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The Effect of Different Orders of Vegetables in Frying on Acrylamide Levels

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Abstract: The aim of this study was to determine the acrylamide level of fried potatoes, peppers, eggplant and zucchini, and to examine the effect different order of vegetables on the acrylamide level of other vegetables in consecutive frying sessions. In this study, in which four different types of vegetables were fried separately and together with sunflower oil, 52 frying sessions were performed in 13 separate frying groups, analyzing a total of 208 samples in LC-MS/MS. The study was carried out at the Eastern Anatolia High Technology Application and Research Center (Erzurum, Turkey) between 20 January and 15 February 2021. Potatoes, peppers, eggplants, and zucchinis were fried consecutively and mean acrylamide levels of they were determined as 1042, 502, 167, and 553 µg kg^{-1}, respectively. Statistically significant results between consecutive frying sessions of all vegetables and acrylamide levels were obtained. The acrylamide levels obtained for all types of vegetables indicate that oil should not be used sequentially. However, there was no difference between the change in the order of vegetables and their acrylamide levels in frying. The results show that the fried foods do not cause an additional load of acrylamide in the oil. More research should be conducted on the effect of consumers' culinary practices on acrylamide levels in foods.

Keywords: Acrylamide, French fries, vegetable fries, consecutive frying, sunflower oil

1. Introduction

Frying is one of the oldest food preparation techniques, widely used in home and food service establishments, with the capacity to give foods having the desired sensory properties (Saguy and Dana, 2003; Orthoefer and List, 2007). Frying foods form a composite structure consisting of a cooked interior and a dehydrated, porous, and crunchy surface (Pedreschi, 2009; Oke et al., 2018; Asokapandian et al., 2020). Fried foods play a significant role in the diet of today's consumers (Wijesinha-Bettoni and Mouillé, 2019). However, many researchers have stated that acrylamide (C_3H_5NO), which causes potential health hazards to humans, may occur in foods, especially in potatoes fried at high temperatures (Dybing et al., 2005; Katz et al., 2012; Mesías et al., 2021).

Acrylamide is a crystalline powdered compound that dissolves in water, ethanol, ether, and chloroform, with no distinctive color and smell. Acrylamide is a chemical agent widely used in dams, tunnels, cosmetics, mining, oil, textiles, and sewage (Anonymous, 1985). The International Agency for Research on Cancer identified acrylamide as a probable carcinogen compound for humans Group 2A in 1994 (Anonymous, 1994). Shortly after this identification, in 2002, a group of Swedish scientists reported that acrylamide exists in foods (Tareke et al., 2002). This discovery has aroused great curiosity in the scientific world and led to the emergence of many studies on acrylamide.

Acrylamide, taken into the organism through contact, digestion, and respiration, is easily absorbed and distributed throughout the body because it has a low molecular weight (71.08 g mol^{-1}). The excretion of acrylamide and its compounds from the body is pretty rapid. It has been declared that acrylamide taken into the organism can be linked to DNA, RNA, and proteins in some tissues and cells by having chemical reactions and that they have a high desire to form compounds with these substances (Abramsson-Zetterberg et al., 2005; Doroshyenko et al., 2009; Anonymous, 2015). The potential risks of acrylamide have also brought up health-related studies.

Acrylamide is an exceedingly toxic compound (Koszucka et al., 2020). Experimental studies on animals have revealed that acrylamide has a genotoxic and neurotoxic effect and causes cancer in multiple organs and tissues (Anonymous, 2005, 2015; Rice, 2005; Park et al., 2021). Studies on humans have reported that dietary acrylamide exposure is associated with some types of cancer (Hirvonen et al., 2010; Pelucchi et al., 2015; Liu et al., 2017; Adani et al., 2020), negatively affects fetal development during pregnancy, such as low birth weight (Duarte-Salles et al., 2013), and also leads to neurological effects such as tingling, pain, and numbness in the hands, arms, legs (Goffeng et al., 2011; Kopanska et al., 2018). The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives defined neurotoxic No Observed Adverse Effect Level (NOAEL) for acrylamide as 0.2 mg kg^{-1} bw/day (Anonymous, 2011).

There is still a great deal of uncertainty about acrylamide formation in foods (Semla et al., 2017). Besides, acrylamide has been reported to be highly formed by heat treatment applied above 120 °C, especially in carbohydrate-rich foods, especially in the existence of aspartic amino acids and reducing sugars (Tareke et al., 2002). Maillard Reaction is admitted as the basic mechanism for the formation of acrylamide. Besides, some studies have shown that pyruvic acid, B-alanine, aspartic acid, carnosine, and acrolein are converted into acrylamide by various reactions (Guenther et al., 2007; Arvanitoyannis and Dionisopoulou, 2014).

Given the literature, potatoes are one of the most researched foods related to acrylamide. The European Food Safety Authority (EFSA) identified potato chips (389 µg kg^{-1}) and French fries (308 µg kg^{-1}) as the second and third foods with the highest average acrylamide levels, respectively, after coffee and its byproducts (522-1499 µg kg^{-1}) (Anonymous, 2015). Many studies have also reported high levels of acrylamide in French fries and highlighted their role in dietary acrylamide exposure (Mojska et al., 2010; Sirot et al., 2012; Esposito et al., 2017; Mesías et al., 2021). Therefore, many studies have been carried out to reduce the level of acrylamide in French fries. In this context, a series of operational procedures such as washing potatoes to reduce the concentration of precursor compounds in acrylamide before frying, soaking in solutions such as lysine, cysteine, glycine, citric acid, acetic acid, and calcium chloride, adding asparaginase enzymes, applying pre-treatments such as pulsed electric fields and ultrasound (Jung et al., 2003; Pedreschi et al., 2008; Medeiros Vinci et al., 2011; Antunes-Rohling et al., 2018; Genovese et al., 2019; Liu et al., 2020; Cerit and Demirkol, 2021), type of oil in frying, frequency of use of oil, frying temperature, and time changes have been introduced (Gökmen and Palazoğlu, 2008; Zhang et al., 2015; Daniali et al., 2016; Chan et al., 2020; Başaran and Turk, 2021).

The European Commission has defined some indicators for certain foods, including French fries. Also, the European Commission recommended monitoring foods with high levels of acrylamide, conducting studies to reduce acrylamide levels, and reporting dietary exposure studies periodically (Anonymous, 2017, 2019). Similarly, the Joint FAO/WHO Expert Committee on Food Additives pointed out that data on acrylamide levels in foods are insufficient, and attracted attention to the importance of future studies in developing strategies for decreasing acrylamide levels in foods (Anonymous, 2011). The EFSA stated that frying practices in homes and restaurants and cooking preferences of consumers at home were not taken into account sufficiently in the studies, causing data loss in dietary exposure studies (Anonymous, 2015). Although there are many studies to reduce acrylamide levels in French fries, it can be said that they do not fully reflect the frying practices in home kitchens. Although it is known in the literature that potatoes are an important source of acrylamide formation, the number of studies showing the acrylamide levels of other fried vegetables is limited. There exists no study on the results of acrylamide levels of potatoes fried with other vegetables. The effect of consecutive frying sessions on acrylamide levels is still a controversial issue. Considering all information, the aim of this study comes under three headings: (i) to determine the acrylamide levels of potatoes, peppers, eggplants, and zucchinis in home fries, (ii) to examine the effect of successive frying sessions of potatoes, peppers, eggplants, and zucchinis on acrylamide levels, and (iii) to examine the effect of different orders of vegetables on the acrylamide levels of other vegetables in consecutive frying sessions.

2. Materials and Methods

2.1. Materials

The material of the study consisted of potatoes (*Solanum tuberosum* L. cv. Agata), sweet peppers

(*Capsicum annuum* L.), eggplants (*Solanum melongena* L.), and zucchinis (*Cucurbita peto* L.) which were purchased from a local grocery (Turkey). Sunflower oil purchased from the grocery was used in frying sessions.

2.2. Experimental design

In this study, vegetables were fried consecutively four times. In four consecutive frying sessions, it was formed a frying group and gave a code to each group using A, B, C, D followed by letters, respectively. First, each vegetable group was fried (potatoes, peppers, eggplants, and zucchinis) consecutively and determined their reference acrylamide values to make comparisons. In the following fryings, the order of potatoes was changed and fried with peppers, eggplants, and zucchinis, respectively, determining other values that would shed light on the study.

2.3. Deep frying experiments

No pre-treatment, such as washing, was applied to the vegetables. Except for potatoes, the vegetables were not peeled. To give a standard shape, each vegetable was chopped in the same way and all slices were mixed according to the vegetable group. An equal amount of samples (5 pieces of each vegetable per session) was randomly selected from this mixture. They have deep-fried in a household fryer (Arçelik, K 1798, Turkey) with a 2.5 L capacity as the heating source and 1800 W electrical resistance. A calibrated temperature was used for measuring to monitor the temperature of the frying oil and reduce temperature variations (Digital thermometer, Interlab). After each frying, the fryer was washed and dried and the oil was changed. The ideal frying temperature and time were optimized before the analysis. The fryer was filled with 500 ml of sunflower oil and the samples were put in it after it reached the desired temperature (170 °C, 5 min). Potatoes, peppers, and zucchinis were fried for 2.5 min and eggplants for 3.0 min. There was a 30-second-pause (for the temperature to reach again to 170 °C) between each frying session. The samples were put on the adsorbent paper for a few seconds to remove excess oil and they were cooled to room temperature.

2.4. Stock solutions and sample preparation

Stock solutions of acrylamide (1000 µg kg^{-1}) and $^{13}C_3$-acrylamide (1000 µg kg^{-1}) were dissolved in High-Performance Liquid Chromatography (HPLC) grade acetonitrile (Merck, Darmstadt, Germany) containing 0.1% formic acid (Merck, Darmstadt, Germany). Working solutions were prepared after the stock solution was diluted with acetonitrile, distilled water (MilliQ system, Millipore, Bedford, MA, USA) with 0.1% formic acid. Stock solutions and working standards were kept at a constant temperature of 4 °C. The rest of the chemicals in the study had an analytical grade.

The acrylamide analysis was carried out by making some modifications to Anonymous (2012). All the samples were crushed in a mortar, which was cleaned before each use, to make them homogenous. In the next step, two different samples were selected to perform two parallel analyses. After 0.2 g of the sample was weighed, we added 950 µL of stock solution and 50 µL of $^{13}C_3$-acrylamide (Sigma-Aldrich, St. Louis, MO, USA) were added. It was mixed with a vortexer for 5 minutes and incubated at 45 °C for 15 minutes in a sonicator. It was then centrifuged (Allegra X-30R was equipped with a C0650 rotor, Beckman Coulter, Palo Alto, CA) at 9000 rpm for 5 minutes. The clear phase was taken with a syringe and the extract was transferred through a 0.45 µm filter (Maxi-Spin PVDF) into a 2 mL autosampler vial. 10 µL was injected into the Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) (Agilent Technologies, model LC-1200 Infinity Series, Englewood, CO, USA). All sample extractions and measurements were performed twice.

2.5. LC-MS/MS analysis

Analytical separation on Zorbax Eclipse XDB-C18 (150 x 4.6 mm, 5 µm) was fulfilled in two mobile phases: Mobile phase A: 0.1% formic acid in water and mobile phase B: 0.1% formic acid in acetonitrile. Conditions: column temperature (30 °C), gas flow (11 L min^{-1}), sheat gas heater (300 °C), gas temperature (350 °C), nebulizer (45 psi), sheat gas flow (7 L min^{-1}). The quantification was performed in multiple reaction monitoring (MRM) mode with the ionic transitions as follows: acrylamide (m/z 72.1 > m/z 55.1, 44.1) and $^{13}C_3$-acrylamide (m/z 75.1 > m/z 58.2, 44.1). All the analyses were performed twice and the results were shown as ng g^{-1} per sample. Acrylamide and $^{13}C_3$-acrylamide peaked at 5.44 minutes.

2.6. Quality control

The linearity of the method was evaluated by constructing seven standard solutions (10-2500 µg kg^{-1}) with 50 µg kg^{-1} of $^{13}C_3$-acrylamide. It was observed that the curve was linear and calculated the coefficient of determination (R^2) by using linear regression analysis (R^2= 0.99974205). The limit of detection (LOD) and the limit of quantification (LOQ) values were calculated as 3.0 and 10.0 µg kg^{-1}, respectively. The performance of the method was evaluated by recovery (R) experiments for

French fries at different spiking levels (50 and 100 µg kg^{-1}). Acrylamide recovery for fried potatoes, sweet peppers, eggplants, and zucchinis was found to be in the range of 91.1-98.4%. RSD_s were <10% for all matrixes.

2.7. Evaluation of data

The study data was analyzed by transferring them to the IBM SPSS Statistics 26 software. First, a test of normality (>50) was applied to the data. The test result revealed that the data did not provide the normality assumption, so non-parametric tests were used in the evaluations. Different letters in the same group indicate statistically significant differences (p<0.05). The sequencing items were followed to evaluate the effect of consecutive sessions of frying and the different orders of vegetables on acrylamide levels as follows:

(i) The correlation (median) between consecutive frying sessions and acrylamide levels in frying groups (A, B, C, D) with the same vegetables were examined through Kruskal Wallis Test and Bonferroni Test (reference acrylamide values).

(ii) The correlation (median) between the change in the order and the acrylamide levels in the frying groups (E, F,……N) in which the potatoes were placed with other vegetables by changing their place in the order was examined using the Kruskal Wallis Test and the Bonferroni Test. The data were compared with those of the consecutive frying sessions of frying groups from which the reference acrylamide values (A, B, C, D) were obtained.

(iii) The reference acrylamide values (A, B, C, D) of acrylamide levels resulting from the change in the order were compared using the Mann Whitney U Test in fry groups (E, F,……N) with which the potatoes were put together by changing their place in the order. The same sessions in the frying groups were taken into account in the comparison.

3. Results and Discussion

3.1. Acrylamide levels of fried vegetables in consecutive sessions

The acrylamide levels and statistical analysis of consecutive frying sessions with the same vegetables are shown in Table 1.

The acrylamide levels (reference values) in consecutive frying sessions of potatoes (A), sweet peppers (B), eggplants (C), and zucchinis (D) ranged from 952 to 1152, from 319 to 655, from 132 to 222, and from 501 to 621 µg kg^{-1}, respectively. In the 1st, 2nd, 3rd, and 4th consecutive frying sessions, the average acrylamide levels were 971, 1034, 1066 and 1097 µg kg^{-1} for potatoes; 336, 473, 571 and 627 µg kg^{-1} for sweet peppers; 136, 148, 172 and 214 µg kg^{-1} for eggplants; and 508, 517, 567 and 618 µg kg^{-1} for zucchinis, respectively. Compared to the average acrylamide levels in the 1st and 4th frying sessions, as the acrylamide levels in potatoes and zucchinis increased by 13.0% and 21.6%, respectively, the increase rate in peppers and eggplants was as high as 86.6% and 57.3%. In the statistical analysis, there existed a significant difference between the consecutive frying sessions of potatoes, sweet peppers, eggplants, and zucchinis according to the acrylamide levels (p<0.05). In fact, while the acrylamide levels in the 1st session of French fries were significantly lower than those in the 3rd and 4th sessions, the acrylamide levels in the 1st session of fried sweet peppers, eggplants, and zucchinis were significantly lower than those in the 4th session (Table 1).

Table 1. Acrylamide levels in the consecutive frying sessions

Vegetables	Frying sessions	Acrylamide level (µg kg^{-1})			x^2	P
		n	Median*	Minimum-Maximum		
Potatoes (A)	A_1	4	971^{b}	952-991	11.051	0.011
	A_2	4	1022^{ab}	1014-1080		
	A_3	4	1071^{a}	1031-1090		
	A_4	4	1090^{a}	1054-1152		
Sweet pepper (B)	B_1	4	329^{b}	319-369	14.118	0.003
	B_2	4	472^{ab}	468-479		
	B_3	4	572ab	556-586		
	B_4	4	622^{a}	610-655		
Eggplant (C)	C_1	4	137^{b}	132-138	14.118	0.003
	C_2	4	148^{ab}	141-155		
	C_3	4	170^{ab}	170-177		
	C_4	4	214^{a}	206-222		
Zucchini (D)	D_1	4	508^{b}	501-516	13.277	0.004
	D_2	4	521^{ab}	507-521		
	D_3	4	571^{ab}	546-578		
	D_4	4	620^{a}	613-621		

n: Number of samples, x^2: Kruskal Wallis test, P: Significance level, *: Different letters in the same group indicate statistically significant differences (p<0.05)

The European Commission has set the indicator acrylamide level for French fries (ready-to-eat) at 500 µg kg^{-1} (Anonymous, 2017). According to the different researchers, the average acrylamide levels in French fries are 750 µg kg^{-1} (Omar et al., 2014), 299 µg kg^{-1} (Elias et al., 2017), 418 µg kg^{-1} (Abt et al., 2019), 550 µg kg^{-1} (Mesías et al., 2020), 709 µg kg^{-1} (Eicher et al., 2020) and 615 µg kg^{-1} (Deribew and Woldegiorgis, 2021).

Takatsuki et al. (2004) reported that the average acrylamide level in baked eggplants and sweet peppers was less than 200 µg kg^{-1}. Besaratinia and Pfeifer (2007) found the mean acrylamide level of 59 µg kg^{-1} in various vegetables (unknown type) fried, baked and grilled. El-Ziney et al. (2009) reported an average acrylamide level of 950 µg kg^{-1} in eggplants cooked on the grill. Biedermann et al. (2010) found the mean acrylamide level was less than 100 µg kg^{-1} in various fried vegetables (type unknown). Guerra-Hernandez et al. (2013) found the average acrylamide level of zucchinis coated with different materials and fried in the range of 50-494 µg kg^{-1}. Wong et al. (2014) found the mean acrylamide levels in fried zucchinis, sweet peppers, and eggplants were 360 (160-480), 140 (94-180), 77 (36-110) µg kg^{-1}, respectively. Omar et al. (2014) stated fried eggplant sample acrylamide content of 330-338 µg kg^{-1}. Jaworska et al. (2019) reported the average acrylamide level in zucchini chips was 78 µg kg^{-1}.

The values in this study generally correspond to a high value compared to both the European Commission and other studies in the literature. The differences between the studies are thought to be due to the lack of a standard frying procedure for consumers and food businesses, the variety of raw materials, the temperature and time applied in frying.

3.2. The effect of vegetables in different orders in frying on acrylamide levels

To evaluate the effect of vegetables in different orders in frying on acrylamide levels, potatoes were subjected to different orders as 1st, 2nd, and 3rd in the frying sessions. The acrylamide levels and statistical analysis of the consecutive frying sessions of vegetables after potatoes are shown in Tables 2, 3, and 4.

The average acrylamide levels were determined as 471, 549, and 639 µg kg^{-1} for the 2nd, 3rd, and 4th sessions of the consecutive frying with sweet peppers after the 1st frying of potatoes (Code: E sessions). The average acrylamide levels were measured as 583 and 638 µg kg^{-1} for the 3rd and 4th sessions, respectively, in the consecutive frying with sweet peppers after the 2nd frying of potatoes (Code: F sessions). The average acrylamide level for the 4th session was found to be 628 µg kg^{-1} in the frying made after frying potatoes in the 3rd frying (Code: G sessions). Compared to the average acrylamide levels of the 1st and 4th frying, in the F and G-coded frying sessions, there was an increase of 78.2 and 61.9%, respectively. In the statistical analysis, the acrylamide levels formed in the 1st frying session of sweet peppers (B_1, F_1, G_1) were significantly lower than those in the 4th frying session (E_4, F_4, G_4) in which potatoes were in different orders ($p<0.05$; Table 2).

The average acrylamide levels for the 2nd, 3rd, and 4th sessions of the consecutive frying with eggplants after the 1st frying of potatoes were determined as 145, 167, and 206 µg kg^{-1}, respectively (Code: H sessions). The average acrylamide levels were measured as 154 and 202 µg kg^{-1} for the 3rd and 4th sessions of the consecutive frying with eggplants after frying potatoes in the 2nd place, respectively (Code: J sessions). The average acrylamide level for the 4th session was found to be 203 µg kg^{-1} in the frying made after frying potatoes in the 3rd place (Code: K sessions). Compared to the average acrylamide levels of the 1st and 4th sessions, in L and K-coded frying sessions, a 50.7% increase and a 62.4% increase were observed, respectively. In the statistical analysis, the acrylamide level formed in the 1st frying session of eggplants (C_1, J_1, K_1) was significantly lower than the acrylamide levels formed in the 4th session (H_4, J_4, K_4) of the fries in which potatoes were in different orders ($p<0.05$; Table 3).

The average acrylamide levels were determined as 517, 561, and 626 µg kg^{-1} for the 2nd, 3rd, and 4th sessions of the consecutive frying with zucchinis after the1st frying of potatoes (Code: L sessions). The average acrylamide levels were measured as 578 and 620 µg kg^{-1} for the 3rd and 4th sessions of the consecutive frying with zucchinis after frying potatoes in the 2nd place, respectively (Code: M sessions). The average acrylamide level for the 4th session was found to be 621 µg kg^{-1} in the frying after potatoes in the 3rd place (Code: N sessions). In the statistical analysis, the acrylamide levels formed in the 1st session of zucchini fries (D_1, M_1, N_1) were significantly lower than those in the 4th session (L_4, M_4, N_4) of fries in which potatoes were in different orders ($p<0.05$; Table 2). Therefore, a significant difference, which was found in terms of the acrylamide levels in the consecutive frying sessions in which only the same vegetable types were fried and the reference acrylamide values were determined and in the consecutive frying sessions in which vegetables were fried together, emerged in the same sessions (1st and 4th sessions).

Table 2. The effect of the change in the frying order of potatoes on acrylamide levels formed in peppers (according to the consecutive frying sessions)

Vegetables	Frying sessions	Acrylamide level (µg kg^{-1})			x^2	P
		n	Median*	Minimum-Maximum		
Potato	E_1**	4	965(B_1**)^b	922-982	14.118	0.003
Sweet pepper	E_2	4	477^{ab}	437-491		
Sweet pepper	E_3	4	551^{ab}	501-593		
Sweet pepper	E_4	4	645^a	607-661		
Sweet pepper	F_1	4	362^b	301-406	13.787	0.003
Potato	F_2	4	995(B_2**)^{ab}	974-1082		
Sweet pepper	F_3	4	574^{ab}	565-578		
Sweet pepper	F_4	4	640^{ab}	617-653		
Sweet pepper	G_1	4	387^b	347-430	13.118	0.003
Sweet pepper	G_2	4	495^{ab}	457-534		
Potato	G_3	4	1116(B_3**)^{ab}	1000-1145		
Sweet pepper	G_4	4	629^a	605-648		

n: Number of samples, x^2: Kruskal Wallis test, P: Significance level, *: Different letters in the same group indicate statistically significant differences ($p<0.05$), **: Since E_1, F_2 and G_3 belongs to potatoes, in the statistical analysis of the consecutive frying sessions in the E, F and G series, B_1, B_2 and B_3 values were taken instead of E_1, F_2 and G_3.

Table 3. The effect of the change in frying order of potatoes on acrylamide levels in eggplants (according to the consecutive frying sessions)

Vegetables	Frying sessions	Acrylamide level (µg kg^{-1})			x^2	P
		n	Median*	Minimum-Maximum		
Potato	H_1**	4	974(C_1**)^b	955-989	12.640	0.005
Eggplant	H_2	4	147^{ab}	127-158		
Eggplant	H_3	4	168^{ab}	155-179		
Eggplant	H_4	4	206^a	197-212		
Eggplant	J_1	4	134^b	130-138	10.324	0.016
Potato	J_2	4	1006(C_2**)^{ab}	979-1057		
Eggplant	J_3	4	155^{ab}	132-176		
Eggplant	J_4	4	202^a	196-208		
Eggplant	K_1	4	126^b	121-128	14.118	0.003
Eggplant	K_2	4	147^{ab}	138-167		
Potato	K_3	4	1050(C_3**)^{ab}	1032-1061		
Eggplant	K_4	4	201^a	195-213		

n: Number of samples, x^2: Kruskal Wallis test, P: Significance level, *: Different letters in the same group indicate statistically significant differences ($p<0.05$), **: Since H_1, J_2 and K_3 belongs to potatoes, in the statistical analysis of the consecutive frying sessions in the H, J and K series, C_1, C_2 and C_3 values were taken instead of H_1, J_2 and K_3.

Table 4. The effect of the change in frying order of potatoes on acrylamide levels in zucchinis (according to the consecutive frying sessions)

Vegetables	Frying sessions	Acrylamide level (µg kg^{-1})			x^2	P
		n	Median*	Minimum-Maximum		
Potato	L_1**	4	968(D_1**)^b	963-986	13.257	0.004
Zucchini	L_2	4	518^{ab}	509-524		
Zucchini	L_3	4	560^{ab}	533-593		
Zucchini	L_4	4	625^a	614-638		
Zucchini	M_1	4	499^b	472-552	12.216	0.007
Potato	M_2	4	1026(D_2**)^{ab}	972-1065		
Zucchini	M_3	4	578^{ab}	570-658		
Zucchini	M_4	4	620^a	574-664		
Zucchini	N_1	4	517^b	466-569	12.658	0.005
Zucchini	N_2	4	577^{ab}	532-582		
Potato	N_3	4	1064(D_3**)^{ab}	1051-1081		
Zucchini	N_4	4	621^a	575-649		

n: Number of samples, x^2: Kruskal Wallis test, P: Significance level, *: Different letters in the same group indicate statistically significant differences ($p<0.05$), **: Since L_1, M_2 and N_3 belongs to potatoes, in the statistical analysis of the consecutive frying sessions in the H, J and K series, D_1, D_2 and D_3 values were taken instead of L_1, M_2 and N_3.

To better explain the effect of the change in the order of vegetables on acrylamide levels, the acrylamide levels measured in frying sessions (E_1, E_2, ……..N_3, N_4) in which potatoes were also fried and the frying order of vegetables was changed (A_1, A_2………..D_3, D_4) were compared to the same sessions of the frying groups where the reference acrylamide values were determined (Table 5).

There was no statistically significant difference ($p>0.05$) between the acrylamide levels formed in the frying sessions of peppers, eggplants, and zucchinis after potatoes in different orders and those in the same sessions of the frying groups in which the reference acrylamide values were obtained. After the fries (E_1, H_1, L_1) in which peppers, eggplants, and zucchinis took the first place, the average acrylamide levels in potatoes were determined as 1011 (F_2), 1012 (J_2), and 1022 (M_2) µg kg^{-1}, respectively. After the fries (F_2, J_2, M_2) in which peppers, eggplants, and zucchinis took the second place, the average acrylamide levels formed in potatoes were determined as 1094 (G_3), 1048 (K_3), and 1065 (N_3) µg kg^{-1}, respectively. In the statistical analysis, no significant difference emerged between the acrylamide level formed in potatoes after frying with peppers, eggplants, and zucchinis in different orders and the acrylamide levels in the same sessions in which potatoes were fried consecutively ($p>0.05$) (Table 5).

Palermo et al. (2016) identified three basic parameters to consider before implementing strategies for reducing acrylamide levels in foods: (i) the reduction rate that is obtained, (ii) assessing potential side effects, and (iii) the feasibility and economic impact of the application. In this study, the reduction strategies outlined in reducing the acrylamide levels in foods have neither any potential adverse effects nor any negative effects in terms of feasibility and economy. The proposed strategies are aimed solely at reducing or limiting the level of acrylamide in frying.

In the literature, there is no study on how the change in the frying order of the vegetables fried together affects the acrylamide levels of the same vegetables. Therefore, the data of this part of the study could not be compared with other studies. Current analyses show that despite the high acrylamide content, different orders of vegetables, including potatoes, in frying do not produce any significant results in reducing acrylamide levels. In other words, frying vegetables with high or low acrylamide do not increase the acrylamide levels of the following vegetables. Therefore, the findings show that fried foods do not cause an additional load of acrylamide in the oil. In this case, consumers can fry potatoes, sweet peppers, eggplants, and zucchinis by ordering them according to their preferences. Mestdagh et al. (2005) and Pedreschi

Table 5. The effect of change in frying order of vegetables on acrylamide levels (according to the same frying sessions)

	Frying sessions	n	Frying session compared (reference values)*	Z	P
The effect of potato (E_1, F_2, G_3) on sweet pepper	E_2^a	4	B_2^a	0.577	0.686
	E_3^a	4	B_3^a	1.155	0.343
	E_4^a	4	B_4^a	0.577	0.686
	F_3^a	4	B_3^a	1.309	0.781
	F_4^a	4	B_4^a	1.309	0.781
	G_4^a	4	B_4^a	0.000	1.000
The effect of potato (H_1, J_2, K_3) on eggplant	H_2^a	4	C_2^a	0.289	0.886
	H_3^a	4	C_3^a	0.289	0.886
	H_4^a	4	C_4^a	1.443	0.200
	J_3^a	4	C_3^a	0.577	0.686
	J_4^a	4	C_4^a	1.732	0.114
	K_4^a	4	C_4^a	1.732	0.114
The effect of potato (L_1, M_2, N_3) on zucchini	L_2^a	4	D_2^a	0.581	0.686
	L_3^a	4	D_3^a	0.289	0.886
	L_4^a	4	D_4^a	0.726	0.486
	M_3^a	4	D_3^a	1.155	0.343
	M_4^a	4	D_4^a	0.000	1.000
	N_4^a	4	D_4^a	0.000	1.000
The effect of sweet pepper, eggplant and zucchini on potato	E_1^a, H_1^a, L_1^a	12	A_1^a	0.728	0.867
	F_2^a, J_2^a, M_2^a	12	A_2^a	1.279	0.734
	G_3^a, K_3^a, N_3^a	12	A_3^a	3.199	0.362

n: Number of samples, P: Significance level, Z: Mann Whitney U test, *: Different letters in the same group indicate statistically significant differences ($p<0.05$)

et al. (2006) reported that asparagine and reducing sugars, which are the precursors of acrylamide, can leach into the frying oil from the surface layer of potatoes. Totani et al. (2007) stated that the oil used in deep fat frying cannot contaminate foodstuffs with acrylamide.

The results in this study show that consecutive use of oil is inconvenient in terms of acrylamide levels. Regardless of whether vegetables are fried on their own or with potatoes, the consecutive use of oil in all fryings significantly increases acrylamide levels. In this study, using sunflower oil more than twice in potatoes and more than three times in peppers, eggplants, and zucchinis was found to be statistically significant in terms of the increase in acrylamide levels. Therefore, not using frying oil in consecutive sessions can be considered as a limiting factor, although it does not reduce acrylamide levels in foods, which will also contribute positively to dietary acrylamide exposure.

The effects of the consecutive use of oils in frying on the formation of acrylamide are still unclear in the literature. The consecutive use of different oil types in French fries does not affect acrylamide levels (Zhang et al., 2015; Kamarudin et al., 2018), and there is a positive and significant relationship (Gertz, 2004; Lim et al., 2014; Kuek et al., 2020) between them. In the frying process, many compounds found in the oil and foods undergo significant changes, and many new compounds are formed (Karakaya and Şimşek, 2011; Wu et al., 2019). It has been reported that the concentration of compounds such as polar compounds, lipid oxidation products, hydroperoxide, and aldehyde, which affect the formation of acrylamide, increase with the consecutive use of oil in frying (Matthäus et al., 2004; Lalas, 2008; Kuek et al., 2020).

4. Conclusions

Frying, one of the commonly used methods of preparing foods for consumption, also causes acrylamide formation in foods. Therefore, it is hard to make acrylamide-free frying or make an arrangement in this context without affecting the sensory properties of the final product. It is possible to reach many studies in the literature that focus on reducing the high acrylamide levels forming with frying and strive to limit the adverse effects on human health. More research needs to be conducted in this area. In the study, many findings were found regarding the acrylamide levels formed in consecutive frying sessions of potatoes, peppers, eggplants, and zucchinis and the effect of the same vegetables fried in different orders on acrylamide levels. It is known that acrylamide is affected by the type of vegetables, their composition, their slice sizes, and their frying conditions like the type of oil, temperature, and time. Therefore, these results may differ from similar studies. This study focused only on specific vegetables and only one of the frying methods. However, consumers use many different frying techniques in home kitchens. Therefore, in the future, more research needs to be conducted to cover the kitchen applications of consumers, contributing to both food safety in the kitchen and thus public health. Therefore, new strategies will be developed to reduce the levels of acrylamide in foods.

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Declaration of Conflicts of Interest

No conflict of interest has been declared by the author.

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