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ARAŞTIRMA MAKALESİ

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A Comparison of Acrylamide Contents of Some Nuts Produced Organically and Conventionally

Organik ve Konvansiyonel Yöntemlerle Üretilen Çeşitli Kuruyemişlerin Akrilamid İçeriklerinin Karşılaştırılması

Baha Aydın YILMAZ¹, Işın AYDIN UNSAL², Murat TAŞAN^{3*}

Abstract

Acrylamide is a carcinogenic and neurotoxic compound defined as a heat treatment process contaminant. Due to its health concern, acrylamide formation needed to be minimized. The objective of this study is to examine how much acrylamide is contained in dried almonds (Prunus dulcis), hazelnuts (Corylus aveilana), pistachios (Pistacia vera), peanuts (Arachishypogaea), sunflower seeds (Helianthus annuus) as well as pumpkin seeds (Cucurbita pepo) that have been cultivated via organically-certified and conventional processes. Frequently and regularlyconsumed nuts - comprising 180 samples that have been cultivated via organically-certified and conventional processes - were studied with UHPLC-MS/MS approach in Turkey. Substantial disparities were statistically found (P<0.05) between the almond, pistachio, peanut and sunflower seed variations that have been cultivated via organically-certified and conventional processes. Conversely, no considerable difference could be found among the variations of hazelnut and pumpkin seeds. It could be observed that the average concentrations of acrylamide in the nuts cultivated via organically-certified and conventional processes were 1.68 ng ml⁻¹ and 266.14 ng ml⁻¹ in almonds, 7.90 ng ml⁻¹ and 6.68 ng ml⁻¹ in hazelnuts, 4.86 ng ml⁻¹ and 9.95 ng ml⁻¹ in pistachios, 14.09 ng ml⁻¹ and 36.27 ng ml⁻¹ in peanuts, 5.96 ng ml⁻¹ and 4.54 ng ml⁻¹ in pumpkin seeds correspondingly. The amount of acrylamide was not ascertained in organically-certified sunflower seeds, while in conventional sunflower seeds, the amount was 16.92 ng ml⁻¹. According to the generally-accepted theory, the production of acrylamide is attributed to the Maillard reaction that takes place during the processing and preparation of high-temperature foods. The data obtained show that consumers should be informed more accurately about the food safety of organic nuts. The effects of organically produced nuts on acrylamide intake from food in daily consumption should also be taken into account. In order to prevent or reduce the formation of acrylamide compounds in organic nuts and to monitor them more effectively, extensive studies should be carried out and food heat treatment methods should be optimized.

Keywords: Acrylamide, Food safety, Organic certified nuts, Heat process contaminant, UHPLC-MS/MS

¹Baha Aydin Yilmaz, Department of Food Engineering, Faculty of Agriculture, Tekirdag Namık Kemal University, Tekirdag, Türkiye. E-mail: aydinbahayilmaz@gmail.com

²Isin Aydin Unsal, Department of Food Engineering, Faculty of Agriculture, Tekirdag Namık Kemal University, Tekirdag, türkiye. E-mail: <u>isin_aydin07@hotmail.com</u> D OrcID: 0000-0002-6005-1315. ³*Sorumlu Yazar/Corresponding Author: Murat Tasan, Department of Food Engineering, Faculty of Agriculture,, Tekirdag Namık Kemal University, Tekirdag,

^{3*}Sorumlu Yazar/Corresponding Author: Murat Tasan, Department of Food Engineering, Faculty of Agriculture,, Tekirdag Namık Kemal University, Tekirdag, Türkiye. E-mail: <u>mtasan@nku.edu.tr</u> (¹⁰) OrcID: 0000-0003-1490-7626.

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Öz

Akrilamid, ısıl işlem proses kirleticisi olarak tanımlanan kanserojen ve nörotoksik bir bileşiktir. Sağlık endişesi nedeniyle akrilamid oluşumunun en aza indirilmesi gerekmektedir. Bu çalışmanın amacı, organik sertifikalı ve konvansiyonel yöntemlerle üretilen badem (Prunus dulcis), findık (Corylus avellana), antepfistiği (Pistacia vera), yerfistiği (Arachishypogaea), ayçiçeği çekirdeği (Helianthus annuus) ve kabak çekirdeğinde (Cucurbita pepo) akrilamid miktarlarını araştırmak ve bulunan değerleri karşılaştırmaktadır. Türkiye'de organik sertifikalı ve konvansiyonel yöntemlerle yetiştirilen ve ülke genelinde yaygın ve düzenli olarak tüketilen kuruyemiş çeşitlerinden 180 adet örnek UHPLC-MS/MS cihazı kullanılarak incelenmiştir. Organik sertifikalı ve konvansiyonel yöntemlerle yetiştirilen badem, antep fıstığı, yerfistiği ve ayçiçeği tohumu çeşitleri arasında istatistiksel olarak önemli farklılıklar bulunmuştur (P<0,05). Buna karşılık, fındık ve kabak çekirdeği çeşitlerinde önemli bir fark bulunmamıştır. Organik sertifikalı ve konvansiyonel yöntemlerle üretilmiş kuruyemişlerde ortalama akrilamid konsantrasyonlarının sırasıyla bademde 1,68 ng ml⁻¹ ve 266,14 ng ml⁻¹, fındıkta 7,90 ng ml⁻¹ ve 6,68 ng ml⁻¹ olduğu, sırasıyla antep fistiğinda 4,86 ng ml⁻¹ ve 9,95 ng ml⁻¹, yerfistiğinda 14,09 ng ml⁻¹ ve 36,27 ng ml⁻¹, kabak çekirdeğinde 5,96 ng ml⁻¹ ve 4,54 ng ml⁻¹ olarak belirlenmiştir. Organik sertifikalı ayçiçeklerinde akrilamid miktarı tespit edilmezken, konvansiyonel yöntemlerle üretilmiş ayçiçeklerinde ise 16,92 ng ml⁻¹ olarak belirlenmiştir. Genel kabul gören teoriye göre akrilamid oluşumu, yüksek sıcaklıktaki gıdaların işlenmeşi ve hazırlanması sırasında meydana gelen Maillard reaksiyonuna bağlanmaktadır. Elde edilen veriler, tüketicilerin organik kuruyemişlerin gıda güvenliği konusunda daha doğru bilgilendirilmesi gerektiğini göstermektedir. Organik olarak üretilen kuruyemişlerin günlük tüketimde gıdalardan akrilamid alımına etkileri de dikkate alınmalıdır. Organik kuruyemislerde akrilamid bilesiği olusumunu önlemek yeva azaltmak ve daha etkin bir şekilde izlemek için kapsamlı çalışmalar yapılmalı ve gıda ısıl işleme yöntemleri optimize edilmelidir.

Anahtar Kelimeler: Akrilamid, Gıda güvenliği, Organik sertifikalı kuruyemişler, Isıl proses kontaminat, UHPLC-MS/MS

1. Introduction

The interest in food products that are believed to be environmentally and health friendly such as natural and organic foods is increasing day by day. Organic farming is a sustainable farming technique that avoids the use of unnatural inputs such as pesticides and synthetic fertilizers and supplies quality, health and environmental standards. Organic food production, on the other hand, is the production in which inappropriate practical application and process are not used, without agricultural residues and chemical food additives, the least processed product and the most appropriate packaging. With the global orientation, an organic market with a rapidly growing trade volume has emerged today (Gok, 2008). Fruits, field crops and vegetables constitute a significant portion of organic herbal products produced in Turkey. Since most of the organic products grown in our country are exported, production is shaped according to the demand from abroad (Demiryurek, 2011).

Nuts and fresh/dry fruit products are organic products exported in Turkey. Production is gathered in nine main categories. These are dried fruits, nuts, fresh and processed fruits and vegetables, legumes, cereals, oil seeds, industrial plants, spices and medicinal plants, and other raw or processed products. Walnut, pistachios, almond, peanut, chestnut are included in the category of hard-shelled fruits. Sunflower and sesame are in the category of oily seeds. Peanuts, poppy seeds, hazelnut flour, apricot kernels etc. are in the other category.

According to studies conducted for consumers, organic foods are perceived as safer, more nutritious and without chemicals that harm health in their content (Karcik and Tasan, 2018). However, the results of studies showing that organic foods are safer than conventional foods are quite contradictory and interesting. The Institute of Food Technologies states that there is a qualitative difference between organic and traditional foods, and more data is needed to state that both production systems are superior to each other in terms of safety or nutritional composition (Turkozu and Karabudak, 2013). It is stated in some studies (Buttriss and Hughes 2000; Magkos et al., 2003; Tosun and Kaya, 2010; Berker, 2012) that various contaminations can be seen in organic foods depending on environmental factors and may involve various risks.

In our country and in the world, heat treatment is applied to nuts at certain temperatures before being offered for consumption. With the application of heat treatments, toxic chemical compounds that may adversely affect the nutritive properties of the food may occur, as well as the formation of the desired properties such as color, taste and structure (Claeys et al., 2005). Compounds such as acrylamide, hydroxymethylfurfural, chloropropanol and its esters, polycyclic aromatic hydrocarbons, N-alkyl N-nitrosamines, trans fatty acids, furan and its derivatives are process contaminants formed as a result of heat treatment. These chemical compounds produce carcinogenic and/or mutagenic effects (Yavuz and Ozcelik, 2013; Mogol, 2014; Tasan and Demir, 2019). Among the mentioned thermal process contaminants, the detection of acrylamide and 5-hydroxymethylfurfural (HMF), its metabolism, its effects on health, and the development of strategies to reduce its levels have been the subject of many studies (Masatcioglu, 2013; Mogol, 2014). Acrylamide is a carcinogenic (Mestdagh et al., 2008) and neurotoxic compound originating from food ingredients and defined as a process contaminant. Although acrylamide is not found in raw foodstuffs, its amounts in heat-treated foods are of concern. Acrylamide formation is associated with the Maillard reaction that occurs as a result of high heat during the preparation and processing of foods. This reaction occurs at temperatures above 100°C. When the required humidity level is provided, acrylamide formation can be observed at temperatures below 120°C (Pedreschi et al., 2007). Although there is a linear relationship between the degree of processing temperature and time and the formation of acrylamide, there are differences in acrylamide level even between different products of the same food varieties or the same products produced on different dates (Can, 2007). The composition, variety, storage conditions and seasonal changes of foodstuffs, especially in terms of asparagine and reducing sugars (especially fructose and glucose), cause differences in acrylamide content (Gokmen et al., 2006). Acrylamide contents have been determined in the vast majority of foodstuffs widely consumed in the world. There are limited studies on the acrylamide content of our traditional products in our country.

In the evaluation of the quality of organic foods, foods not only should be questioned in terms of harmful compounds that are transmitted as a result of environmental effects such as heavy metals, pesticides or incorrect applications but also, antinutrient compounds that can be formed by food processing should be considered (Finotti et al., 2006). Some varieties of organic nuts can also be heat treated. In Turkey, dried nuts are produced mostly by traditional methods and by applying minimum processing technologies (Tezer et al., 2015). In industrial

applications, for example, hazelnuts are processed between 100-180°C for 5-10 minutes. Nuts variety, air circulation and similar factors are decisive in these processes (Suvari et al., 2017).

When the studies on acrylamide are examined in the literature, it is seen that the studies focus on the effects of different heat treatment methods (Zhang and Zhang, 2008), parameters (Nizamlioglu, 2015; Unver, 2016; Asadi et al., 2020), storage time (Amrein et al., 2005), food categories (Sayaslan et al., 2008; Karasek et al., 2009). In this study, the acrylamide content that can be formed by heat treatment in organic nuts was determined and compared with nuts produced by conventional methods, it contributed significantly to the literature with the results of specific food quality in the field of organic food safety.

2. Materials and Methods

2.1. Materials

Organic certified nut varieties were determined as almond (*Prunus dulcis*), hazelnut (*Corylus avellana*), pistachio (*Pistacia vera*), peanut (*Arachishypogaea*), sunflower seeds (*Helianthus annuus*) and pumpkin seeds (*Cucurbita pepo*). In the study, samples of each organic certified nut variety were obtained from five different brands in the market. In order to make comparisons and evaluations, samples from five different brands were obtained from the same dried nut varieties produced by conventional methods. In the sample procurement, attention was paid to ensure that there were samples from all brands with three different batch numbers, and logo and certificate numbered ones were selected on the label. Acrylamide analyzes were applied to the dried nuts samples, which were obtained in shell, after the shells were removed.

2.2. Chemicals and reagents

Magnesium sulfate anhydrus (MgSO₄) (purity>99.5%), acrylamide analytical standard (purity> 99.8%) and formic acid Sigma-Aldrich (UK) company, sodium chloride, aluminum oxide (Al₂O₃) and n-hexane were obtained from Merck (Darmstadt, Germany), acetonitrile (ACN) was obtained from PanReac (Barcelona, Spain). Ultrapure water (MilliQ system, Millipore, Bedford, MA, USA) was used in analysis.

2.3. Samples preparation

1 g of dried nut samples (grinded in a grinder (Siemens MC 23 200) and homogenized) were weighed and placed in a 50 ml centrifuge tube. 5 ml of n-hexane was added and shaken with vortex (Heidolph Reax Top) for 1 minute. Then, a mixture of 10 ml of ultrapure water, 10 ml of acetonitrile (ACN), 5 g of anhydrous magnesium sulfate (MgSO₄) and 1 g of sodium chloride (NaCl) was added into the tube. After the salts were added, the tubes were vortexed again for 1 minute to prevent crystallization. The tubes were centrifuged at 4500 rpm for 6 minutes and separated into layers. 3 ml of liquid from the middle chamber containing acrylamide was transferred to 15 ml small centrifuge tubes containing 150 mg of aluminum oxide. The mixture was vortexed for 30 seconds and then centrifuged at 4500 rpm for 3 minutes. After centrifugation, 2 ml of the upper liquid was drawn with a syringe, passed through 0.45 μ m Macherey-Nagel (Chromafil AO 1745/25) filters, transferred to glass tubes and evaporated under nitrogen gas to ensure dryness with a light stream. The residue was diluted by adding 200 μ l of ultrapure water to the dried glass tubes and mixed with vortex for 1 minute. The samples taken from here into mini centrifuge vials were centrifuged at 14800 rpm for 10 minutes and transferred from these vials to vials with glass inserts. The amount of acrylamide was measured by injecting the samples twice into the UHPLC-MS/MS (Ultra High-Pressure Liquid Chromotography Mass Specthrometry, AB Sciex, 3200 QTrap) and the mean values were reported as ng ml-1 with their standard deviations (Ali Omar et al., 2015).

2.4. Preparation of the calibration curve

In order to detect acrylamide quantitatively, calibration standards at different concentrations were injected into the UHPLC-MS/MS device and a linear calibration curve (R^2 =0.9997) was drawn. 100 ppm main stock solution was obtained and stored at -18°C. By diluting the main stock with ultrapure water, 2 ppm intermediate stock acrylamide was obtained. Then, by making dilutions to the amounts taken from the intermediate stock solution, calibration standards were obtained at concentrations of 0-1-5-10-25-50-75-100 ppb. The prepared intermediate stock solutions were stored at +4°C.

2.5. Chromatographic conditions

Studies were carried out on the UPLC-MS/MS device according to the following conditions;

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Injection volume: 20 µl, Column: Venusil AQ C18 3 µm 100 Å (2.1 mm x 50 mm), Detector: MS/MS detector

Mobil Phase A: Ultrapure water containing 0.1% formic acid (90%), Mobile Phase B: Acetonitrile with 0.1% formic acid (10%), Flow rate: 0.25 ml mobile phase/minute, Pump: Turbo pump, Column temperature: 40°C

Source parameters; Gas temperature: 550°C, Ionization type: Turbo ion spray positive polarity, Capillary voltage: 5500 V, Curtain Gas (CUR): 20 psi, Nebulizer pressure 1 (GS 1): 40 psi, Nebulizer pressure 2 (GS 2): 60 psi, Infer face heater (Ihe): On

2.6. Recovery, limit of detection (LOD) and limit of quantitation (LOQ)

Almonds, sunflower seeds, peanuts, hazelnuts, pistachios and pumpkin seeds without acrylamide were studied in raw samples. After separating the oil by adding n-hexane on 1 gr sample. 500 μ l of 100 ppm stock acrylamide standard solution was added. Extraction continued. The necessary standards for the calibration curve were prepared by diluting the extract obtained as a result of this process with the mobile phase. According to these standards the detection (LOD, Limit of Detection) limit of the samples was 0.33 ng ml⁻¹ (ppb), the detection (LOQ, Limit of Quantitation) limit was 1 ng ml⁻¹ (ppb). The % recovery values of sunflower seeds, almonds, peanuts, pistachios, hazelnuts and pumpkin seeds were determined as 143%, 136%, 137%, 135%, 118% and 128%, respectively.

2.7. Statistical analysis

Analyzes were performed in triplicate for each sample. The results were calculated as the arithmetic mean and standard errors (\pm) of the replications. Statistical analyzes of the obtained data were performed with ANOVA and Duncan test using STATISTICA Software. The significance of the difference between the mean data in the tables is shown with the lettering system.

3. Results and Discussion

Acrylamide content of almond, hazelnut and pistachios produced via organically certified and conventional process are shown in the *Table 1*. Acrylamide contents of peanuts, sunflower seeds and pumpkin seeds produced via organically certified and conventional process are shown in the *Table 2*.

Table 1. Acrylamide contents of almonds, hazelnuts and pistachios produced by conventional methods and
certified organic (ng ml ⁻¹)

Samples	Almond		Hazelnut		Pistachios	
(Company number)	Organic ¹	Conventional ²	Organic	Conventional	Organic	Conventional
1	2.47±0.07b	76.81±3.16e	17.54±0.77a	9.49±0.17a	4.51±0.11c	4.44±0.20d
2	0.33±0.04e	241.08±15.27c	8.86±0.06b	3.34±0.09d	5.22±0.12b	25.38±0.63a
3	3.22±0.11a	231.16±4.50d	6.40±0.12c	8.94±0.09b	6.33±0.42a	5.22±0.20c
4	1.35±0.10c	318.62±21.43b	3.50±0.12d	3.22±0.10d	3.09±0.14d	5.07±0.16c
5	1.02±0.04d	463.05±36.03a	3.18±0.15d	8.43±0.10c	5.14±0.04b	9.66±0.14b
Overall average	1.68±1.16B	266.14±140.74A	7.90±5.87	6.68±3.13	4.86±1.19B	9.95±8.87A

¹: They are the results of organic certified company samples and are the average of three replications

²: They are the results of the company samples produced by the conventional method and are the average of three replications. The differences between the averages shown with different letters for the company samples were found to be statistically significant, and the lettering was done in vertical alignment, a, b, c, d (\downarrow), (P<0.05). The differences between the averages of the same type of nuts shown with different letters for the general average were found to be statistically significant, and the lettering was made in horizontal alignment, A, B (\rightarrow), (P<0.05).

Samples	Peanut		Sunflower		Pumpkin seeds	
(Company number)	Organic ¹	Conventional ²	Organic	Conventional	Organic	Conventional
1	26.64±3.42a	33.64±4.92b	ND	37.75±4.99a	12.61±2.21a	2.41±0.50c
2	19.17±1.40b	26.17±1.11b	ND	29.25±3.47b	1.18±0.08b	4.65±0.49b
3	7.33±0.42c	65.61±7.98a	ND	NDd	0.95±0.12b	4.76±0.36b
4	9.40±0.92c	15.09±0.10c	ND	NDd	0.99±0.12b	3.89±0.73b
5	7.89±0.73c	40.82±0.62b	ND	17.58±1.75c	14.06±1.78a	6.98±0.93a
Overall average	14.09±8.51B	36.27±18.97A	NDB	16.92±17.02A	5.96±6.75	4.54±1.66

 Table 2. Acrylamide contents of peanuts, sunflower seeds and pumpkin seeds produced by conventional methods and certified organic (ng ml⁻¹)

¹: The results of the organic certified company samples and the average of three replications.

²: It is the results of the company samples produced by the conventional method and is the average of three replications. ND: Not at detectable level.

The differences between the averages shown with different letters for the company samples were found to be statistically significant, and the lettering was done in vertical alignment, a, b, c, d (\downarrow), (P<0.05). The differences between the averages of the same type of nuts shown with different letters for the general average were found to be statistically significant, and the lettering was made in horizontal alignment, A, B (\rightarrow), (P<0.05).

The acrylamide values of almonds produced by organic certified and conventional methods were 1.68 ± 1.16 ng ml⁻¹ (range 0.33-3.22 ng ml⁻¹) and 266.14 ± 140.74 ng ml⁻¹ (range 76.81-463.05 ng ml⁻¹), were determined, respectively. Since it is known that almond contains acrylamide precursors, free asparagine and reducing sugar, at significant levels, the roasting temperature and time parameters should be adjusted appropriately. On the other hand, although acrylamide values determined in organic certified almonds in the study remained at a very low level, there is a possibility that short-term heat treatment was carried out. The data showed that it is important to evaluate almonds and almond products that may contain almonds in terms of acrylamide content.

The acrylamide values of hazelnuts produced by organic certified and conventional methods were 7.90 ng ml⁻¹ (range 3.18-17.54 ng ml⁻¹) and 6.68 ng ml⁻¹ (range 3.22-9.49 ng ml⁻¹), were determined, respectively. Although the average acrylamide values of organic certified hazelnuts were found to be higher, this difference is not statistically significant. In the study, acrylamide values determined in hazelnuts produced by organic certified and conventional methods remain at a very low level compared to the data in the literature. Olmez et al. (2008) found 10-421 μ g kg⁻¹ in roasted hazelnut samples and 128 μ g kg⁻¹ on average, Jagerstad and Skog (2005) determined it in the range of 64-457 μ g kg⁻¹ in hazelnut and hazelnut paste samples. Amrein et al. (2005) determined low levels of acrylamide (16-56 ng g⁻¹) in roasted hazelnuts and this was due to the low levels of asparagine amino acids in hazelnuts. (Xu et al., 2013) determined acrylamides at the level of 150 ng g⁻¹ in roasted hazelnuts.

Our data showed, it is possible that these products were subjected to short-term heat treatments at very low temperatures and under similar conditions.

The acrylamide values of the organic certified and conventionally produced pistachios were determined 4.86 ng ml⁻¹ (range 3.09-6.33 ng ml⁻¹) and 9.95 ng ml⁻¹ (range 4.44-25.38 ng ml⁻¹), respectively. The average of acrylamide values in organic certified pistachios was lower and the effect of the production methods that created this difference is statistically significant. Acrylamide values were quite low compared to the data in the literature. Schlörmann et al. (2015) found that acrylamide contents changed depending on the roasting conditions and acrylamide compounds were found in the range of 14-88 μ g kg⁻¹ in pistachio samples, which were heated at 140.8-185.1°C for 21-25 minutes. Ozer (2012) determined 318-462 ng g⁻¹ acrylamide compound in pistachio, while Otles and Otles (2004) determined it as <30 ng g⁻¹ in pistachio powder product. In our study, it is considered that pistachios were applied at short-term and relatively low temperatures for drying and/or roasting purposes.

The acrylamide values of organic certified and conventionally produced peanuts were determined 14.09 ng ml⁻¹ (range 7.33-26.64 ng ml⁻¹) and 36.27 ng ml⁻¹ (range 15.09-65.61 ng ml⁻¹), respectively. Olmez et al. (2008) determined acrylamide compounds in the range of 10-120 μ g kg⁻¹ (average 66 μ g kg⁻¹) in roasted peanuts and between 45-63 μ g kg⁻¹ (average 54 μ g kg⁻¹) in peanut butter. Suvari et al. (2017) found 21.4-60.5 ng ml⁻¹ (mean 34.69 ng ml⁻¹). Yates (2012) reported the acrylamide content in roasted unsalted peanuts as 28 μ g kg⁻¹. Acrylamide content of 15 roasted peanuts analyzed according to FDA data (Anonymous, 2005) varies between less than detectable value and 36 ppb. Cressey et al. (2012) determined 9-84 μ g kg⁻¹ (average 42 μ g kg⁻¹). These results are close or very similar to the values contained in the samples produced by conventional methods in our study. In our study the effect of organic and conventional methods can be seen. The acrylamide values of peanuts were higher than the content of hazelnuts and pistachios, but considerably lower than the content of almonds produced by conventional methods.

The acrylamide values of pumpkin seeds produced by organic certified and conventional methods were determined 5.96 ng ml⁻¹ (range 0.95-14.06 ng ml⁻¹) and 4.54 ng ml-1 (range 2.41-6.98 ng ml⁻¹), respectively. Although organic certified pumpkin seeds had higher acrylamide content, this difference is not statistically significant. On the other hand, although organic certified pumpkin seeds, hazelnuts, pistachios and peanuts contained higher levels of acrylamide precursors compared to their acrylamide contents, it is remarkable that organic certified almonds had a low acrylamide content.

Acrylamide compound was not detectable in organic certified sunflower seeds. On the other hand, it was 16.92 ng ml⁻¹ on average in sunflower seeds produced by conventional methods. This result, determined in organic certified sunflower seeds, differs from other examined organic certified pumpkin seeds, hazelnuts, pistachios, peanuts and almonds.

4. Conclusion

It is important to investigate the acrylamide compound, which has negative effects on human health, in organic nut varieties and to evaluate the results in the context of organic food safety. The data obtained in this study stated that similar processing conditions were applied to pumpkin seeds and hazelnut varieties, which were dried nut varieties produced by organic and conventional methods, while relatively higher temperature and/or time were used in the conventional method in peanut and pistachio varieties. It is remarkable that the organic certified almond variety had the lowest acrylamide content and the organic certified sunflower seeds were free of acrylamides. On the other hand, it is considerable that there were higher rates of acrylamides in organic certified pumpkin seeds and hazelnuts than those produced by conventional methods. Eventually this study provides significant data that enables comparison of suspected acrylamide compounds in both organic and conventional production systems which fulfils the gap of research in organic product area.

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