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Effect of Roasting Conditions and Storage on Acrylamide Content and Colour of Almonds

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ABSTRACT

In this study, the effect of roasting temperature (130, 155 and 180°C), roasting time (5, 25 and 45 minutes) and storage (0., 6. and 12. month) on the acrylamide content of almonds was determined, and the relation between acrylamide formation and changes in colour (CIE *L*, a** and *b**) was investigated. Acrylamide contents of roasted almonds determined by liquid chromatography coupled to diode array detector (LC–DAD) ranged from 24.61 to 882.10 ppb. Acrylamide formation increased by an increase in roasting temperature and time; however, acrylamide contents of roasted almonds reduced during storage up to 12 months. The degree of this reduction in the first 6 months of storage was higher than that the second 6 months of storage. At high roasting temperatures and for long roasting times, the lightness values of almonds (*L**) decreased while colour values of *a** and *b** increased. Colour *L** value lower than 50 and *a** value higher than 12 were found in roasted almonds while acrylamide contents of almonds were higher than 200 ppb. Colour *L** and *a** values may be recommended as rapid quality control parameters for the estimation of the adequate roasting time and temperature during roasting processing of almonds.

Keywords: Acrylamide, Almond, Heat treatment, Storage, Colour

Kavurma Şartları ve Depolamanın Bademlerin Akrilamid İçeriği ve Rengi Üzerine Etkisi

ÖZ

Bademe uygulanan kavurma sıcaklığı (130, 155 ve 180°C), kavurma süresi (5, 25 ve 45 dakika) ve kavrulmuş bademlerin depolama süresinin (0., 6. ve 12. ay) akrilamid içeriğine etkisi incelenmiş ve renk değişimi ile akrilamid oluşumu arasındaki ilişki de belirlenmiştir. Kavrulmuş bademlerin akrilamid içeriği sıvı kromatografisi (LC-DAD) ile tespit edilmiş ve örneklerin akrilamid içeriğinin 24.61 ile 882.10 ppb aralığında olduğu bulunmuştur. Bademlerin akrilamid içeriğinin kavurma sıcaklığı ve süresinin artışı ile arttığı, fakat 12 aylık depolama süresinde ise düştüğü belirlenmiştir. Altı aya kadar olan depolama süresinde akrilamid içeriğindeki düşüşün kalan zaman aralığına göre daha fazla olduğu gözlenmiştir. Yüksek kavurma sıcaklıkları ve uzun sürelerde düşük parlaklık (*L**) değerleri elde edilmiştir. Yüksek sıcaklık ve süre uygulamalarında daha yüksek *a** ve *b** değerleri elde edilmiştir. *L** değeri 50'den düşük ve *a** değeri 12'den yüksekken, bademlerin akrilamid içerikleri 200 ppb'den yüksek bulunmuştur. *L** ve *a** değerleri bademlerin kavrulması esnasında, kavurma sıcaklık ve sürelerinin sınırlandırılmasının öngörülmesi amacı ile hızlı kontrol parametreleri olarak önerilebilir.

Anahtar Kelimeler: Akrilamid, Badem, Isıl işlem, Depolama, Renk

INTRODUCTION

Thermal processing of foods has some advantages but also induces the reduction of nutritional and functional properties of some compounds. It also induces the formation of some unexpected harmful compounds [1]. Swedish National Food Administration and the University of Stockholm reported in April 2002 that the heat application in high temperatures to starch-rich foods causes the formation of acrylamide which responsible of some toxic effects [2, 3]. Because acrylamide is classified as a probable carcinogenic in humans, many researchers have focused on the formation mechanisms of acrylamide, stability of acrylamide, levels of acrylamide in foods and strategies for reduction of acrylamide levels in foods [3, 4]. Also, Gökmen [5] suggested the usage of Bigelow's procedure for providing a basis for thermal process safety calculations by considering the hottest point of foods to evaluate the risk assessment of heat treatment.

In recent literature, researchers reported some pathways for acrylamide formation in foods. The most probable one was reported to be the Maillard reaction which is the major formation pathway for a reaction between reducing sugars and asparagine above 120°C [1, 6-9]. Other acrylamide formation mechanisms were also studied and described by researchers [6, 10-16].

Acrylamide exposure of humans may be due to external sources or from the diet. Tareke et al. [17] reported acrylamide content of some foods and classified the heated protein-rich foods as moderate level, which contains acrylamide between 5–50 ppb, and the carbohydrate-rich foods as higher content ranged from 150 ppb to 4,000 ppb, such as potato, beetroot, and selected commercial potato products. Acrylamide contents of French fries, biscuits and crackers were classified as median levels, ranging from 410 to 450 ppb.

Zhang and Zhang [18] reported that the formation pathways of acrylamide have been nearly clarified in model systems, which contain asparagine and carbohydrate but the effectiveness of food matrices as a factor on formation was also expressed. There are many studies to find the effective factors in different food matrixes, including potato [11, 20, 21], cereal [22, 23], bread [19, 24], almond [6, 25-28], coffee [16, 29] and other food matrixes [18].

Seron et al. [26] reported the content of asparagine in almond in the range of 2000-3000 mg/kg. Ruggeri et al. [25] reported glucose and fructose levels between 500-1300 mg/kg, and sucrose as 2500-5300 mg/kg [25]. Means that almond contains the precursors of acrylamide. Amrein *et al*. [28] detected lower acrylamide in almond species from European origin in comparison with the species from U.S. and stated the due to the lower amount of asparagine in the species from European.

Amrein et al. [27] showed that the percentage acrylamide decrease in 100 days stored roasted almonds was from 20 to 57%, at room temperature. In addition, no correlation was reported between the decrease and the initial acrylamide content or the degree of roasting. Acrylamide reduction by storage was also reported for coffee and cocoa [29-31]. Amrein *et al*. [27] reported that there was no available data on how storage affects acrylamide and on the cause of reduction and, assumed that the reactive compounds which formed during roasting may be effective for the acrylamide degradation, during storage period.

The first aim of the present study was to determine the effect of roasting temperature and roasting time on acrylamide formation and storage on its degradation. The second aim was to evaluate the potential use of colour values for the estimation of acrylamide formation during roasting.

MATERIALS and METHODS

Materials

In this study, Gülcan II '101/13' almond (*Prunus dulcis*) [32] cultivar was used as sample and was collected from Aegean region of Turkey. 250 grams of almond were roasted in each batch in an oven, have a rotating chamber. The roasting temperatures were 130, 155 and 180°C, and the roasting times were 5, 25 and 45 minutes. Then, each sample was stored in plastic bags for 6 and 12 months at 0°C in a refrigerator.

HPLC grade acrylamide (>99%) was obtained from Aldrich (Milwaukee, WI, USA). All organic solvents were of HPLC-grade unless otherwise stated. Water was purified by a Millipore Direct-Q 3 UV water purification system (Millipore, Bedford, MA, USA) with a resistivity higher than 18 MΩ cm. 2 mL SPE and Oasis HLB 60 mg cartridges were obtained from Waters (Milford, MA, USA). Amber glass auto-sampler vials with septum screw caps were obtained from Agilent Technologies (Wilmington, DE, USA). The analytical column was Inertsil ODS-4 column (4.6 mm i.d. × 25 cm x 5µm) obtained from GL Sciences, (Tokyo, Japan**).**

Acrylamide stock solution (2.0 mg/mL) was prepared by dissolving 2.0 mg of acrylamide in 1.0 mL of purified water. The acrylamide stock solution was diluted to prepare calibration standards at 2, 4, 6, 10, 25, 50, 100, 200, 300, 400, 500, 1000 and 2000 µg/L.

Methods

Sample Preparation

Two grams of ground almond were weighed, and 10 mL of methanol was added in a centrifuge tube. The suspension was homogenized for 3 min with a homogenizer. Then, it was centrifuged at 10000 rpm (11 180×*g*) and 10°C for 10 min. The clear supernatant was treated with 100 µL of Carrez I and II solutions. The mixture were centrifuged again at 10000 rpm (11 180×*g*) and 10°C for 5 min. Then, 2.5 mL of clear supernatant was placed in a water bath at 40°C and evaporated to dryness under nitrogen. The residue was dissolved in 1

mL of water. For the SPE clean-up, Oasis HLB cartridge was preconditioned consequently with 1ml of methanol and 1 mL of water at rate of two drops per second using a syringe. Then, 1 mL of the extract was passed through the cartridge at a rate of one drop per second using a syringe. The first seven to eight drops of the effluent were discarded to prevent any dilution of sample by replacing water held in the sorbent void fraction with the sample effluent. The forthcoming drops were collected and filtered through a 0.45µm syringe filter [1]. Eighty µL of the final solution was injected to HPLC with an Inertsil ODS-4 column.

HPLC-DAD Analysis

Acrylamide was determined with the HPLC coupled to DAD, described by Gökmen et al. [1], with some minor modifications. HPLC-DAD analysis was performed on an Agilent 1200 Infinity series HPLC instrument equipped with a vacuum degasser, a quaternary pump. The injection volume of the calibration standards and sample extracts were 80 µL. HPLC separation was conducted on an Inertsil ODS-4 column. The detection wavelength was 198 nm. Mobile phase was water and the rate of it was 1.0 mL/min. The column oven temperature was 25°C. A calibration curve was prepared in the range of 2–2000 µg/L and was linear (R^2 = 0.999). The limit of detection (LOD) and limit of quantification (LOQ) were estimated to be 5.04 ppb and 16.81 ppb, respectively.

Determination of Colour

Almonds were ground in a household coffee mill and evenly spread on a Petri dish. The colour was determined with a Minolta colorimeter, measuring colour according to the CIE *L*, a*, b** system. The lightness was used as a measure for the degree of roasting. *a** and *b** values were also evaluated for colour changes during roasting, where *L**=100 means white and *L**=0 means black [1] .

Statistical Analysis

Data for dose related effects of almond administration was analysed by one-way ANOVA followed by Tukey's test for post-hoc analysis. Results for the training and test sessions obtained from EPM and MWM were analysed by Bonferroni test following three-way (almond × scopolamine × sessions) ANOVA with repeated measure design. Recognition index and acetylcholine concentration were statistically estimated by two-way ANOVA with Tukey's post hoc test to compare the different groups. One-sample t test was used to determine whether the exploration ratios in NOR test under each condition were significantly different from chance levels. Furthermore, various correlations were also determined by Pearson correlation test. Values of $p<0.05$ was considered as a statistically significant difference.

RESULTS and DISCUSSION

The precursors of acrylamide in almond were reported to be in enough amounts. Seron et al. [26] detected free asparagine in the range 2000 – 3000 mg/kg. Ruggeri *et al*. [25] reported the glucose and fructose contents as 500–1,300 mg/kg, and sucrose contents ranged from 2,500 to 5,300 mg/kg. Therefore, the formation of acrylamide over 120°C is an expected result as in our observations. Acrylamide in roasted almonds were reported from 260-1530 ppb [6, 33]. In our study, the acrylamide content of the roasted almonds were found to be between 24.61-882.10 ppb.

The results on acrylamide content of roasted almonds were as shown in Figure 1. By the increase of roasting temperature, from 130 to 155 and to 180°C, acrylamide formation increased. The increase of the roasting time also increased acrylamide formation. But, the storage of the roasted almonds was negatively affected acrylamide content and a time dependent decrease was observed. The roasting temperature, roasting time and the storage were found to be significant ($P>0.05$) parameters. The highest acrylamide content was found to be in the beginning of the storage for the samples roasted at 180°C for 45 minutes. The acrylamide formation rate was also found to be more in the samples roasted at 180°C up to 45 minutes. Amrein et al. [27] reported that prolonged roasting can result in lower acrylamide concentrations which were similar in roasted coffee beans [34] and gingerbread [19]. Our observations were not in accordance with Amrein et al. [27] in terms of prolonged roasting. The acrylamide formation increased by the increase of the roasting time up to 45 minute. These contrast results may be due to the species of the almonds used in our experiment.

The observations on acrylamide content for the first day were higher in comparison to stored samples. It is apparent that the content of acrylamide in the samples reduces by the storage. For instance, acrylamide contents were 174.2, 416.5 and 882.1 ppb after frying at 130, 155 and 180°C, respectively, in the case of almonds roasted at 45 minute. After 180 days, the content of those samples were reduced approximately 50%. Amrein et al. [27] also expressed that the acrylamide in roasted almonds decreases during storage at room temperature

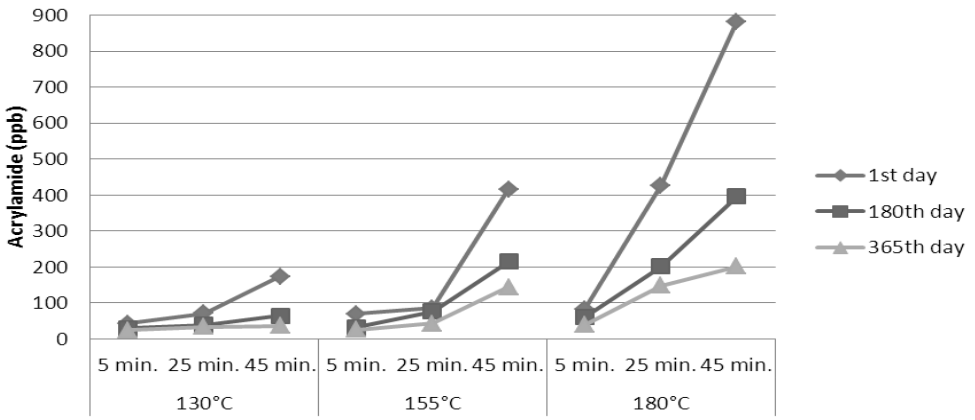

Figure 1. Influence of roasting time, roasting temperature and storage on acrylamide content of almonds

French fries, potato chips and crisps exhibit relatively higher values of acrylamide as 424 and 1739 ppb, respectively [35]. Acrylamide contents of French fries, biscuits and crackers were classified as median levels, were ranged from 410 ppb to 450 ppb [17]. Only the almonds, roasted at 180°C for 45 minute were found to be higher than the classification of Tareke et al. [17] as median level.

*L** value is a brightness parameter, shows the degree of roasting. Melanoidins formed in Maillard reaction were reported to be effective on colour of heat-treated foods. Amrein et al. [27] reported that *L** value of almonds (cultivar Price), roasted from 130°C up to 180°C for 2.5 to 40 minutes time combinations, were reported to be from 46 to 71. The *L** values obtained in our study were ranged from 40.94 to 67.10. The lowest *L** values were detected for the almonds roasted at 180°C, while the highest values were for the samples roasted at 130°C (Figure 2). The decrease of the lightness of the almonds for higher roasting temperatures and for the higher roasting time was an expected result. In general, by the storage, *L** value was also decreased. The decrease of *L** value was more apparent up to 180th days of storage. The decrease of *L** value for the samples stored for 180 days was more than the decrease obtained for the samples stored between 180th and 365th days. More acrylamide formation were detected in the samples which had lower *L** values. When acrylamide content was over 200 ppb, the L* value was found to be lower than 50 which is apparent for the samples roasted at 180°C and for the stored almonds roasted at 155°C for 45 minute. So, *L** value may be referred as a control parameter for the limitation of roasting time and temperature for the rapid control of formation of acrylamide.

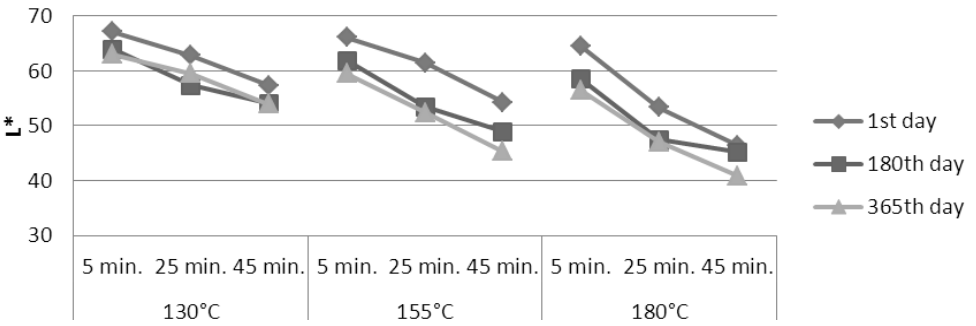

Figure 2. The effect of roasting time, roasting temperature and storage on CIE *L** value of almonds

Colour *a** value of roasted almonds ranged from 5.08 to 16.95 (Figure 3). According to the obtained data, when *a** value was detected to be over 12, the acrylamide content of roasted almonds were found to be over 200 ppb. The highest *a** value was obtained for the highest acrylamide containing roasted almonds. *a** value may also referred to be a rapid control parameter in thermal processing of almond for limitation of acrylamide formation as mentioned for *L** value. Storage also influenced a* value and caused to its decline. *a** value of the samples roasted for 5 minutes for all applied roasting temperatures were found to be similar.

During storage, *b** value of the samples were declined (Figure 4). By the increase of the roasting time, b* value was also increased except for the samples roasted at 130°C and 155°C for 25 minutes. The roasting temperature differentiation slightly affected *b** value. *b** value was ranged in between 13.82-32.37. In general, acrylamide content of samples were increased, by the increase of *b** value.

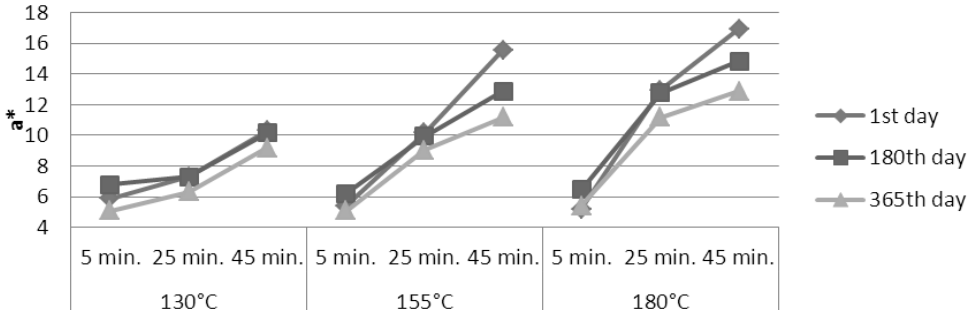

Figure 3. The effect of roasting time, roasting temperature and storage on CIE *a** value of almonds

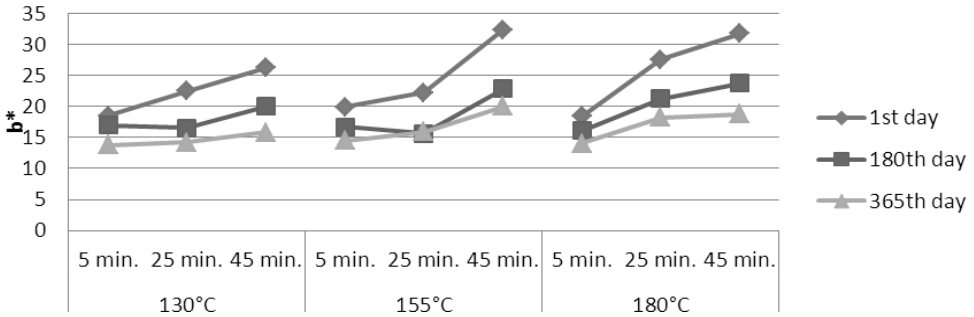

Figure 4. The effect of roasting time, roasting temperature and storage on CIE *b** value of almonds

Pearson correlation test was performed to determine a correlation between acrylamide content and colour values of almond samples and shown in Table 1.

Table 1. Correlation results between acrylamide content and colour values of almond samples

Variables	Acrylamide (ppb)	*a**	*b**
*a**	-0.035		
*b**	0.836**	-0.164	
*L**	-0.512**	-0.021	-0.327**

Colour *b** (0.836**, p<0.01) and *L** (0.512**, p<0.01) values and acrylamide content of roasted almonds seems to be linearly related ($R^2 \approx 0.92$). The correlation between *a** value and acrylamide content of the samples were non-significant. As a result of the correlation test, *a** value may not a suitable acrylamide prediction parameter for the almonds in roasting process whereas *L** and b* values may be preferable.

CONCLUSION

The acrylamide content of the studied almonds (cultivar Gülcan II) was ranged from 24.61 to 882.10 ppb. By the increase of roasting temperature from 130 to 180°C and roasting time from 5 to 45 minutes, acrylamide content of almonds was increased. Acrylamide content declined up to 365 days of storage at 0°C in a refrigerator. Except for the almonds roasted at 180°C for 45 minutes, the acrylamide content of all analysed samples was not too much and may be evaluated as median level in comparison with potato chips and crisps.

Acrylamide is classified as probably "carcinogenic in humans". After the discovery of acrylamide in foods many studies have been done for getting information about its formation mechanisms and levels. Many analytical procedures have been developed for its determination in heat-treated foods. However, the methods for determination of acrylamide are expensive and take too much time. In processing conditions, secondary easy quality control parameters may be useful for rapid decisions for limitation of heat treatment such as colour. Our observations shows that *L** (higher than 50) and *a** (lower than 12) values may be utilizable for termination of time of heating process, if 200 ppb limitation needed for acrylamide for the almond cultivar Gülcan II. *L*, a** and *b** values may be modelled for other foodstuffs. Therefore, producers may save time and easily control the process with a cheaper and rapid alternative analysing method.

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