005; Shipp *et al.*, 2006; Jannek *et al.*, 2008). WHO provided guidance on water quality and its content of acrylamide resulting from the use of polyacrylamide in the purification of drinking water with a concentration of only 0.5 g/l, while the European Union (EU) determined polyacrylamide levels of 0.1 g/L of water. The FAO/WHO Expert Committee on Food Additives (JECFA) has identified the oral dose of acrylamide leading to acute toxicity above 100 µg/kg of body weight, daily intake of acrylamide should not exceed 0.3-0.8 µg/kg of body weight (Muccil *et al.*, 2003; FAO/WHO, 2011). Therefore, the study aimed to conduct some treatments using additives that

may affect the reduction of acrylamide formed during the frying process.

Materials and Methods

Sample of the study

Potato tubers were selected from the local market of Baghdad governorate based on their sizes and equal weights and then they were cleaned thoroughly to remove soil and dirt using tap water. The tubers were cut using a sharp knife into thin slices of equal size and immersed with 0.25% Sodium metabisulphite for five minutes to prevent enzymatic bleaching. The study samples were divided into treatments as indicated in table 1. Rosemary powder was added to C1 treatment, which was obtained from local markets and pre-licensed in the Faculty of Science for Girls, University of Baghdad after grinding using a laboratory mill to obtain homogeneous powder. Then, placed in a clean, sealed glass container and coated with aluminum foil to avoid being affected with light until use, in addition to the treatment of potato slices with citric acid, salt and food salt in the proportions indicated (table 1). In addition, the treatment of control, samples of non-treated potato chips were implemented. Treatments mixtures were prepared manually and separately after being immersed in the additives (table 1) using sterile medical gloves for homogeneous treatment. Then, potato slices were deep-fried including additive in sunflower oil using deep frying pan to fully cover the slices with frying oil at 170°C for 5 minutes constantly stirring to ensure uniform heat distribution on all slices. Treatments were kept in polythene bags and sealed well after they were placed on plates with drying paper to remove excess oil and then freeze them at -20°C and writing details on them until the extraction process is done with two replicates for each treatment (Granda and Moreira, 2005).

Preparation of standard solutions

The method used as mentioned by Wang *et al.* (2008), preparing standard solutions for the study sample using standard 1 mg/ml acrylamide from Sigma Deisenhofer, Germany, while the internal standard solution (d3-acrylamide) was prepared 10 µg/ml, which is supplied by the same company with a solution of methanol-water (20-80 v/v) using free ions water. Mix well to obtain a homogeneous mixture and leave for three minutes, then filtered through filter syringe 0.45 mm to get rid of remaining residue.

Extract the acrylamide from the samples

The method in Gökmen *et al.* (2005) was implemented to obtain the extraction through the homogenizing samples in a ceramic mortar weighing 2g.

Table 1 : Added percentages of rosemary powder, citric acid, and salt to potato slices.

Treatment code	Treatment type and additives percentage %
C0	Non-treated potato slices
C1	Potato slices + rosemary powder 0.1, 0.2, 0.3
C2	Potato slices + Citric Acid 0.5, 1.5, 2
C3	Potato slices + table salt 1.5, 2, 2.5
C4	Potato slices + rosemary powder + citric acid + table salt 0.2, 1.5, 2
	Potato slices + rosemary powder + citric acid + salt 0.3, 2, 2.5

The 125 microliters of d3-acrylamide were added as an internal standard in glass tubes, centrifuged and left for 10 minutes to homogenize and then 18 ml of standard water HPLC grade was added then 1 mL of each solvent was added sequentially (Potassium hexacyanoferrate 150 g/l) and carrez 2 (zinc sulfate 300g/l). The tubes were placed in an ultrasonic bath, blended for 15 minutes and then were put into the centrifuge for 10 minutes at 15,000 rpm. The fat was removed by adding 15 mL of n-hexane per tube and left for 1 min then, upper layer of the tubes was removed while retaining the bottom layer to complete the process of estimating acrylamide using HPLC technology.

Standard conditions for HPLC

High performance liquid chromatography (HPLC) (RF-20A) supplied by Shimadzu, Japan was used for estimating the level of acrylamide in the examined samples according to the standard conditions below and according to Gökmen *et al.* (2005):

1. **Colum** : C18(250 × 4.6 mm), Mobile phase: aqueous phosphate buffer.
2. **Flow rate** : 1.1 ml/min, Detector: UV 202 nm, Injection loop: 200 µL,
3. **Temperature**: 25°C, Pressure: 38 atm

The concentration of acrylamide in the samples was determined according to the following equation :

Concentration of sample (µg/kg) = The area of the sample / area of the standard × standard concentration × Dilution factor.

Sensory evaluation

Specialized evaluators, which included odour, carried out the sensory evaluation of the fried potato coefficients referred to in table 1 and taste characteristics, texture and colour according to the sensory assessment score (table 2).

Table 2 : Potato chips quality assessment form.

Sample	Taste and odour*	Texture **	Color ***

* Grade of odour and taste assessment 01234,

** Grade of texture assessment,

*** Colour acceptable / unacceptable.

Statistical analysis

The Statistical Analysis System- SAS (2012) program (SAS 2012) was used to effect of difference factors in study parameters. Least significant difference-LSD test was used for significant compare between means in this study.

Results and Discussion

Table 3 shows the ratio of acrylamide in the potato slices treated with the rosemary powder C1 calculated on the basis of mg/kg and µg/kg at 50, 31 and 7 µg/kg, respectively, compared to the control treatment of 186 µg/kg. The potato varieties play a large role in the formation of this toxic substance during the frying process and even within the same class. For example, in low-sugar potatoes, the formation of acrylamide is less than the potato with high sugar, and the storage has the same role. On the other hand, the risk of the formation of the acrylamide increases if the potato were stored gradually in low temperature less than 8°C. Returning to table 2, there is a significant shorthand of rosemary powder in the decreasing of acrylamide, which can be produced in potato chips. However, the act of oxidation by the rosemary through the volatile oils within its internal components as excellent natural antioxidants, reduced concentrations of this substance. The following compounds were identified by GC-MS by Wang *et al.* (2008), which are 1,8-cineole (27.23%), α-pinene (19.43%), camphor (14.26%), camphene (11.52%) and β-pinene (6.71%). The other researcher was pointed out that the rosemary extract is more effective than the industrial antioxidants BHA/BHT in preventing the increase in the value of the thiobarbituric acid reactive substance (TBARS) or the loss of red color in frozen sausages (Sebraneka *et al.*, 2005).

Results of the study were compared with Pedreschi (2004), 35.28 and 38.55 µg/kg respectively (table 4). Their studies showed that heating the immersed potato slices in concentrations of 10-20 g/L of citric acid gave a significant indication of acrylamide reduction by 70% compared to control sample. Another study (Jung *et al.*, 2003) recorded a decline in this percentage by 73.1% - 79.7% after the potato slices were also covered with the same concentrations of citric acid (Pedreschi *et al.*, 2004).

Table 3 : Effect of the addition of different percentages of the rosemary powder C1 in the ratio of acrylamide to potato fries.

Percentage of added rosemary (%)	Ratio of acrylamide ppm(mg/kg)	Ratio of acrylamide (mg/kg) ppm
0.0	0.186	186
0.1	0.050	50
0.2	0.031	31
0.3	0.007	7

Table 4 : Effect of adding different percentages of citric acid C2 in the ratio of acrylamide in potato fries.

Addition of citric acid (%)	Ratio of acrylamide(ppm) (mg/kg)	Ratio of acrylamide (ppm) (mg/kg)
0.0	0.186	186
0.5	0.436	43.66
1.5	0.03528	35.28
2	0.0385	38.55

and coated at 190°C due to the effect of citric acid in reducing the pH of both dipping water and potato slices. Significantly reduced the amount of acrylamide and prevented its formation during frying (Pedreschi *et al.*, 2004). In addition, the best pH for acrylamide formation during frying is between 7-7.5 although some studies have indicated that the ideal pH for this substance is 8 (Rydberg *et al.*, 2003).

The results in table 5, which include the treatment of table salt C3 by its role in reducing the ratio of acrylamide to less than the treatments (C1, C2) preceded it, corresponded to the results of our study which were 60, 44 and 33 µg/kg. In addition, these results also corresponded to the result of the study conducted by Gökmen and Senyuva (2007), where they confirmed that prolonging the immersion period of potato slices in table salt (sodium chloride) and calcium chloride will reduce the amount of formed acrylamide compared to the same slides immersed in water only (Pedreschi and Zuniga, 2009). Studies have included the addition of equal amounts of amino acid asparagine with fructose sugar using some cations such as Ca_2^+ and found that the formation of acrylamide was not complete, while monocationic ions such as Na^+ will reduce half the amount of formed acrylamide (Kolek *et al.*, 2006). This is due to a significant reduction in water efficiency on the surface layer of the potato slices, which inhibits the mechanical act of acrylamide forming during frying. The results were consistent with their study of equal amounts of asparagine, glucose and concentrations of sodium chloride

Table 5 : Effect of adding different proportions of table salt C3 in the ratio of acrylamide of potato fries.

Addition of table salt (%)	Ratio of acrylamide (ppm) (mg/kg)	Ratio of acrylamide (ppm) (mg/kg)
0.0	0.186	186
1.5	0.06	60
2	0.044	44
2.5	0.033	33

Table 6 : Effect of adding different proportions of potato slices + rosemary + citric acid + C4 table salt in the ratio of acrylamide in fries.

Adding ratios of rosemary + citric acid + salt (%)	Ratio of acrylamide(ppm) (mg/kg)	Ratio of acrylamide(ppm) (mg/kg)
0.0	0.186	186
0.2, 1.5, 2	0.02	2
0.3, 2, 2.5	0.02	2

from 0-10 on a percentage basis and then frying the potato slices at 171°C for 10 minutes. The percentage of the removed acrylamide was 40%, but the actual percentage of the removal is at a concentration of 0-1 on a percentage basis of the decrease is not linear, as well as the effective effect of salt in preventing the polymerization of acrylamide and thus reduce its composition. While, Abou-Zaid (2015) pointed out that table salt spread over the surfaces of potato chips would significantly affect the amount of oil absorbed and thus, reducing the temperature of the slices and finally, reducing the proportion of acrylamide formed.

The synergistic action of treatment C4 (table 6) has the same effect in preventing the formation of acrylamide due to the effect of citric acid, which plays a role in lowering pH, as well as the presence of monatomic ion of sodium chloride, with the ground rosemary and its effects. Acrylamide was 2 mg/kg. These results were consistent with what is reported in Stavros and Vassilis (2003) regarding the mutual action of 0.5% table salt and 3% citric acid with tomato juice in preventing acrylamide forming during frying.

Previous studies have pointed to the breakdown of food by its concentration of acrylamide, for example, foods rich in carbohydrates, such as potatoes, (high) ranging between 150-4000 ppm (0.015 - 4.0 ppm) and medium levels 1,200 ppm in Potato slices (1.20 ppm) while acrylamide levels in foods rich in protein (moderate) ranged between 5-50 ppm (0.005 - 0.050 ppm) (El-Shawaf *et al.*, 2014).

Table 7 : Sensory evaluation grades for fried potato treatments with the addition of materials in the indicated proportions.

Sample		Odour and taste **	Texture**	Colour***
Non-treated potato slices (C)		4	1	Unacceptable
C1 Potato slices + rosemary	0.1%	3	2	acceptable
	0.2%	2	2	acceptable
	0.3%	1	3	acceptable
C2 Potato slices + citric acid	0.5%	3	1	Unacceptable
	1.5%	1	2	acceptable
	2%	0	3	acceptable
C3 Potato slices + table salt	1.5%	3	2	acceptable
	2%	1	2	acceptable
	2.5%	1	4	acceptable
C4 Potato slices + rosemary + citric acid + table salt	0.2%	4	1	acceptable
	1.5%	1	3	acceptable
	2%	1	3	acceptable
C5 Potato slices + rosemary + citric acid + table salt	0.3%	0	4	acceptable
	2%	1	4	acceptable
	2.5%	1	4	acceptable
LSD Value		1.638 *	1.844 *	—

* ($P < 0.05$).

Table 7 shows the results of the evaluation of fried potatoes treatments. C4 treatment (slices of potatoes, rosemary, citric acid and table salt) 0.3, 2.2.5%, which showed superiority in characteristics of textures similar to the treatment of C3 (slices of potato and table salt) 2.5% compared to the rest of the treatments for the combined effect of these substances. While the percentage of odor and taste characteristics but by 0.2% was equal to 4 with the treatment of control C, which differed in terms of non-acceptance of color for the latter (McCarthy *et al.*, 2001; Stavros and Vassilis, 2003).

Table 7 shows the results of the sensory evaluation (color, texture, odor, and taste) of the fried potato slices for C1, C2, C3 and C4 treatments at different concentrations for each treatment. A significant difference $p < 0.005$ was observed in the color of the fried potato slices for all treatments and for all the ratios added for each treatment compared to the control treatment. It was observed that with the increase in the added ratios of each treatment, the color of the fried potato slices was acceptable compared to the control treatment and the second treatment when adding citric acid to potato chips by 0.5%. It was found that the addition of rosemary, citric acid, individually or in combination, led to the improving

of the fried potato slices color and was more acceptable comparing to the control treatment without any additives.

On the other hand, significant $p < 0.05$ was observed in the texture of the fried potato slices compared to the control and all the other treatments (C1, C2, C3 and C4) and for all the different added percentages. It was noted that with the increase of the added ratios of all rosemary, table salt and citric acid separately and in a mixed manner, there was a noticeable improvement in the texture of the fried potatoes, where the highest value of the first treatment was 3 when adding the rosemary by 0.3%, where the lowest value was 2 when added ratios were 0.1 and 0.2%, respectively.

As for the second treatment, the highest value of tissue was 3 with the addition of 2% citric acid

and the lowest value was 1 at the ratio of addition of 0.5%, which means that when increasing the added proportion of citric acid, led to clearly improve the texture of fried potato chips. There was a slight difference $p < 0.05$ for the third treatment, with the highest value being 4 with the addition of 2.5 table salt while the lowest value was 2 with the addition of 1.5 and 2%, respectively, indicating that the increase in concentrations of added table salt to the fried potato slices, improves the texture compared to control treatment, which reached 1.

The fourth treatment showed a significant increase $p < 0.05$ when adding a mixture of rosemary, citric acid and table salt to the fried potato slices with different concentrations. This synergistic effect of the additives resulted in a noticeable improvement in the texture of the fried potato slices, which was best at concentrations of 0.3 and 2 and 2.5% respectively, with a value of 4 compared to the addition of 0.2%, which amounted to 1 while the it was 3 at the addition of 1.5 and 2% respectively. Furthermore, table 6 shows a significant increase $p < 0.05$ in the taste and odor of fried potato slices for all treatments (C1, C2, C3 and C4) and for all the different additive ratios compared to the control treatment. It was found that in the first treatment the

best value for taste and odor of slices was 3 when adding 0.1% of the rosemary, which was the highest, however, it began to decrease gradually after the addition of rosemary at 0.3 and 0.2%, which was 1 and 2 respectively. It is noticed in the second treatment that there was a gradual decrease in the taste and odor of fried potato slices when adding different percentages of citric acid, which reaches 0, which means no odor and taste at the addition of 2%. While the best value was at the addition of 0.5%. In addition to the above, it has been shown that the greater the concentration of citric acid added, led to the lack of taste and odor significantly. The second treatment also showed a decrease in the taste and odor of the fried potato slices when adding different proportions of table salt, the best of which valued to 3 at the addition of 1.5% and was 1 at the addition of 2 and 2.5%, respectively. In addition, the higher the addition of table salt to the potato slices, the lower the taste and odor of the slices.

Conclusion

Acrylamide is present in many food products. The addition of rosemary, citric acid and salt to the food, as well as the synergistic action of these substances, combined with potato chips, played an important role in reducing the formation of acrylamide during the frying process compared to the control group. The results of all the treatments under study showed low rates of acrylamide somewhat compared to the limits recommended by the World Health Organization and the US Food and Drug Administration, which is 0.3-0.8 mg / kg per kg body weight per day. If we consider the average body weight to be 75 kg, the daily intake rate of acrylamide will be 60 mg/kg. In addition, additional tests need to be carried out to verify the genetic toxicity of the rosemary to ensure its safety.

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