Acrylamide in almond products

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ORIGINAL PAPER

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Abstract Acrylamide was determined in 86 different almond products, such as roasted almonds, almond-containing bakery products, raw almonds, and marzipan. The highest acrylamide concentrations were found in dark roasted almonds, while only moderate acrylamide contents were determined in bakery products. Roasting experiments under different process conditions showed that acrylamide increases with time and that temperature has a much stronger effect on acrylamide formation than time. During roasting reducing sugars are consumed faster and to a larger extent than free asparagine, suggesting that the content of reducing sugars may be a critical factor for acrylamide formation in roasted almonds. Acrylamide was found to decrease in roasted almonds during storage at room temperature.

Keywords Acrylamide · Almond · Sugars · Free amino acids · Roasting

Introduction

The detection of acrylamide in a broad range of heated foods at concentrations sometimes exceeding 1,000 μg/kg [1] led to worldwide activity to reduce the acrylamide content of foods because of its neurotoxic and carcinogenic properties [2, 3]. Acrylamide is formed at elevated temperatures and medium to low moisture contents concurrently to the Maillard reaction between reducing sugars and the free amino acid asparagine, which provides the backbone of the acrylamide molecule [4–6]. Exceedingly high amounts of acrylamide were detected in strongly heated potato products such as crisps, French fries and hash browns [1, 7, 8], which is at least partially explained by the high content of free asparagine and reducing sugars in raw potatoes [9]. It has been shown that in potatoes the reducing sugars largely control the extent of acrylamide formation, whereas the more abundant free asparagine does not correlate with acrylamide content [9–11]. In contrast, free asparagine is often limiting in sweet bakery products and therefore plays a key role together with the baking agent ammonium hydrogencarbonate, which strongly promotes acrylamide formation [12–14].

Almonds contain both acrylamide precursors in appreciable amounts: the content of free asparagine is reported in the range 2,000 - 3,000 mg/kg [15]. Glucose and fructose contents were determined to be 500–1,300 mg/kg, and sucrose contents ranged from 2,500 to 5,300 mg/kg [16]. As a consequence, the detection of acrylamide in roasted almonds in concentrations from 260 to 1,530 μg/kg [7, 14] was not surprising. It was shown that the physical form of the almond (whole kernel versus cut versus ground) has an impact on the amount of acrylamide formed during heating [14].

The aim of the present study was to gain an overview of the acrylamide content of various almond products in Switzerland and to identify the products with the highest acrylamide concentration. Over 80 samples of raw, intermediate, and final products containing almonds were collected and their acrylamide content was determined. Acrylamide formation during roasting was monitored and sugars and free amino acids in raw and roasted almonds were measured.

Materials and methods

Almonds (raw, salted, smoked, roasted, and caramelized), and almond-containing products, such as biscuits, cookies, cakes, bars, breads, pastry, and marzipan, were obtained from supermarkets and Swiss food manufacturers. The almonds used for the roasting experiments were all of Californian origin and were supplied by Almond Board of California (Modesto, CA, USA). The following cultivars were investigated: Non Pareil, Price, Monterey, and Mission. Roasting experiments were performed as pile roasting (batch size 200 g) using a fluidized-bed hot-air laboratory roaster

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Table 1 Acrylamide content of various almond products

Product	*n*	Mean (μg/kg)	Median (μg/kg)	Max. (μg/kg)	Min. (μg/kg)	RSD (%)
Roasted almonds	36	443	424	2,147	nd	100
Bakery products with almonds	34	196	103	1,574	nd	168
Marzipan plus raw almonds	16	4	0	42	nd	290
All products	86	250	91	2,147	nd	149

RSD relative standard deviation, *nd* not detected

(G.W. Barth, Freiburg, Germany). All samples were homogenized in a household cutter (Moulinette; Moulinex, Paris, France) before being subjected to further analysis.

Analysis of acrylamide

Acrylamide was determined with the gas chromatography (GC)–mass spectrometry (MS) method described by Biedermann et al. [17]. The internal standards were $^{13}C_3$-acrylamide (CIL, Andover, MA, USA) and methacrylamide (Fluka, Buchs, Switzerland), both dissolved in methanol (Fluka) and 500 μg of each was added per kilogram of sample. Extraction and clean-up was performed as described in Ref. [17]. Measurement of acrylamide was done with an 8000 series gas chromatograph with an on-column injector (Fisons Instruments, Milan, Italy) coupled to an SSQ 710 quadrupole mass spectrometer (Finnigan Mat, San Jose, USA). The precolumn (TSP deactivated, inner diameter 0.53 mm) and the separation column (BGB-Wax, 12 m, inner diameter 0.25 mm) were both from BGB Analytik (Böckten, Switzerland). The GC and MS conditions were as described in Ref. [17]. Spiking experiments were made by adding10 μl of an aqueous solution of acrylamide (500 mg/l; Sigma-Aldrich, Steinheim, Germany) to 10 g of homogenized sample.

Determination of free amino acids in almonds

Homogenized almonds (approximately 10 g) were mixed with deionized water (1+2) and further homogenized to a slurry (Polytron; Kinematica, Lucerne, Switzerland). About 7.5 g of the slurry was transferred to a 100-ml flask and 1 ml of an aqueous solution of norleucine (5 mg/ml; Fluka) was added as an internal standard. About 60 ml 0.1 M HCl (Fluka) and 5 ml of each Carrez I (150 g $K_4[Fe(CN)_6]\cdot 3H_2O$ per liter, Fluka) and Carrez II (300 g of $ZnSO_4\cdot 7H_2O$ per liter, Fluka) solution were added and the mixture was thoroughly shaken. Foam was broken with 50 μl 1-octanol (Fluka) and the volume was adjusted to 100 ml with 0.1 M HCl (Fluka). After filtration (602 H 1/2, Schleicher & Schuell, Dassel, Germany), the samples were diluted 1+4 with 0.16 M lithium citrate buffer (pH 2.2, PVP physiological; Laborservice Onken, Gründau, Germany) and thoroughly mixed. Diluted samples were filtered through a 0.45-μm high-performance liquid chromatography membrane filter (Titan; Infochroma, Zug, Switzerland) and subjected to analysis by cation-exchange chromatography followed by postcolumn derivatization with ninhydrin (Biochrom 30, Biochrom, Cambridge, UK) by using the physiological system (Biochrom) as described by the producer. An aliquot of 50 μl was injected and quantification was done both by comparison with an external standard and with the internal standard.

Measurement of sugars in almonds

Homogenized almonds (10.00 g) were mixed with deionized water (1+2), further homogenized (Polytron) and about 10 g of the slurry was weighed into a 100-ml flask. After Carrez clarification (5 ml of each Carrez I and II solution), foam was broken with a few drops of 1-octanol (Fluka), the pH adjusted to 7 with 4 M KOH (Fluka), and the volume adjusted to 100 ml with deionized water. After filtration (Schleicher & Schuell) fructose, glucose, and sucrose were determined enzymatically using the kit from Scil Diagnostics (Martinsried, Germany).

Determination of color and moisture content

Almonds were ground in a household coffee mill (Mio-Star, Migros, Zurich, Switzerland) and evenly spread on a Petri dish. The color was determined with a Minolta CR310 colorimeter (Konica Minolta Photo Imaging, Dietikon, Switzerland) measuring color according to the L^*, a^*, b^* system. The lightness was used as a measure for the degree of roasting, where $L^*=100$ means white and $L^*=0$ means black.

Dry matter was determined gravimetrically: About 1 g of homogenized (Moulinette) almonds was weighed in a predried metal dish and thoroughly mixed with 4 g of dried quartz sand (Fluka) and then dried in an oven at 103 °C for 4 h.

Results and discussion

Acrylamide in almonds and almond products

The acrylamide content of various almond-containing products or prefabricates (total $n=86$) was determined. Spiking samples with acrylamide resulted in recoveries (r) in the range $90\% \leq r \leq 110\%$. The products were split into three groups: roasted almonds, bakery products with almonds, and marzipan plus raw almonds. The results are shown in Table 1.

The data in Table 1 demonstrate that the highest acrylamide concentrations were found in roasted almonds, with most of the values being around 400 μg/kg. The highest concentration was found in the dark roasted almonds used as an ingredient in the manufacture of biscuits. Most of the values for roasted almonds are within the range reported by other groups [7, 14]; however, acrylamide levels in roasted almonds exceeding 2000 μg/kg were not found before. The median acrylamide content of roasted almonds was 4 times higher than that of bakery products with almonds, which demonstrates that roasted almonds generally contain more acrylamide than almond-containing bakery products. For the bakery products, only eight values exceeded 200 μg/kg. However, there was one biscuit product (from two batches) that contained 1,307 and 1,574 μg/kg, which is extraordinarily high. The main reason for this high amount was the presence of ammonium hydrogencarbonate (E 503) and a large amount of reducing sugar. This baking agent was shown to strongly enhance acrylamide formation in bakery products [12–14, 18]. This is pointed out by the fact that five out of eight bakery products containing more than 200 μg/kg acrylamide were prepared with ammonium hydrogencarbon-

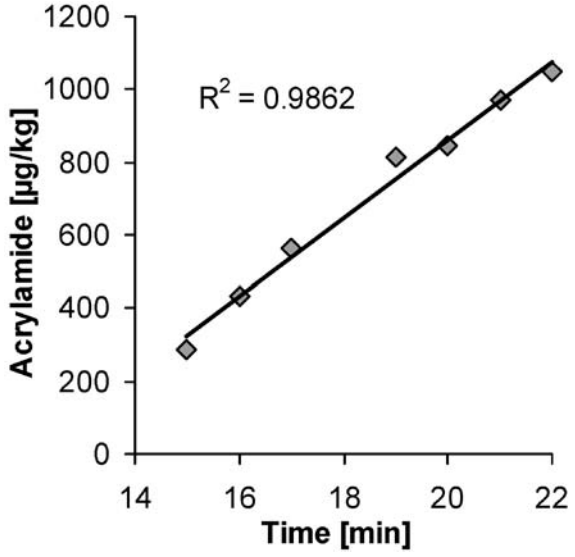

Fig. 1 Influence of roasting time on the acrylamide content of almonds during roasting at 150 °C

ate. In marzipan, cooked, blanched, and raw almonds usually no acrylamide (limit of detection 20 µg/kg) or very low amounts were found. Acrylamide is formed at temperatures above 100 °C [19] and even some slightly roasted almonds contained no detectable acrylamide (Table 1), which explains these results. The relative standard deviation was high in all three categories, showing that there is considerable variation in the acrylamide content of almond products. Because roasted almonds contained the most acrylamide, the focus of the present study was directed to this category and some further experiments were carried out.

Acrylamide formation during roasting of almonds

The data for roasted almonds in Table 1 show a considerable variation, suggesting that the roasting process may have an important influence on acrylamide formation. Therefore, samples from an industrial roasting experiment, performed at 150 °C, were taken between 15 and 22-min roasting time and analyzed for acrylamide.

Figure 1 shows that the acrylamide content of roasted almonds increased in a linear manner with time. However, a nonlinear or a decreasing course of the acrylamide content has to be expected at longer process times as observed in model systems [20] as well as in foods such as gingerbread [12], coffee [19], and potatoes [8, 21, 22].

Besides the roasting time, the roasting temperature is assumed to influence acrylamide formation during roasting, too. Therefore, roasting experiments using various temperature/time combinations were performed with a laboratory roaster, and the results are shown in Table 2. The reproducibility of the roasting process was checked with four samples roasted at 180 °C for 4 min: The mean acrylamide content was 1,834 µg/kg with a relative standard deviation of 8.5%, which is well within the range of other process variations reported [12, 18].

Table 2 Acrylamide content of almonds roasted under different temperature and time combinations

Temperature (°C)	Time (min)	Acrylamide (µg/kg)
130	2.5	nd
130	11.0	nd
130	16.5	44
130	22.5	94
130	40.0	236
150	15	715
150	25	1,547
150	30	1,044
180	4.0	1,834[a]
180	7.0	1,718

n=1
[a] *n*=4

The results in Table 2 show that only small amounts of acrylamide were formed at 130 °C. Acrylamide was not detected in the first 11 min of roasting and even after 40 min only 236 µg/kg was measured. In contrast, at 150 °C acrylamide formation was much greater and faster: After 15 min 35 times more acrylamide was found than after 16.5 min at 130 °C. At 150 °C 6.5 times more acrylamide was formed after 25 min compared with roasting at 130 °C for 40 min. This clearly shows that temperature has a much stronger influence on acrylamide formation than time has. Experiments performed at 150 and 180 °C indicated that prolonged roasting can result in lower acrylamide concentrations. Almonds roasted at 150 °C for 25 min contained substantially more acrylamide compared with the sample roasted for 30 min. Prolonged roasting at higher temperatures seems to favor the elimination of acrylamide relative to its new formation. A similar effect was also observed in roasted coffee beans [19] and gingerbread [12]. Altogether, these results suggest that the optimization of the roasting process in terms of temperature and time may be a way to reduce the acrylamide content of roasted almonds, and detailed investigations are in progress to find optimal process conditions.

Changes of sugar and asparagine content in almonds during roasting

In parallel to the acrylamide analysis, the changes of the concentrations of sugars and free asparagine during roasting were determined. Table 3 shows the contents of sugars and free asparagine in raw almonds of different cultivars.

The main sugar in raw almonds was sucrose which accounted for about 3–4% of the fresh weight. Glucose and fructose were determined in the ranges 1,500–2,300 and 900–1,500 mg/kg, respectively, which are similar to values reported from Italian almond varieties [16]. On a molar basis free asparagine is roughly as abundant as the reducing sugars: The ratio of the molar

Table 3 Content of sugars and free asparagine in raw almonds

Cultivar	Glucose (mg/kg)	Fructose (mg/kg)	Sucrose (mg/kg)	Free asparagine (mg/kg)
Non Pareil	1,566	867	32,700	2,175
Mission	2,130	1,151	38,650	2,041
Price	2,339	1,482	43,430	2,238
Monterey	2,101	1,103	40,950	2,474
RSD (%)	16	22	12	8

n=2; values refer to fresh weight

Table 4 Reducing sugars, free asparagine, and acrylamide in raw and roasted almonds (cultivar Price, $n \geq 2$; values referring to dry matter)

Roasting conditions	Glucose (mg/kg)	Fructose (mg/kg)	Free asparagine (mg/kg)	Acrylamide (μg/kg)
Raw	2,860	2,010	2,200	nd
130 °C, 22.5 min	700	900	2,100	79
180 °C, 7.0 min	110	140	1,600	1,718

content of free asparagine to reducing sugars ranged from 0.8 to 1.2. Thus, the situation of the acrylamide precursors in almonds is different from that for potatoes, where reducing sugars are limiting [9, 23, 24], or that for sweet bakery products, where asparagine is limiting [12, 18]. As can be seen from the relative standard deviation, the reducing sugars varied somewhat more between the cultivars than free asparagine. Therefore, more extensive investigations will be undertaken in another study to check if there are significant differences between the different almond cultivars in terms of sugars, free amino acids, and acrylamide formed during roasting.

Since reducing sugars and free asparagine are considered to be the main precursors for acrylamide formation in foods, their changes during two different roasting experiments were monitored (Table 4). Almonds from the same batch were roasted at 130 °C for 22.5 min ("light roast") and at 180 °C for 7.0 min ("dark roast").

Glucose and fructose were consumed faster than free asparagine: After roasting at 180 °C for 7 min only 4% of the initial glucose content and 7% of the initial fructose content was found, whereas the free asparagine content had decreased only by 27%. The effects were likewise but less pronounced for the roasting experiment carried out at 130 °C: reducing sugar decreased by more than 50%, whereas still 95% of the initial asparagine content was found. Similar behavior of these compounds was also observed in heated potatoes [24]. Glucose and fructose react much faster than free asparagine upon heating and therefore their content could directly influence acrylamide formation. This was tested with three samples of raw almonds with a different content of reducing sugars. They were roasted at 160 °C for 10 min and the results are shown in Fig. 2. A strong correlation was found between the glucose content before roasting and the acrylamide content determined after roasting. The correlation was similar for fructose (R^2=0.9474) and glucose plus fructose (R^2=0.9802), whereas sucrose correlated only very weekly with acrylamide (R^2=0.3336). This indicates that besides the roasting process the content of reducing sugars in the raw almonds may be a critical factor for acrylamide formation in almonds. However, the data are limited and further experiments are needed to fully corroborate and understand these interrelations.

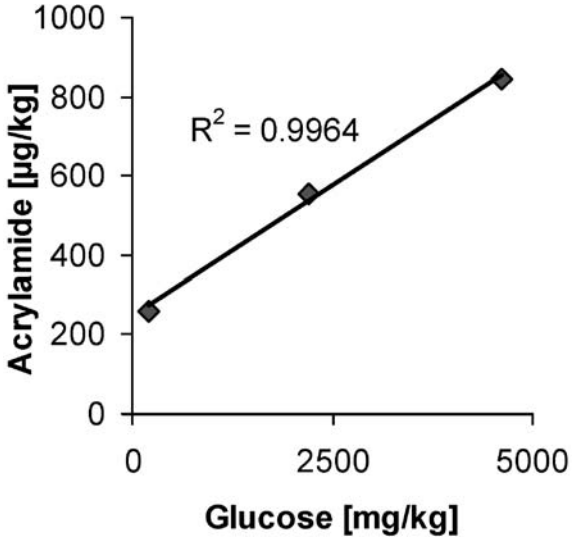

Fig. 2 Interrelation between glucose content in raw almonds and acrylamide determined in these almonds after roasting at 160 °C for 10 min

As both acrylamide and color (melanoidins) are formed in the Maillard reaction [4, 20, 25], the color of the roasted almonds was compared with their acrylamide content. The L^* value (brightness) of roasted almonds (cultivar Price) ranged from 46 to 71. The L^* value and the acrylamide content showed a fairly close correlation with R^2=0.8384: The darker the almonds were the higher the acrylamide content was. The brown skins of the almonds were a major cause for uncertainty in the color determination because they differ between varieties and their change in color during roasting is different compared with that of the kernel. A similar correlation between browning (L^* value) and acrylamide content was observed in gingerbread [12] and fried potato slices [21].

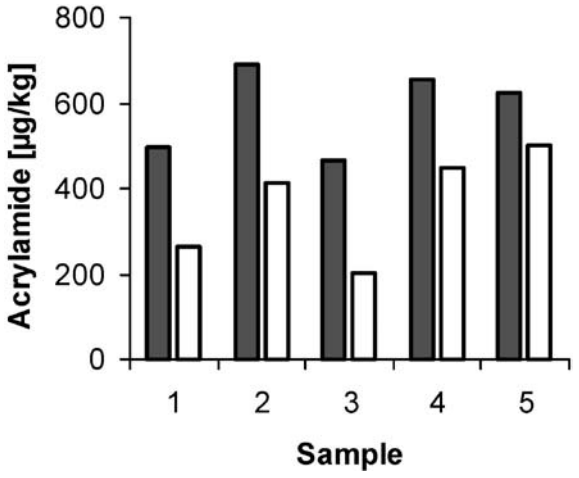

Fig. 3 Acrylamide content in samples of roasted almonds before (*black bars* first analysis) and after storage (*white bars* second analysis) for 100 days

Stability of acrylamide in roasted almonds during storage

The stability of acrylamide in foods during storage is another point of interest. It was shown that acrylamide is stable in starchy foods such as breakfast cereals, biscuits, and potato crisps during storage, whereas the content of acrylamide significantly decreased in coffee products and cocoa powder [26–28]. Data for roasted almonds were not available and, therefore, some samples of roasted almonds stored in a sealed container were reanalyzed after 100 days of storage at room temperature (Fig. 3). In all samples a decrease in the acrylamide content was observed and it ranged from 20 to 57%. So far, no correlation between the decrease and the initial acrylamide content or the degree of roasting has been found. Interestingly, decreasing acrylamide contents were mainly reported for roasted products such as coffee and cocoa [26–28]. It can be assumed that reactive compounds formed during the roasting process may be responsible for the decrease of acrylamide in these products during storage.

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