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RESEARCH ARTICLE

Reducing the Acrylamide Concentration in the Potato Chips Product by Using Sea Salt, Green Pepper and Thyme

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ABSTRACT

The study was conducted to show the effect of adding different percentages of Sea Salt solution and aqueous extracts of green pepper and thyme individually or in combination, it reduces the Acrylamide content in the potato chips product when exposed to high temperatures during processing, potato slices were treated by soaking before frying with C1 (0.1, 0.2, 0.3) % Sea Salt solution and the aqueous extract of Green Pepper C2 and thyme C3 in proportions (1, 2,3)% and a mixture of the three additives C4, the treatment C was left to be the control, High performance liquid chromatography technology (HPLC) was used to estimate the concentration of acrylamide in the treatments, sensory evaluation to the parameters of taste, color, odor, texture and general acceptance was conducted, the results showed a clear decrease in the acrylamide concentration in the potato chips treatments by increasing the concentration of the additives, C4 treatment with the synergistic action of Sea Salt, thyme and Green Pepper outperformed all other treatments in decreasing the acrylamide concentration, The results were descending C4 > C2- 3% C3- 3% > C1- 3% amounting to 0.013, 0.024, 0.034 and 0.084, respectively, compared to the control treatment 0.522, the results of sensory evaluation recorded the highest degree of receptivity to the treatments by increasing the percentage of additives, and the results were descending C4 > C3> C2 compared to the control treatment.

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Introduction

Potatoes are one of the most preferred strategic foods around the world because they contain a high nutritional value in addition to the calories that they provide to the body it is included in the various types of foods for most peoples to add to it a desirable taste, a distinctive flavor and a preferred texture (Fao, 2005), potato chips product is at the forefront of the popular and desirable snacks that are consumed by millions of people of different cultures and nationalities.

The unique flavor and preferred texture of this product comes from the use of the deep frying method of potato chips, whether in food factories or at home (Lingnert et al., 2002), Frying the product depends on the heat transfer from the hot oil, which causes the water to be displaced from the potato chips to be replaced by the oil (El-Shawaf et al., 2014).

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The reaction that occurs during the cooking of starchy foods, especially potato chips, at high temperatures is known as the Maillard reaction, by reducing sugars with amino acids, the most important of which is asparagine, at temperatures higher than 120° C and under low humidity conditions (El-Desouky et al., 2015).

The formation of acrylamide in potato chips depends on several external factors, such as the type of cooking process, temperature, cooking time, pH value, and surface area exposed to heat and oil, in addition to the internal factors, which include the percentage of amino acids, especially asparagine, alanine, arginine, and others, as well as the presence of carbohydrates represented by monosaccharides and disaccharides such as reduced glucose sugar and fructose, in addition to sources of non-reducing sugars, genetic and environmental factors play a large role in the formation of acrylamide, such as the type of potato used in cultivation, cultivation system, harvest time,

duration and storage temperature (Rydberg et al., 2003 and Zhang and Zhang, 2007).

The Swedish National Food Administration and Stockholm University announced the presence of elevated levels of acrylamide up to 1000 mcg/kg in starchy foods treated at high temperatures, which was directly supported by the US Food and Drug Administration FDA, where it showed that chips and fingers of fried potatoes contain high levels of it ranging from 17 - 2762 mcg / kg, the International Agency for Research on Cancer classified acrylamide as one of the possible compounds in causing cancer in humans, based on biological experiments that showed carcinogenic effects to many tissues in mice, especially kidney and lung cancer, it is classified among substances that affect the nervous system, especially peripheral nerves, when exposed to long-term periods, it has negative effects on the growth and development of fetuses, and has mutagenic toxicity and toxicity affecting genes (Shipp et al., 2006, Friedman, 2005, Jannek, et al., 2008).

Studies have recently tended to try to reduce the formation of acrylamide by breaking the Maillard reaction in several ways, many of which proved successful by reducing its percentage in potato chips between 20-60% (Kita et al., 2004).

Various strategies have been adopted to deal with foods treated with high temperatures, and studies have focused on reducing the acrylamide content in potato chips product depends on three main aspects they are modifying the raw materials used, improving the heat treatment conditions and using additives that control the reactions that lead to the formation of acrylamide (Franke et al., 2005).

In recent years, there has been significant evidence in the field of study the acrylamide formation in potato chips product that proved the effectiveness of many natural extracts of antioxidants in reducing its percentage, this is due to the ability of these extracts, with their various structures and functional groups, to interact with food components affecting the formation of acrylamide or with acrylamide itself, which leads to a decrease in its concentration in the product (Capuano et al., 2010, Cheng et al., 2010), studies have shown that the addition of extracts of oregano, wild thyme, cinnamon, pepper, rosemary and some phenolic compounds helped to restrict the effect of reducing sugars as they are the ideal mediator for Maillard reaction and thus reduce the concentration of acrylamide in potato chips (Kotsiou et al., 2010; Yuan et al., 2011), the JECFA Joint Expert Committee on Food Additives has determined the oral dose of acrylamide leading to acute toxicity to be greater than 100 µg/kg body weight, while it was determined that the daily percentage taken from acrylamide should not exceed 0.3-0.8 µg/kg of body weight (FAO/WHO, 2011)

This study aimed to reduce the concentration of acrylamide in potato chips product by using different concentrations of sea salt solution and aqueous extracts of green pepper and thyme individually and in combination.

Materials and Methods

Dried thyme was purchased from the local market and was diagnosed in the herbarium of the College of Science for Girls/University of Baghdad, fresh green pepper (Capsicum annuum) was used after being thoroughly washed and dried in the electric oven. As for sea salt, the brand (BADIA) packed in the United States of America was used, thyme, dried green pepper and sea salt were ground and kept in the refrigerator in clean containers at 4°C until use.

Preparation of Extracts

The method presented in El-Shawaf et al., (2014) and modified by Hwang and Do, (2014) in the preparation of extracts of Thyme and Green Pepper, 40 g of the powder was placed in a clean flask with 800 ml of methanol, and placed in a shaker incubator for an hour at 25° C, then it was filtered with filter paper (Whatman No. 1) and the filtered solution was placed in a distilled Vacuum at 40°C in a Rotary evaporator, the final extracts were stored in clean, opaque glass bottles at 4°C until use.

Preparing and Frying Potato Chips

Local Potatoes (Solanum tuberosum L.) were purchased, after washing, drying and peeling, they were cut into 1.5 mm thick slices and washed 5 times with distilled water, before frying: divided into groups and soaked for 20 minutes at 30 ° C in a mixture of distilled water with Sea Salt and aqueous extracts of thyme and green pepper with the concentrations shown in Table (1).

Table 1. Added percentages of Sea Salt, Green Pepper and Thyme powders to the soaking water of potato slices

Treatments	Treatment type and percentage to add (%)
C (Control)	Untreated potato slices
C1	Potato slices + Sea Salt 1, 1.5, 2
C2	Potato slices + Thyme 1, 2, 3
C3	Potato slices + Green Pepper 1, 2, 3
C4	Potato slices + Sea Salt + Thyme + Green Pepper 1.5, 2, 2

They were taken out of the soaking mixture and filtered from the solution well, then they were fried separately in hot sunflower oil in a deep frying pan to covering the slices with frying oil at a temperature of 170°C for 5 minutes and stirring them constantly to ensure that the heat is distributed homogeneously on all the slices, spread in containers on blotting paper to get rid of the excess oil. Each treatment was divided into two groups, the first was used directly for sensory evaluation, while the second group was homogenized for the estimation of acrylamide, these transactions were kept in polyethylene bags, closed well, and then frozen at -20°C, the information was written on them until the extraction process was performed, with two repetitions for each treatment according to the method mentioned by Granda and Moreira, (2005).

Preparation of Reagents and Standard Solutions

The method mentioned was followed by Khoshnam et. al, 2010 where 0.1 g of standard acrylamide was dissolved (for HPLC) supplied by Sigma Deisenhofen, Germany by using non-ionic water in a volumetric vial to bring the volume to

100 ml, in order to prepare the standard solution of acrylamide (1000 µg ml^{-1}), This solution was used to prepare successive standard concentrations (100 µg ml^{-1} and 2µ g ml^{-1} the working standard solutions were (20, 40, 80, 160, 240, 320, and 400 ng ml^{-1}) prepared by mixing an appropriate dilution of 2 µg ml^{-1} of standard solution with water. All the standard and stock solutions were kept at 4° C.

Acrylamide Extraction

4 gm of potato slices were weighed after grinding well and placed in a closed flask, the fat was removed by adding 10 ml of hexane and shaken the flask well for 5 minutes, then the mixture was dried under pressure and 20 ml acetone and 100 µl of distilled water were added to the samples to extract the acrylamide from them. Then the mixture was filtered by filter paper, the extract was dried by evaporating 10 ml of the filtered mixture under pressure, then 2 ml of water was added to it and shaken well to dissolve the remaining sediment, the aqueous solution was filtered again by filter paper (Khoshnam et al., 2010).

Determination of Acrylamide Concentration in Potato Chips Samples

High performance liquid chromatography (HPLC) RF-20A device supplied by Shimadzu, Japan was used to estimate the acrylamide concentration in the tested samples as indicated by Gökmen et al., (2005), where the conditions of the experiment were adjusted according to the following: Column: C18 (250 x 4.6 mm), Mobile phase: aqueous phosphate buffer, Flow rate: 1.0 ml/ min, Detector: UV 254 nm, Injection loop: 200 µL, Temperature: 40°C, Pressure: 38 atm.

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

Concentration of sample (mg/kg) = (the area of the sample/ area of the standard) × standard Concentration× Dilution factor

Sensory Evaluation

The sensory evaluation of the fried potato chips treatments referred to under study was conducted by 10 experienced persons, where the color, flavor, taste, texture and general acceptability of the samples were evaluated after 30 minutes of frying according to Zhang and Zhang, (2007), and on a scale consisting of 10 degrees, where the number 10 represents a degree Excellent and grade 1 poor.

Statistical Analysis

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

Results and Discussion

Table (2) shows the concentration of acrylamide in potato chips treated with sea salt solution and aqueous extracts of Thyme and Green Pepper, the results showed a clear decrease in the concentration of acrylamide in samples treated with soaking for 20 minutes at 30° C in Sea Salt solution at 1%, 1.5% and 2% which amounted to 0.395, 0.107 and 0.084 mg/kg respectively, compared to its percentage in the control treatment, 0.522 mg/kg, these results were close to what was found by Alemzadeh, (2016) when treating potato slices by soaking them in a solution of table salt at concentrations 1%, 1.5% and 2.25% for 30 minutes at room temperature and then frying them at 165° C for 4 minutes, the concentrations of acrylamide in the treatments decreased from 0.351 mg/kg in the control treatment to 0.242, 0.107 and 0.083 mg/kg respectively, for the concentrations used in his treatments.

Bakhtiary, (2014)) indicated that treating potato chips by soaking them with NaCl, KCl and $CaCl_2$ salts before frying led to a reduction in acrylamide formation in potato chips to 46%, 32% and 55%, respectively, a study by Mestdagh et al., (2008) showed that the reason for the decrease in the concentration of acrylamide in potato chips product may be due to the fact that additives such as NaCl and MgCl salts and others reduce the available oil content relative to the total surface of potato slices, where the salts act as an obstacle to the penetration of the oil into the potato tissues, thus reducing the heat transfer from the oil to the potato slices, thus reducing the chance of acrylamide formation, therefore, it seems that less oil absorption is an acceptable mechanism to reduce the concentration of acrylamide when treating potato slices by soaking in salt solutions before frying, in addition, these additives are likely to change the structural properties of potato tissues, which leads to a difference in the percentage of oil absorption and a decrease in heat transfer from it to the potato, and in the end, a decrease in the concentration of acrylamide formed.

The aqueous extracts of thyme and green pepper (Table, 2) showed a high efficiency in reducing the concentration of acrylamide in the fried potato chips that were soaked with their extracts, the treatments C4 and C3- 3% and C2 - 3% recorded low concentrations of acrylamide 0.013, 0.034 and 0.024 mg/kg respectively, compared to the control treatment 0.522 mg/kg, It is clear from these results that the synergistic action of sea salt, thyme and green pepper in treatment C4 was the best in reducing the concentration of acrylamide in potato chips, While the two treatments C3 for green pepper extract by 3% and C2 for thyme by 3% were in the second degree in terms of reducing acrylamide formation, meaning that the chance of its formation in potato chips decreased with the increase in the percentage of adding these extracts.

The results of El-Desouky et al., (2015) approach to what was found by this study, where the concentration of acrylamide decreased in fried potato slices pre-soaked in hibiscus aqueous extract 3% for 10 minutes to 0.026 mg/kg compared to the control treatment 1.25 mg/kg.

Zhu et al., (2009) indicated that the process of soaking potato slices in 2% and 3% of green tea extract before frying led to a decrease in acrylamide concentration from 1.07 mg/kg in the control treatment to 0.075 and 0.033 mg/kg respectively.

Mekawi et al., (2019) studied the effect of nanoparticles of pomegranate peel extract on reducing acrylamide concentration in potato chips soaked for 10 minutes and fried at 175°C.

This treatment reduced acrylamide concentration to 0.019 mg/kg compared to the control treatment 1.67 mg/kg, the study of El-Shawaf et al., (2014) came to show that extracts of clove 3% and rosemary 3% significantly reduced the concentration of acrylamide in potato chips treatments from 0.126 mg/kg to 0.006 and 0.014 mg/kg respectively.

The addition of natural antioxidants, such as extracts of vegetables, fruits and herbs, because of their phenolic compounds, resins and others, helps reduce the concentration of acrylamide in food products in general and in potato chips in particular, the great effectiveness of these antibiotics has been recorded in their trapping effect of the deoxy-2-3 hexulose sugars, which are intermediate compounds in Maillard reactions and thus inhibit or limit the formation of the products of these reactions, which are mainly acrylamide (Cheng et al., 2010; Lotfy et al., 2018), natural extracts may play the role of antioxidants, which helps reduce free radicals, which contributes to reducing the chance of acrylamide formation in the potato chips product, as indicated by Morales et al., (2014) when they studied the effect of natural extracts of wild oregano, thyme, cinnamon and green tea in reducing the concentration of acrylamide in potato chips, they explained that these extracts inhibited and reduced the activity of free radicals by a rate ranging between 48-98% and thus reduced the concentration of acrylamide.

Table (3) shows the results of sensory evaluation of potato chips samples that were treated with Sea Salt solution and extracts of thyme and Green Pepper, it appears that there are no clear differences for the characteristics of taste, color, odor, texture and general acceptance between the control treatment and the Sea Salt treatment C1-1% but soon, the evaluations of these traits start to rise with the increase in the percentage of addition of all extracts to reach the highest values of sensory receptivity in the C4 treatment with the synergistic action of the additions together, It reached 9.75 for the taste, 9.58 for the color, 9.82 for the smell, and 9.51 for the general acceptance compared to the control 7.88, 7.93, 7.75 and 7.85 respectively, for these Characteristics, except for texture trait, treatment C3-30% had the highest rate of 8.92 compared to control treatment 8.02, the treatments C1-30%, C2-30% and C3-30% recorded high sensory evaluation results that come in the second degree after treatment C4, superior to the control and the rest of the concentrations of additives.

El-Desouky et al., (2015) showed that the addition of hibiscus extract at 1%, 2% and 3% to potato chips treatments improved the sensory characteristics of the product and that 3% was the best in the two characteristics: taste 8.77 and color 8.5 compared to the control treatment 8.06 and 7.89, respectively for the two characteristics.

The 2% treatment was better in odor characteristic 8.43 compared to control 8.02.

El-Shawaf et al., (2015) indicated that 3% rosemary extract was better than 3% clove extract in all the sensory evaluation characteristics of potato chips product.

The overall acceptance rates were 9.71 for rosemary and 9.37 for cloves, while the study of Lotfy et al., (2018) reported that there are no significant differences in the taste and odor characteristics of bread products when adding Thyme, Cumin and Anise compared to the control, but the degrees of color and texture evaluation of those baked improved when adding Thyme 3%, which reached 7.80 and 8.30, respectively, for the two qualities, and for cumin 3%, which reached 8.05 and 8.10, respectively.

Bunger et al., (2003) found that soaking potato chips in a 3% Salt solution did not significantly affect the color and odor characteristics, but it significantly affected the texture and general acceptance, and that soaking in 7% Salt made most of the sensory characteristics of potato slices unacceptable.

Table 2. Acrylamide concentration in potato slices treated with Sea Salt solution and aqueous extracts of thyme and Green Pepper

Treatments of Potato Chips		**Concentrations of aqueous extract**	**Acrylamide Content* (mg/kg)**
Control	**C**	0	0.522
Sea Salt	**C1**	1%	0.395
		1.5%	0.107
		2%	0.084
Thyme	**C2**	1%	0.236
		2%	0.075
		3%	0.024
Green Pepper	**C3**	1%	0.283
		2%	0.093
		3%	0.034
Sea Salt 1.5% + Thyme 2% + Green Pepper 2%	**C4**	--	0.013
LSD value		---	0.0782 *
* (P≤0.05).			

Table 3. Effect of sea salt and aqueous extracts of Thyme and Green Pepper on the sensory characteristics of potato chips treatments

Treated samples		**Characteristics**				
		Taste (10)	**Color (10)**	**Odor (10)**	**Texture (10)**	**Overall acceptability (10)**
C	0	7.88	7.93	7.75	8.02	7.85
C1	0.1 %	7.92	7.81	8.04	8.15	8.06
	0.2 %	8.05	8.11	8.45	8.13	8.22
	0.3 %	8.17	8.23	8.54	8.26	8.43
C2	1%	8.56	8.25	8.43	8.25	8.53
	2%	8.82	8.85	8.97	8.95	8.91
	3%	9.03	8.76	9.27	9.35	9.38
C3	1%	8.88	8.87	8.65	8.45	8.53
	2%	8.92	8.97	8.74	8.83	8.97
	3%	9.25	8.89	8.97	8.92	9.88
C4		9.75	9.58	9.82	8.43	9.51
LSD value		1.037 *	1.188 *	1.086 *	0.772 *	1.169 *
* (P≤0.05).						

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