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Effect of Different Roasting Temperatures on Acrylamide Formation of Some Different Nuts

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Abstract: *Consumers may prefer double roasted nuts as snack food since roasting process result in formation of aromatic compounds, taste and development of color in nut products. However, applying of high temperatures (above 120°C) to foods which include proteins and carbohydrates causes some toxic compounds such as acrylamide. In this study, acrylamide formation during roasting process was investigated at three different temperatures on almonds, sunflower seeds and peanuts that consumed in common. For that reason, total 12 samples which include raw samples of each nut were investigated. Acrylamide contents of all samples were determined by UHPLC-MS/MS. The method's limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0,33 ng mL^{-1} and 1 ng mL^{-1}, respectively. Despite no acrylamide content was observed in all unroasted nuts, increasing of temperature degrees promoted the occurrence of acrylamide. The highest concentration of acrylamide was determined in almonds (273 ng mL^{-1}), the lowest in peanuts (60.5 ng mL^{-1}) under different roasting conditions.*

Keywords: *Acrylamide, Almonds, Peanuts, Sunflower seeds, UHPLC-MS/MS*

I. Introduction

Heat treatments including cooking, frying, grilling, roasting and sterilization, usually applied at 90-220°C for food production and preservation. Heat treatment at high temperatures may result in formation of some toxic compounds, which can decrease the nutrition value and put food safety in danger. Those compounds are heterocyclic amines, polycyclic aromatic hydrocarbons, N-alkyl-N-nitrozamines and acrylamide which are known as carcinogenic and mutagenic components [1,2]. Acrylamide has been specified as 'likely carcinogen for humans' and included in Group 2A by the International Agency for Research on Cancer [3].

Acrylamide which forms because of heat treatment on high temperatures (120°C) of food containing carbohydrates and protein, is not a compound naturally found in foods [4]. There are many studies indicating that acrylamide forms in many foods such as potato chips, French fries, bread, biscuits, cakes, cereals, coffee, cocoa, roasted nuts and cornflakes, because of production conditions [5,6,7,8]. There are many major and minor pathways causing acrylamide formation. Acrylamide is generally formed in parallel to the Maillard reaction from the free amino acid asparagine and reducing sugars [9]. Addition to Maillard reaction, it is reported that 3-aminopropionamide compound, decarboxylated Amadori product, acrylic acid and acrolein forms acrylamide with different mechanisms [2,10,11,12,13]. Nuts as snack food are processed with heat treatments at different temperatures before going to market both in Turkey and in the World. Roasting process result in removal of raw taste and decreasing water activity which bring long shelf together.

Some consumers may demand or prefer double roasted nut products because of taste which is enhanced by aromatic compounds forming during roasting process. Regarding the consumer preferences, food producers put double roasted products on market. Double roasted products are prepared by setting either higher temperature or longer time parameters. The aim of this study was to investigate the effect of heat treatment at different temperatures on snacks including sunflower seeds, almonds and peanuts which are consumed commonly in Turkey. In this content, raw sunflower seeds, almonds and peanuts were roasted at three different temperatures under laboratory conditions and then, the dry matter, pH, ash, protein and fat contents, color change, sensory evaluation were performed. The results were evaluated statistically to determine the correlations between acrylamide formation and other parameters.

II. Materials and Method

There were 12 nut samples which were raw and roasted at 3 different temperatures from each kind of nuts including sunflower seed, almond and peanuts. Raw samples were packaged under controlled atmosphere and kept at laboratory conditions. All chemicals including acetonitrile (Panreac, Barcelona-Spain), anhydrous $MgSO_4$, acrylamide standard, formic acid (Sigma Aldrich-UK), and n-hexane, NaCl, Al_2O_3 (Merck-Darmstadt, Germany) were analytical grade. Dry matter, ash and protein contents, pH, color (L*,a*,b*) values of all samples were analyzed. Optimum roasting time and temperature parameters were determined with preliminary test. Tested time-temperature combinations were decided based upon the commercial applications. In order to

evaluate the temperature effect, it was gradually increased for every nut type's time was constant for each process. Analysis of acrylamide formation and other parameters were repeated 3 times with 2 parallels. In Table 1, roasting time and temperature parameters of each kind of nuts are shown.

Table 1 Roasting process parameters (time-temperature)

Type of nuts	Roasting Time (min)	1. roasting temperature (°C)	2. roasting temperature (°C)	3. roasting temperature (°C)
Sunflower seeds with shell	23	160	165	170
Almonds	23	140	145	150
Peanuts	40	150	155	160

2.1 Sample preparation procedure for acrylamide analysis

The sample preparation procedure for acrylamide analysis was performed according to Ali Omar et al. with some minor modifications [14]. Homogenized 1 gram of nut sample was put into 50 mL centrifuge tube. 5 mL n-hexane is added to the tubes for separating fat part and then it was shaken for 1 minute. Afterwards 10 mL of ultrapure water, 10 mL of acetonitrile, 5 g of anhydrous $MgSO_4$ and 1 g of NaCl mixture were added into the tubes. Samples were immediately mixed for 1 minute to avoid crystallization after addition of salt mixture. All samples were centrifuged at 4500 rpm for 6 minutes. After that 4 separate layers were observed. The top layer of hexane is removed. 3 mL of acetonitrile layer which contains acrylamide was transferred into 10 mL of small centrifuge tubes which contained 150 mg aluminium oxide. Later the samples including acrylamide were vortexed for 30 seconds and centrifuged at 4500 rpm for 3 minutes. 1 mL of supernatant was taken with injector and filtered through 0,45 µm Nacherey-Nagel filter, then transferred into glass tubes to evaporate under light stream of nitrogen gas. Dried residues were diluted with 200 µL ultrapure water and then vortexed for 1 minute. The samples were transferred into mini centrifuge vials and centrifuged at 10.000 rpm for 8 minutes. After this stage the supernatant was injected into a UHPLC auto sampler vial. Quantative analysis of acrylamide formation were determined at ng mL^{-1} level by using Ultra High Pressure Liquid Chromotography Mass Specthrometry (UHPLC-MS/MS).

2.2 Calibration curve preparation for acrylamide analysis

To prepare calibration curve, standart solutions at 7 different concentaration (ranged 0.1-100.0 ppb) were prepared. The linearity of calibration curve was shown in Fig. 1. (R= 0,9998). The seperation of acrylamide was done at UHPLC AB Sciex 3200 QTrap LC-MS/MS. Venusil AQ 3 µm C18 100 Å (2,1*50 mm) colon was used as chromatographic colon. Mobile phase A (% 90) was % 0,1 formic acid in ultrapure water and mobile phase B (%10) was % 0,1 formic acid in acetonitrile at flow rate 0,4 mL min^{-1}. Aliquots of 20 µL of the sample extract were injected into the chromatographic system using the autosampler. The ionization type was turbo ion spray positive polarity. Temperatures were 40˚C and 550°C oven and gas, respectively. Capillary voltage was 5000 V and press of nebulizer 1:2 were 40:60 psi.

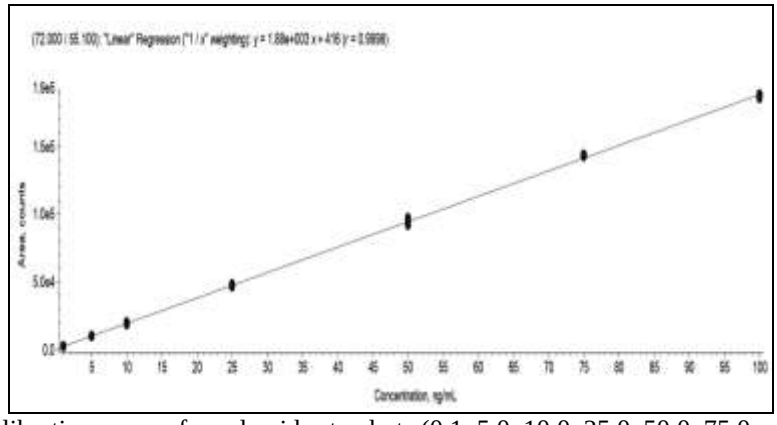

Figure 1. Calibration curve of acrylamide standarts (0.1, 5.0, 10.0, 25.0, 50.0, 75.0 and 100.0 ppb)

2.3 Determination of recovery values, LOD and LOQ for acrylamide analysis

For recovery study, unroasted nut samples including sunflower seeds, almonds and peanuts were used. After fat seperation of samples , they were spiked with acrylamide standards at different concentrations. Extraction of acrylamide was performed as explained before. The extract which was obtained from this process was diluted by mobile phase and calibration curve points were prepared. LOD and LOQ values were calculated with these equilibrium:

LOD = 3,3* s/S and LOQ = 10* s/S [14].

In specific concentration; s indicates the lowest concentration and S shows the signal/noise rate in the lowest concentration. According to these equilibrium the sample's limit of detection (LOD) and limit of quantification (LOQ) values were determined as 0,33 ng mL^{-1} and 1 ng mL^{-1} respectively.

III. Result and Discussion

3.1 The physicochemical properties and acrylamide content of sunflower seed roasted at different temperatures

The physicochemical properties which are dry matter, ash, protein and fat content, pH and color parameter of sunflower seed are indicated in Table 2. As seen in Table 2, % dry matter, % ash, % protein and % fat content and a* value were increased with rising temperature. The differences of each samples were statistically significant ($p<0,05$). The difference of between pH values of raw samples and samples roasted at 170°C were not important ($p>0,05$) statistically, whereas others were confirmed as significantly different ($p<0,05$). While the roasting temperature increased, the L* value of samples initially rose and then decreased. Rising temperaure caused to increase a* value of sunflower seeds. In a different study, Özdemir et. al. reported the highest a* value (4,07) of hazelnuts in the minimum water activity [15]. All the b* value of the samples increased gradually while temperature increasing, it decreased slightly at highest roasting temperature (170°C). The differences between b* values of each sunflower seed samples were significantly important ($p<0,05$).

Table 2 Dry matter, ash, protein and fat content, pH and color parameter of sunflower seed roasted at 160, 165 and 170°C for 23 minutes (n=6)

THE ANALYSES AND RESULTS OF SUNFLOWERSEEDS (AVERAGE RATIOS)								
ROASTING TEMPERATURE (°C)	DRY MATTER (%)	ASH (%)	pH	PROTEİN (%)	FAT (%)	COLOR		
						L*	a*	b*
Unroasted sample	95,17 ±0,18[a]	3,4991 ±0,29[a]	6,08 ±0,27[c]	10,961 ±0,31[a]	40,673 ±0,55[a]	52,28 ±0,04[a]	-0,22 ±0,14[a]	9,57 ±0,08[a]
160	98,40 ±0,35[b]	3,5860 ±0,42[b]	5,67 ±0,21[b]	20,66 ±0,01[b]	44,227 ±0,34[b]	58,42 ±0,15[d]	1,04 ±0,08[b]	15,07 ±0,02[b]
165	98,67 ±0,39[c]	3,6360 ±0,31[c]	5,56 ±0,36[a]	21,942 ±0,28[c]	48,334 ±0,42[c]	57,78 ±0,13[c]	2,14 ±0,20[c]	15,35 ±0,20[d]
170	99,32 ±0,34[d]	3,7391 ±0,25[d]	6,05 ±0,35[c]	22,5581 ±0,25[d]	48,527 ±0,39[d]	54,00 ±0,14[b]	3,17 ±0,19[d]	15,24 ±0,13[c]

[a-d:] The values with same letter are not significantly different for each parameters ($p>0.05$)

In acrylamide analysis, recovery value of sunflower seeds was calculated as % 112,6. Acrylamide concentration showed positive correlation with increasing roasting temperature in sunflower seeds and the highest amount of acrylamide (61,5 ng mL^{-1}) detected at the highest (170°C) roasting temperature ($p<0,01$, $R=0,970$). Comparably to our study, Jägerstad and Skog reported the concentration of acrylamide as 66 µg kg^{-1} in sunflower seeds [16]. Additionally, lower acrylamide contents were observed in sunflower seeds when compared with almonds and peanuts even though it roasted at 170°C which is higher than roasting temperature of other samples. Since sunflower seeds roasted with shell, it is thought that acrylamide formation may be prevented by shell providing protection effect from roasting process. However there is no enough research about the acrylamide content of sunflower seeds. The acrylamide concentrations of sunflower seeds with rising temperature is shown in Fig. 2.

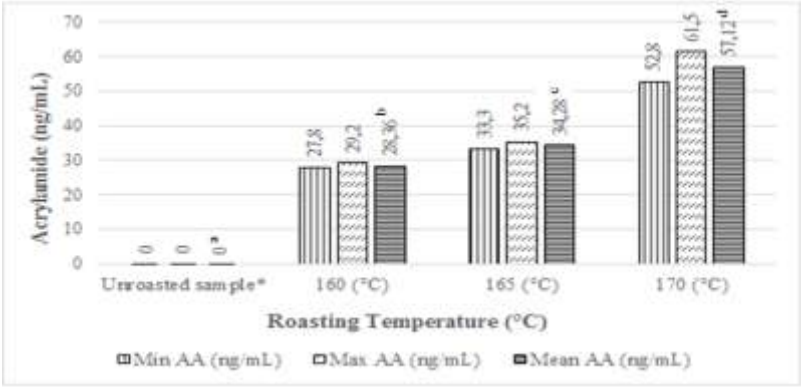

Figure 2 The minimum (Min), maximum (Max) and average (Mean) acrylamide (AA) concentrations of sunflower seeds roasted at different temperatures (n=6)

*Under the detection limit (LOD=0,33 ng mL^{-1})

[a-d:] The values with same letter are not significantly different for each parameters ($p>0.05$)

3.2 The physicochemical properties and acrylamide content of almond roasted at different temperatures

Investigated properties of almond including % dry matter, % ash, % protein, % fat and a* value increased with rising temperature. Contrary to this situation, rising temperature caused a decrease in pH, L* and b* values of the samples. Also, it was found that the physicochemical properties of all samples processed at different temperatures were significantly different ($p<0,05$). The physicochemical properties which are dry matter, ash, protein and fat content, pH and color parameter of almonds are indicated in Table 3.

Table 3 Dry matter, ash, protein and fat content, pH and color parameter of almond roasted at 140, 145 and 150°C for 23 minutes (n=6)

THE ANALYSES AND RESULTS OF ALMONDS (AVERAGE RATIOS)								
ROASTING TEMPERATURE (°C)	DRY MATTER (%)	ASH (%)	pH	PROTEİN (%)	FAT (%)	COLOR		
						L*	a*	b*
Unroasted sample	96,00 ±0,28[a]	2,9435 ±0,25a	6,148 ±0,03d	19,945 ±0,35[a]	34,88 ±0,02[a]	37,728 ±0,04[d]	10,32 ±0,09[a]	16,393 ±0,01[d]
140	97,61 ±0,30[b]	2,9664 ±0,16[b]	5,873 ±0,02[c]	20,256 ±0,35[b]	42,19 ±0,03[b]	36,928 ±0,02[c]	10,975 ±0,10[b]	15,531 ±0,14[c]
145	97,77 ±0,33[c]	3,0397 ±0,34[c]	5,826 ±0,03[b]	20,416 ±0,27[c]	43,98 ±0,03[c]	35,706 ±0,01[b]	11,166 ±0,05[c]	15,36 ±0,02[b]
150	98,21 ±0,27[d]	3,0912 ±0,12[d]	5,71 ±0,03[a]	20,57 ±0,33[d]	45,02 ±0,03[d]	35,593 ±0,01[a]	11,07 ±0,08[b,c]	15,033 ±0,005[a]

[a-d:] The values with same letter are not significantly different for each parameters ($p>0.05$)

The highest acrylamide concentration was determined as 273 ng mL^{-1} in almonds roasted at 150°C which was the highest temperature applied (Fig.3). A positive correlation was detected between acrylamide formation and increasing roasting temperature (R=0,905). In acrylamide analysis, recovery value of almond samples was calculated as % 108,2. Similar roasting conditions were applied to almonds in the study of Lukac et. al. and the acrylamide contents were determined as an average 494 µg kg^{-1} [17]. Tekkeli et. al. reported that acrylamide content of almonds as 95,5 µg kg^{-1} after they applied stacking technique[18]. This differences may based on variety of almonds, and process conditions.

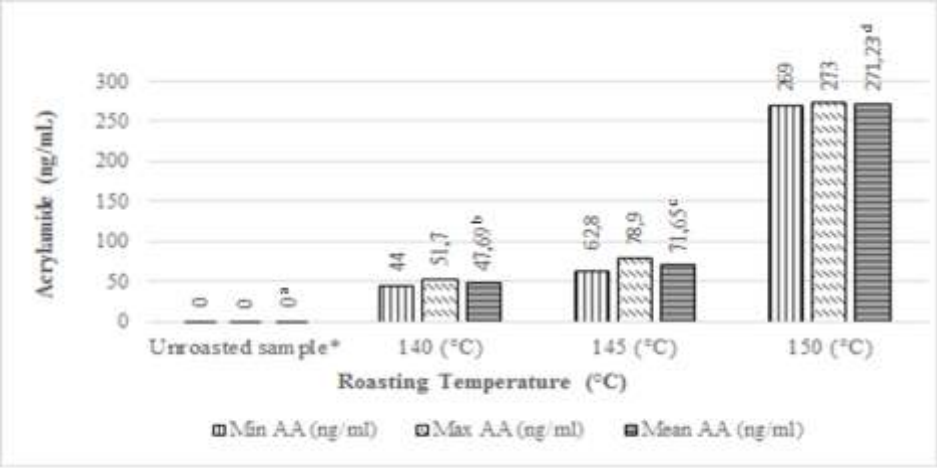

Figure 3. The minimum (Min), maximum (Max) and average (Mean) acrylamide (AA) concentrations of almonds roasted at different temperatures (n=6)

*Under the detection limit (LOD=0,33 ng mL^{-1})

[a-d:] The values with same letter are not significantly different for each parameters ($p>0.05$)

Friedman stated that the acrylamide concentration in almond samples was as 260 µg kg^{-1} in his review including various foods [19]. Similarly acrylamide content of almond ranged from 207-313 µg kg^{-1} (mean 260 µg kg^{-1}) in Ölmez et al. 's study [8]. There is a review about acrylamide content of the food products including almonds roasted with salt reported the concentration as average 657 µg kg^{-1} [20]. In different almond products the acrylamide contents were determined by Amrein et. al. as follows: 443 µg kg^{-1} in roasted almonds, 196 µg kg^{-1} in bakery products containing almonds. According to Amrein et. al. the mean acrylamide concentration in all almond products was reported as 250 µg kg^{-1} [21]. As seen in the literature acrylamide contents are similar to result of this study. Heating process can cause increase in redness parameter of almond samples initially, however, a* value decreased subsequently after it reached the highest level. It also observed in this study a*

value of almond samples increased initially but it decreased at the highest roasting temperature (150°C). Similarly, Gökmen and Şenyuva reported that a* value increased initially and than decreased in the highest roasting temperature in green coffee [22].

3.3 The physicochemical properties and acrylamide content of peanut roasted at three different temperatures

The physicochemical properties including dry matter, ash, protein and fat content, pH and color parameters, of peanut are indicated in Table 4. As a result of applying different roasting temperatures to peanut samples some changes were observed in nutrition values. While roasting temperature rising, % dry matter, % ash, % protein, % fat content, a* and b* values were increased. pH and L* value decreased despite the high roasting temperatures. The differences between all peanut samples were determined significantly ($p<0,05$).

Table 4 Dry matter, ash, protein and fat content, pH and color parameter of peanut roasted at 150, 155 and 160°C for 40 minutes (n=6)

THE ANALYSES AND RESULTS OF PEANUTS (AVERAGE RATIOS)								
ROASTING TEMPERATURE (°C)	DRY MATTER (%)	ASH (%)	pH	PROTEİN (%)	FAT (%)	COLOR		
						L*	a*	b*
Unroasted sample	91,30 ±0,02[a]	2,0101 ±0,38[a]	6,5 ±0,02c	23,5073 ±0,02[a]	43,877 ±0,02[a]	62,251 ±0,04[d]	-0,151 ±0,20[a]	21,03 ±0,03[a]
150	99,00 ±0,03[b]	2,2260 ±0,29[b]	6,32 ±0,03[b]	24,879 ±0,03[b]	45,463 ±0,02[b]	54,766 ±0,02[c]	5,91 ±0,11[b]	22,41 ±0,04[d]
155	99,14 ±0,03[c]	2,2682 ±0,03[c]	6,26 ±0,07[b]	25,625 ±0,02[c]	46,59 ±0,03[c]	54,078 ±0,12[b]	5,95 ±0,05[b]	22,19 ±0,43[c]
160	99,20 ±0,02[d]	2,3062 ±0,03[c]	6,19 ±0,06[a]	25,713 ±0,02[c]	47,433 ±0,03[d]	52,903 ±0,11[a]	6,97 ±0,12[c]	22,03 ±0,07[b]

[a-d:] The values with same letter are not significantly different for each parameters ($p>0.05$)

In acrylamide analysis recovery value of peanuts was determined as % 105. When the acrylamide contents of peanuts were investigated, the lowest (21,4 ng mL^{-1}) and the highest (60,5 ng mL^{-1}) concentrations were determined at 150°C and 160°C respectively as seen in Fig. 4. The roasting temperature is the key factor for acrylamide formation in peanut samples, since there is a positive correlation between temperature and acrylamide concentration (R=0,934). A study conducted by Ölmez et. al. stated that acrylamide level of peanuts were determined as 66 µg kg^{-1} ,similarly to this study [8]. There is another study in New Zeland by Cressey et. al. to search the acrylamide content of various snack foods reported acrylamide concentration of dry roasted peanuts as in the range of 9-84 µg kg^{-1} and average 42 µg kg^{-1} [23]. Paola et al. reported that the acrylamide content of peanuts roasted at different temperatures between 1,08-42,86 µg kg^{-1} which is lower than the results of this study, since they used salted peanuts in shell [24]. Moreover a review of Bureau of Chemical Safety presented that acrylamide contamination of three different peanut butter as 122, 99, 85 µg kg^{-1} and of salted peanuts as 31 µg kg^{-1} [20]. It is seen that salted peanuts have relatively lower acrylamide content, because salting process provide monovalent cations to prevent acrylamide formation like in study of Gökmen and Şenyuva [25]. Peanut butter showed relatively higher acrylamide concentrations than peanut kernels because of free fat content. It is thought that free fat content may promote acrylamide formation via acrolein pathway in peanut samples [20].

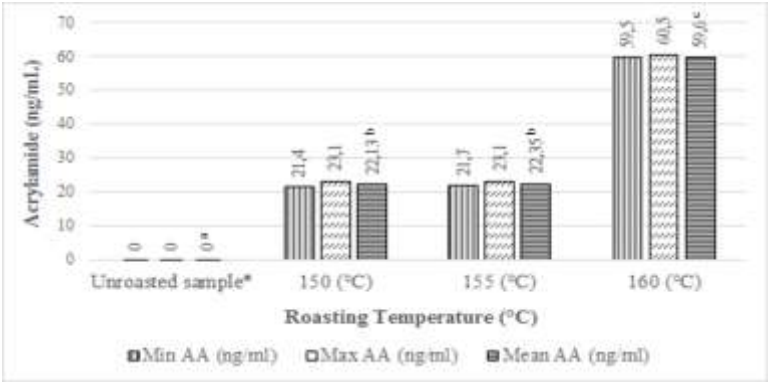

Figure 4. The minimum (Min), maximum (Max) and average (Mean) acrylamide (AA) concentrations of peanuts roasted at different temperatures (n=6)

*Under the detection limit (LOD=0,33 ng mL^{-1})

[a-d:] The values with same letter are not significantly different for each parameters ($p>0.05$)

IV. Conclusion

In this study the effect of roasting conditions (time-temperature) on acrylamide formation were investigated because of high preference of double roasted nut products by consumers. Usually consumers choose to eat nuts as a healty snack food because of beneficial components of them. Although when they prefer double roasted nuts, they are exposed to higher acrylamide contents. From this study, it is concluded that the maximum acrylamide contents were determined in the nuts roasted at highest temperatures. In order to minimize acrylamide content, it should be avoided from very high roasting temperatures. It is determined that there is no acrylamide formation in all unroasted nut samples, therefore some nuts especially almonds should be consumed without roasting in order to prevent hazardous health effect of acrylamide.

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