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Acrylamide in Roasted Almonds and Hazelnuts

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The influences of composition and roasting conditions on acrylamide formation in almonds and hazelnuts were investigated. Eighteen samples of almonds originating from the U.S. and Europe were analyzed for sugars and free amino acids, and acrylamide formed during roasting was determined. Asparagine was the main free amino acid in raw almonds and correlated with the acrylamide content of dark roasted almonds. Roasting temperature was another key factor and had a very strong influence on acrylamide formation. Almonds of European origin contained significantly less free asparagine and formed significantly less acrylamide during roasting as compared to the almonds from the U.S. Roasted hazelnuts contained very little acrylamide because of the low content of free asparagine in the raw nut. Reducing sugars, although being consumed much faster than free amino acids in both types of nuts, were not decisive for the extent of acrylamide formation during roasting.

KEYWORDS: Acrylamide; roasting; almonds; hazelnuts; asparagine; reducing sugars

INTRODUCTION

Acrylamide has neurotoxic and carcinogenic properties (*1*) and is classified as probably carcinogenic to humans (group 2A) (*2*). Therefore, its detection in a broad range of heated food products (*3*) led to an impressive number of research activities to investigate its formation, to monitor its sources in food, and to find ways for mitigation (*4*, *5*). Although the relevance of human exposure to acrylamide through the diet is not fully clarified yet, the world health organization (WHO) stated that acrylamide levels in food should be reduced because of public health concern (*6*). Acrylamide is formed in the Maillard reaction by the reaction of the free asparagine and reactive carbonyls (e.g., reducing sugars) at temperatures above 120 °C (*7*−*9*). Studies with stable isotope compounds have shown that the backbone of the acrylamide molecule purely originates from asparagine (*8*, *10*). However, the type of carbonyl has a strong influence on the amount of acrylamide formed from asparagine during heating (*10*, *11*).

Because potato products such as chips, hash browns, and French fries showed the highest acrylamide contents (*3*, *12*), and thus may contribute a major part to human exposure (*13*), many research activities were conducted in this field. The optimization of the raw potatoes in terms of amounts of reducing sugars (*14*, *15*) as well as the control of the frying temperature (*15*) are the main approaches to reduce the acrylamide content of these products. In contrast, for sweet bakery the baking agent ammonium hydrogencarbonate, the content of free asparagine, and the baking process were identified as critical factors for acrylamide formation and may provide options for mitigation strategies (*16*−*18*).

Comparatively little information is available on acrylamide in roasted nuts, although the acrylamide content of roasted almonds can exceed 1000 μg/kg (*12*, *19*, *20*). Almonds contain free asparagine and reducing sugars in appreciable amounts (*19*, *21*), and the roasting temperature as well as the physical form (whole, sliced, cut) of the almond kernel strongly influence the acrylamide formation (*19*, *20*). The purpose of this study was to continue the investigations of our first study on almonds (*19*), particularly to obtain an overview of the composition of almonds and hazelnuts, to identify the critical components for acrylamide formation, and to monitor the formation of acrylamide as well as the consumption of its precursors during roasting. Almonds of 14 different cultivars originating from the U.S. and Europe were analyzed for free amino acids and sugars, roasted under different conditions, and analyzed for their acrylamide content. Interrelations between acrylamide, its precursors, and the process conditions are shown and discussed.

MATERIALS AND METHODS

Samples of raw almonds of the 2003 and 2004 harvest were obtained from the Almond Board of California (ABC, Modesto, CA) and from various Swiss food companies. The investigated cultivars were as follows. (1) U.S.: Butte, Carmel, Fritz, Mission, Monterey, Neplus, Nonpareil, Padre, Price, and Sonora. (2) European (Spain and Italy): Avola, Longuettes, Larguetta, Valencia, and bitter almonds. Raw hazelnuts from Turkey and Italy and roasted hazelnuts (14−18 min at 150 °C) were obtained from Wernli AG (Trimbach, Switzerland). Raw nuts were stored at 5 °C in the dark until analysis and roasting, while roasted samples were stored airtight at −24 °C until analysis. All

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samples were homogenized in a household cutter (Moulinette, Moulinex, Paris, France) before analysis.

Roasting experiments were carried out in batches of 200 g by pile roasting using a fluidized-bed hot air laboratory roaster (G. W. Barth AG, Freiburg, Germany). Hot air was directed through the pile from bottom to top with a flux of 3.5 m/s. Temperatures of hot air below, within, and above the pile of nuts were monitored with temperature sensors (T51.1, Rotronic, Bassersdorf, Switzerland). After roasting, samples were cooled with air until the temperature within the pile stayed below 30 °C. Standard roasting experiments were performed at 145 °C for 14 min (light roast) and 165 °C for 12.5 min (dark roast), and roasting curves were obtained at 145 °C (0−32 min), 165 °C (0−30 min), and during roasting for 10 min (120 °C − 220 °C).

Analysis of Sugars and Free Amino Acids. Homogenized samples were mixed with deionized water and (1 + 4, e.g., 15 g of almonds + 60 g of water) and further homogenized (Polytron, Kinematica, Lucerne, Switzerland). For the determination of glucose, fructose, and sucrose, 12 to 40 g of the slurry was weighed into a graduated flask (100 mL). After addition of some water, 5 mL each of Carrez I (150 g of $K_4[Fe(CN)_6]\cdot 3H_2O$ per liter, Fluka, Buchs, Switzerland) and Carrez II (300 g of $ZnSO_4\cdot 7H_2O$ per liter, Fluka) solution were added, and the mixture intensively shaken. The pH was adjusted to 7 with a few drops of 4 M KOH solution (Fluka), the foam was broken with 1-octanol (Fluka), and the volume was adjusted to 100 mL with deionized water. Samples were shaken and filtered (Schleicher and Schuell, Dassel, Germany) and then analyzed using an enzymatic kit as described by the producer (Scil, Martinsried, Germany). For the determination of free amino acids, about 12 g of slurry was weighed into a 100 mL flask (graduated), and 1000 μL of a norleucine solution (internal standard, 5 mg/mL in deionized water, Fluka) as well as about 60 mL of 0.1 M HCl (Fluka) were added. After Carrez clarification (see above) foam was broken with 1-octanol (Fluka), the volume adjusted to 100 mL with 0.1 M HCl, and the samples thoroughly shaken. Samples were filtered (Schleicher and Schuell), diluted 1 + 4 (almonds) or 1 + 1 (hazelnuts) with 0.16 M lithium citrate buffer (pH = 2.2, PVP physiological, Laborservice Onken, Gründau, Germany), and mixed (Vortex, Bender and Hobein, Zurich, Switzerland). Samples were filtered through a 0.2 μm HPLC membrane filter (Titan, Infochroma, Zug, Switzerland) and subjected to analysis by cation-exchange chromatography followed by postcolumn derivatization with ninhydrin (Biochrom 30, Biochrom, Cambridge, U.K.) by using the physiological system (Biochrom) as described by the producer. The injection volume was 50 μL, and quantification was done both by comparison with an external standard and the internal standard. The external standard was an amino acid standard solution (Sigma, Steinheim, Germany) to which asparagine, glutamine, and nor-leucine (all from Fluka) were added. The *t*-test was performed as a two-tailed test (homoscedastic) by using Microsoft Excel 2002.

Determination of Acrylamide. Acrylamide was determined with a GC−MS method (*22*) using $^{13}C_3$-acrylamide (CIL, Andover, MA) and methacrylamide (Fluka), both dissolved in methanol (Fluka), as internal standards. GC−MS involved a 2000 series "TRACE GC" gas chromatograph with on-column injector (Thermo Quest CE Instruments, Milan, Italy) coupled to a TSQ quadrupole mass spectrometer (Finnigan Mat, San Jose, CA). The precolumn (TSP deactivated, i.d. 0.53 mm) and the separation column (BGB Wax, 12 m, i.d. 0.25 mm) were both from BGB Analytik (Böckten, Switzerland). GC and MS conditions were as described in ref 22 with the following modifications: The pressure of the reagent gas methane was set to 2000 mTorr only to allow for a much lower background giving better sensitivity and lower detection limits. To minimize undesired fragmentation of analytes, the ion source was held at 120 °C which demanded frequent cleaning of the ion volume. The signal-to-noise ratios for the determination of the limit of detection (LOD) and the limit of quantification (LOQ) were set to larger than 3:1 and larger than 10:1, respectively. ***Caution**: Acrylamide (CAS 79-06-1) is classified as toxic and may cause cancer. Wear suitable protective clothing, gloves, and eye/face protection when handling acrylamide.*

Table 1. Sugars and Free Amino Acids in Individual Almond Kernels of Cultivar Butte[a]

value	glucose [mg/kg]	fructose [mg/kg]	sucrose [mg/kg]	free Asn [mg/kg]	total free amino acids [mg/kg]
mean	596	220	38274	1334	3655
median	586	232	38429	1114	2836
min	482	152	28067	445	1796
max	733	286	51121	3463	8011
RSD [%]	12	23	21	75	58

[a] Data refer to fresh weight, $n = 10$. RSD: relative standard deviation.

RESULTS AND DISCUSSION

Reproducibility of Analyses and Laboratory Roasting Process. The method for the determination of free amino acids in almonds was tested with a homogenized sample of Nonpareil almonds in a small interlaboratory test with four Swiss laboratories (different techniques for extraction, separation, and detection). The mean content of free asparagine was 1837 mg/kg with a relative standard deviation (RSD) of 2.0%; the concentration determined by our method was 1854 mg/kg which demonstrates the good performance of the method. In-house validation showed recoveries of added asparagine of 96% and relative standard deviations ($n = 4$) of 5% (one homogenized sample) and 9% (four individual samples), respectively. Results were calculated based both on internal and external standards and differed usually only a little (maximum 10%); the results shown below were calculated with the external standard.

The reproducibility of the roasting process was checked with four individual roasting experiments at equal conditions (180 °C, 5 min) of Nonpareil almonds. The mean acrylamide content was 582 μg/kg with an RSD of 9.7% which is within the range of other process variations reported (*16*, *18*). Analysis of a homogenized sample of roasted almonds ($n = 4$) gave a mean acrylamide content of 494 μg/kg with an RSD of 2.7% demonstrating that the analysis varied less than the roasting process. On the basis of these results, differences between two samples larger than 20% (i.e., 2 times the variation of the roasting process) were considered to be significant. The analysis of a sample of roasted almonds spiked with different amounts of acrylamide ($n = 5$, 100−1000 μg/kg) resulted in a linear correlation between measured acrylamide contents and added acrylamide with $R^2 = 0.9996$, showing the good linearity of the analysis. On the basis of the signal-to-noise (s/n) ratios with standards and real samples, the LOQ was estimated to be 10 μg/kg (s/n > 10) and the LOD was estimated to be 4 μg/kg (s/n > 3); e.g., a sample of roasted hazelnuts (injection volume of 2 μL) gave a s/n of 37:1 for the acrylamide peak (*m*/*z* 72) corresponding to an acrylamide content of 14 μg/kg.

Variation of Acrylamide and Its Precursors between Individual Almond Kernels. It has been shown that sugars and free amino acids vary considerably between individual potato tubers from the same lot, which also affected the potential for acrylamide formation (*14*). To test if this fact applies also to raw almonds, individual kernels of the cultivars Butte, Nonpareil, and Monterey (harvest 2003) were analyzed separately for sugars and free amino acids. Results for cultivar Butte are shown in **Table 1**. The content of free asparagine varied considerably and to a larger extent than that of the sugars. A similar pattern was observed for cultivar Monterey (RSD = 68% for the free asparagine content), whereas for Nonpareil the content of free asparagine varied less (RSD = 30%). The RSD for glucose and fructose contents in all three cultivars ranged from 12% to 33%. The content of total free amino acids varied

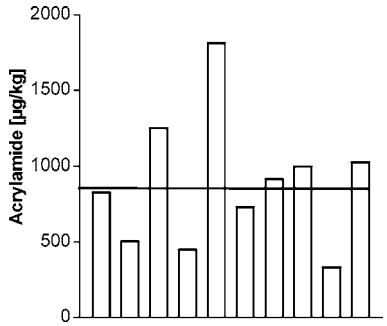

Figure 1. Acrylamide content of individual kernels from the same batch of roasted almonds (white bars, individual kernels; horizontal line, mean value; data refer to fresh weight, $n = 1$).

less in Nonpareil and Monterey as compared to that of Butte. These differences could be due to different stages of physiological maturity of the kernels (*23*) and are likely a major cause for the different degrees of browning between individual nuts from the same roasting lot (*24*, *25*).

Altogether, these data show that individual almond kernels of the same batch can vary considerably in their content of acrylamide precursors, especially in free asparagine. Therefore, the acrylamide content of roasted almonds was determined to check its variability between individual kernels (**Figure 1**). A sample of roasted almonds (150 °C, 22 min) obtained from industry was used for this purpose. The acrylamide content of individual roasted kernels varied strongly: The mean and median acrylamide content were 885 μg/kg and 872 μg/kg, respectively, but the values ranged from 334 μg/kg (minimum) to 1811 μg/kg (maximum), and the RSD was 47%. These differences reflect the individual composition of the almond kernels. As a consequence, batches of 200 g of almonds (170 to 200 kernels) were used for all roasting experiments, and for analysis of precursors in raw almonds two samples of about 50 g each were analyzed per lot.

Sugars and Free Amino Acids in Raw Almonds and Acrylamide in Roasted Almonds. Sugars and free amino acids were determined in 18 samples of raw almonds (14 different cultivars) grown in California and Europe. These almonds were subjected to two standard roasting processes, 14 min at 145 °C and 12.5 min at 165 °C, corresponding to a medium and a dark roasting degree, respectively, and the acrylamide content was determined (**Table 2**). Sucrose was the most abundant sugar in all samples with values ranging from 12 000 to 50 000 mg/kg. The mean glucose and fructose contents were about 2000 mg/kg (not including bitter almonds) and 1100 mg/kg, respectively, which is in the range of other published values (*19*, *26*). The sample of bitter almonds contained a very large amount of glucose, while the other components were in the range of the other European samples. There was a considerable variation of all parameters determined in the raw almonds indicating their different composition which is also noticeable within samples from the same cultivar (e.g., Valencia and Nonpareil).

Asparagine was the major free amino acid in all samples (500−2760 mg/kg), accounting for 20 to 50% of the pool of free amino acids. The other free amino acids were (ranges of all samples in parentheses) as follows: glutamic acid (270−700 mg/kg), aspartic acid (230−720 mg/kg), proline (120−630 mg/kg), alanine (110−210 mg/kg), valine (nd−230 mg/kg), glutamine (40−250 mg/kg), γ-amino butyric acid (nd−200 mg/kg), serine (40−120 mg/kg), threonine (30−430 mg/kg), glycine (40−100 mg/kg), phenylalanine (nd−120 mg/kg), isoleucine (40−90 mg/kg), and leucine (40−70 mg/kg). In total, almonds contained a large pool of free amino acids: The mean value was 3560 mg/kg and values ranged from 2200 to 5500 mg/kg. Our present data on free amino acids are in the range of or slightly below published data (*19*, *21*).

Light roasting at 145 °C resulted in low to moderate amounts of acrylamide (20−360 μg/kg), while during dark roasting at 165 °C up to 1500 μg/kg of acrylamide was formed, confirming previous findings that the roasting temperature has a very strong influence on acrylamide formation in almonds (*19*). For both roasting processes a wide variation of the acrylamide contents was observed which probably reflects the different composition of the almonds. Part of these variations might also be due to inhomogeneities in samples and variation of analysis (see above). The RSD of the acrylamide contents of almonds roasted at 145 °C and 165 °C were 60% and 53%, respectively, which is close to the RSD of the contents of free asparagine (RSD = 56%) in the raw samples.

Table 2. Sugars and Free Amino Acids in Raw Almonds and Acrylamide Formed in Two Standard Roasting Processes[a]

cultivar	origin	Glc [mg/kg]	Fru [mg/kg]	Suc [mg/kg]	Free Asn [mg/kg]	TFAA [mg/kg]	AA [μg/kg] 145 °C 14 min	AA [μg/kg] 165 °C 12.5 min
Butte	USA	1610	920	38100	1040	3000	44	712
Carmel	USA	1180	700	32100	2760	5390	200	1681
Nonpareil A	USA	1500	1020	41650	1380	3690	226	1110
Nonpareil B[b]	USA	1260	660	23760	1460	4060	251	1081
Nonpareil C	USA	450	210	45810	1500	4080	356	1265
Fritz	USA	1800	720	35210	2370	5160	67	1556
Monterey	USA	1510	880	39310	1940	4690	166	1333
Padre	USA	2870	1800	50630	1260	3890	185	1243
Neplus	USA	2320	1520	36930	720	2770	19	504
Mission	USA	1730	1210	47100	1710	4220	234	1462
Price	USA	1380	740	35970	2640	5510	163	1381
Sonora	USA	2160	1250	39460	1330	3660	73	1200
Bitter almonds	Spain	35830	1210	26750	590	2640	105	605
Valencia A	Spain	1230	700	38430	820	2880	129	460
Valencia B	Spain	1660	780	17390	560	2280	73	250
Valencia C[b]	Spain	4010	2580	16110	490	2520	73	312
Largueta[b]	Spain	3320	2290	12380	520	2240	32	202
Longuettes	Spain	1570	790	30900	860	2640	66	664
Avola	Italy	4260	1560	15510	570	2350	95	353

[a] Glc, glucose; Fru, fructose; Suc, sucrose; Asn, asparagine; TFAA, total free amino acids; AA, acrylamide; $n \geq 2$, except for acrylamide ($n = 1$). Data refer to fresh weight; variation of analyses within a certain cultivar was smaller than 12 % on average. [b] Almonds from harvest 2003 (all other samples were from harvest 2004).

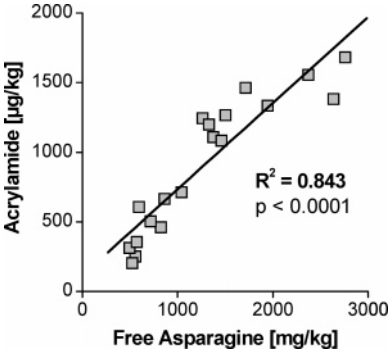

Figure 2. Correlation between the acrylamide content of roasted almonds (165 °C 12.5 min) and the content of free asparagine in raw almonds ($n = 1$; data refer to fresh weight).

Interestingly, almonds of European origin contained on average about 2.7 times less free asparagine than almonds from the U.S., which in turn contained somewhat less reducing sugars and 1.7 times more sucrose than the samples from Europe. A similar but less pronounced pattern of free asparagine in U.S. and European almonds was reported by Seron et al. (*21*). They found on average 1.6 times more free asparagine in U.S. almonds (3660 mg/kg, $n = 4$) than in European samples (2240 mg/kg, $n = 11$)which turned out to be a significant difference in the *t*-test ($p < 0.05$). In our study, free asparagine accounted for 39% of the free amino acids in U.S. almonds but only 25% in European samples. As for acrylamide in dark roasted almonds, the comparison between U.S and European samples gave the same picture as for free asparagine. U.S. samples contained on average 3.0 times more acrylamide than European almonds. The *t*-test revealed a highly significant difference between European and U.S. samples for both criteria: p (free asparagine) < 0.001 and p (acrylamide, 165 °C 12.5 min) < 0.0001. The situation was similar but less pronounced in medium roasted almonds (145 °C, 14 min): U.S. almonds again contained significantly more acrylamide than European almonds ($p < 0.05$). Furthermore, U.S. samples contained significantly more total free amino acids ($p < 0.0005$), free glutamine ($p < 0.00001$), isoleucine ($p < 0.0001$), proline ($p < 0.0001$), and sucrose ($p < 0.001$) than European samples, while the differences were less clear for the reducing sugars. However, to corroborate these differences between almonds from the U.S. and Europe, more samples from further harvests have to be compared. Due to the limited number of samples it was not possible to identify statistical differences between almond varieties. To identify such differences much more samples per variety will have to be analyzed.

The results presented above suggest that the content of free asparagine in raw almonds determined the acrylamide formation. This hypothesis was tested by checking various combinations of acrylamide content and components determined in raw almonds. A strong correlation was found between acrylamide and the content of free asparagine (**Figure 2**), confirming the key role of free asparagine for acrylamide formation in roasted almonds. Acrylamide correlated also with total free amino acids ($R^2 = 0.877$), which can be explained by the correlation between free asparagine and total free amino acids ($R^2 = 0.971$), respectively. In contrast, no correlation was found between acrylamide and glucose, fructose, or sucrose. This is different from potatoes, where reducing sugars largely determine the acrylamide formation and free asparagine does not correlate with acrylamide (*14*, *27*). Furthermore, the earlier observed connection between the content of reducing sugars and acrylamide based on few almond samples (*19*) has to be considered as coincidental rather than causal. Similar correlations between

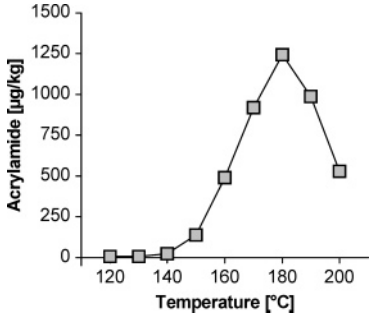

Figure 3. Acrylamide content in almonds roasted for 10 min at different temperatures (cultivar Butte, $n = 1$; data refer to fresh weight).

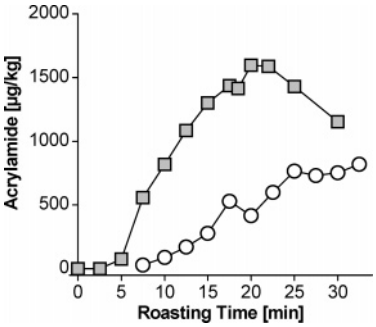

Figure 4. Development of acrylamide contents during roasting at 145 and 165 °C for different times (circles, 145 °C; squares, 165 °C; cultivar Nonpareil, $n = 1$; data refer to fresh weight).

acrylamide in the heated product and free asparagine in the raw product were also observed in cereal model systems, breads, and gingerbread (*16*, *28*).

Almonds roasted to a medium degree (145 °C 14 min) showed no correlation between the acrylamide content and any of the sugars and amino acids determined. This might be explained by the fact that after 15 min of roasting at 145 °C asparagine and amino acids in general had hardly been consumed and were thus not limiting, as will be discussed in the next paragraph.

Formation of Acrylamide and Consumption of Its Precursors during Roasting. Temperature and time are known to influence acrylamide formation (*11*). To investigate this interrelation in detail roasting experiments at different conditions were carried out. **Figure 3** shows the influence of roasting temperature on the acrylamide content of almonds roasted for 10 min. Acrylamide formation was observed from 140 °C onward and increased strongly from 140 °C to 180 °C, confirming the dominant impact of temperature on acrylamide formation in almonds (*19*). At 190 °C and 200 °C the acrylamide content decreased again showing that elimination of acrylamide exceeded the new formation which was also observed in a model system (*7*).

In our previous study (*19*) we reported a seemingly linear relationship between acrylamide content and roasting time at a constant temperature of 150 °C. However, data did not cover the full time range, and thus, extended roasting experiments were performed at 145 °C and 165 °C **(Figure 4)**. During roasting at 165 °C, acrylamide formation started after about 5 min only, and between 7.5 and 15 min an almost linear course was observed, confirming previous results (*19*). The delay of the onset of acrylamide formation was probably due to the time to heat the almonds to a temperature beyond 100 °C. After about 20 min of roasting the acrylamide content leveled off and decreased afterward indicating that elimination exceeded net new formation. Similar patterns of time dependency of acry-

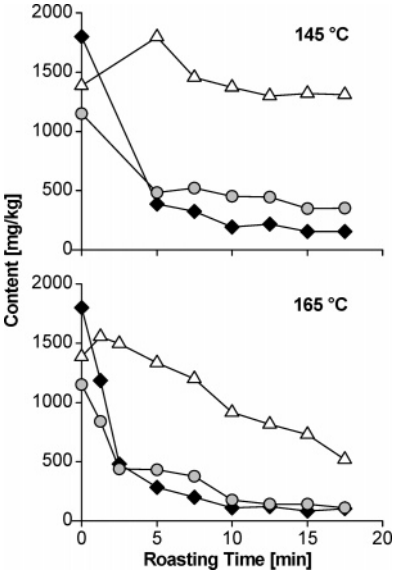

Figure 5. Consumption of free asparagine and reducing sugars during roasting at 145 and 165 °C (cultivar Nonpareil, $n = 1$; values refer to fresh weight; triangles, free asparagine; circles, fructose; diamonds, glucose).

lamide formation were also observed in model systems (*29*), roasted coffee beans (*4*), grated potato (*30*), and gingerbread (*16*). In practice, roasting at 165 °C is an upper limit for almonds, and samples roasted for longer than 10−15 min are considered "over-roasted" and bitter and therefore hardly suitable for direct consumption. Therefore, it seems unlikely that approaches based on elimination are feasible to reduce the acrylamide content of almonds. At 145 °C acrylamide formation started only after about 7 min and was much slower as compared to that at 165 °C. After 32 min the acrylamide contents were still below 900 μg/kg, and new formation was yet larger than elimination as indicated by the still increasing acrylamide contents.

The consumption of sugars and free amino acids during roasting presents another point of interest (**Figure 5**). After 15 min of roasting at 145 °C still 95% of the initial content of free asparagine and 93% of the initial content of total free amino acids were found, while less than 20% of the reducing sugars were left. Therefore, asparagine was not limiting at all at this roasting temperature, which explains why no correlation between acrylamide and any amino acid was found for this roasting temperature (see above). Although the content of free asparagine determined the acrylamide formation in dark roasted almonds, its depletion was much slower compared to that of the reducing sugars (**Figure 5**). At both temperatures the content of free asparagine slightly increased in the first minutes of roasting. A similar increase was also observed in rye and potato cakes (*29*) but can only partially be explained by the loss of water. After 5 min roasting at 165 °C still 96% of the initial content of free asparagine was found, while only 16% of the glucose and 38% of the fructose were left. After 15 min still half of the free asparagine was present while about 90% of the reducing sugars had already reacted. This might explain why no correlation was found between reducing sugars and acrylamide formation in standard roasting processes. The reducing sugars were depleted long before the acrylamide content reached its final level. Glucose was consumed somewhat faster than fructose, while the content of sucrose did not decrease to a significant extent in the first 15 min, which is well in line with previous findings in roasted almonds (*19*) and with results from cereal and potato model systems (*14*, *29*). Among the free amino acids glutamine was depleted fastest (not detectable anymore after 5 min), while 60 to 80% of the other free amino acids were still present after 10 min. After 15 min 50% of the initial content of total free amino acids was found, and the recovery of the main free amino acids were as follows: asparagine, 47%; glutamic acid, 19%; aspartic acid, 67%; proline, 54%; alanine, 74%; valine, 40%; serine, 53%. The different extent of consumption of the individual amino acids at least partially reflects their different chemical reactivity which was also observed in potatoes and cereals (*14*, *29*). The fast depletion of glutamine in roasted almonds can have various reasons: If glutamine is heated with reducing sugars large amounts of 2-pyrollidinone and traces of 3-buteneamide are found (*31*). Glutamine can also depleted through the loss of ammonia which takes place even under mild conditions (*32*) and/or by the formation of pyrrolidone carboxylic acid.

Interestingly, after 5 min 75% of the reducing sugars had disappeared, although only 80 μg/kg acrylamide were formed corresponding to only 5% of its maximal content (see **Figure 4**). Reducing sugars were consumed very fast in the first 5 min of roasting, while asparagine decreased more uniformly. The loss of free asparagine was negatively correlated with acrylamide formation ($R^2 = -0.9836$) which was also observed in cereal and potato model systems (*29*). The molar concentrations of reducing sugars and free amino acids in raw almonds at least partly explain these observations: The median content of reducing sugars was 14 mmol/kg, while it was 42 mmol/kg for total free amino acids and 7 mmol/kg for free asparagine. Thus, the reducing sugars were confronted with about 2.5 times more free amino acids, which would explain their fast degradation. A similar effect was also observed in heated potato cakes, and the authors suggested that the greater loss of reducing sugars was due to the excess of free amino acids compared to the amount of the reducing sugars (*29*).

However, these different behaviors give also raise to the following hypotheses: (i) Formation of intermediates, e.g., Schiff base or decarboxylated Amadori product (*9*), did not require a large amount of energy, since sugars were rapidly consumed in the first part of the roasting process when the temperature was still increasing. (ii) The release of acrylamide from such intermediates required more energy, whereby the acrylamide formation was delayed compared to the consumption of reducing sugars. (iii) Sugars were degraded to fragments (e.g., desoxysones, glyoxal, etc.) under moderate conditions with amino acids acting as catalysts. Once these fragments were formed they readily reacted with free asparagine, whereby acrylamide was formed. Hypothesis iii is supported by the findings that sugar fragments were formed under mild conditions from glucose and amino acids (*33*) and that sugar fragments such as glyoxal and hydroxyacetone formed more acrylamide than glucose when heated with asparagine (*10*, *11*). The determination of intermediates and sugar fragments was out of the scope of the project, and thus, these hypotheses cannot be verified or rejected. However, monitoring intermediates and/or fragments of sugars and asparagine could be useful tools to understand the formation of acrylamide in food matrices.

Stability of Acrylamide in Roasted Almonds. The stability of acrylamide in foods was unclear for some time and was supposed to contribute to the wide range of reported acrylamide levels for a certain group of foods. Five samples of roasted almonds (medium roasting degree) were thus stored in sealed containers at room temperature for up to 300 days and reanalyzed. **Figure 6** shows that in all five samples the

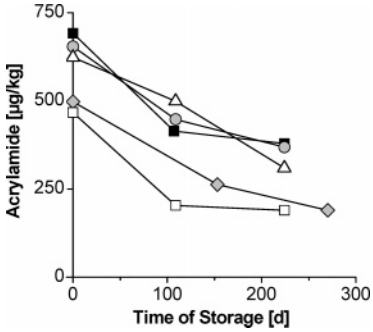

Figure 6. Changes of the acrylamide content in five samples of roasted almonds during prolonged storage at room temperature ($n = 1$; data refer to fresh weight).

Table 3. Sugars and Free Amino Acids in Raw Hazelnuts and Acrylamide in Roasted Hazelnuts[a]

compound	content
Glc [mg/kg]	970
Fru [mg/kg]	890
Suc [mg/kg]	29260
free Asn [mg/kg]	30
free Glu	450
free Ala	240
free Asp	170
TFAA [mg/kg]	1610
AA (145 °C 14 min) [µg/kg]	16
AA (165 °C 12.5 min) [µg/kg]	56

[a] Origin, Turkey. Glc, glucose; Fru, fructose; Suc, sucrose; Asn, asparagine; Glu, glutamic acid; Ala, alanine; Asp, aspartic acid; TFAA, total free amino acids; AA, acrylamide; $n = 2$, except for acrylamide ($n = 1$). Variation of duplicate analyses: RSD < 15 %. Data refer to fresh weight.

acrylamide content decreased over the storage period. Between the first and last analysis the acrylamide contents had decreased significantly by 44% to 62%, which confirms that acrylamide is not stable in roasted almonds (*19*). Therefore, roasted almonds with a similar production date must be compared and roasted samples should be analyzed quickly. A similar effect was recently reported for coffee and cacao where the acrylamide contents decreased for some 30% during storage (*34*). As losses through evaporation or UV-induced polymerization can most likely be excluded, a reaction of acrylamide with Maillard reaction products from the roasting process, e.g., $-SH$ or $-NH_2$ compounds (*34*), seems to be a reasonable explanation since acrylamide is known to easily react with thiol compounds through a Michael addition (*1*).

Acrylamide and Its Precursors in Hazelnuts. Roasted hazelnuts are widely used as ingredients in the manufacture of bakery and chocolate products, but information on the acrylamide content of roasted hazelnuts is scarce. Therefore, samples of raw and roasted hazelnuts were obtained from industry and analyzed. Roasted hazelnuts as typically used for biscuits contained very little acrylamide (14 to 22 µg/kg). This can be explained by the very low content of free asparagine in the raw hazelnuts (**Table 3**). Hazelnuts contained about 40 times less free asparagine as compared to that in almonds, whereas the sugar contents were similar. In hazelnuts free asparagine contributed only 2% to the pool of free amino acids in which glutamic acid (24% of total free amino acids), alanine (15%), and aspartic acid (10%) were the major free amino acids. Similar sugar contents were found by other groups (*26*, *35*) while Alasalvar et al. reported somewhat higher contents of free amino acids, but asparagine contributed again only little (about 7.5%) to the pool of free amino acids (*35*). Hazelnuts subjected to the same roasting processes as the almonds (see **Table 2**) contained only a little acrylamide: roasting at 165 °C for 12.5 min produced dark roasted hazelnuts, but even in this sample the acrylamide content stayed below 60 µg/kg. This is about 15 times less compared to the average acrylamide content in almonds roasted under the same conditions which underlines the difference between these two nuts.

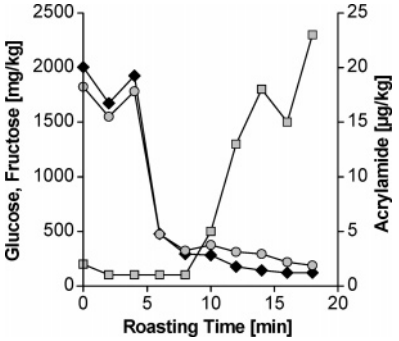

Figure 7. Consumption of reducing sugars and formation of acrylamide during roasting of hazelnuts at 150 °C (origin, Italy; $n = 1$; data refer to fresh weight; squares, acrylamide; circles, fructose; diamonds, glucose).

Formation of acrylamide in hazelnuts roasted at 150 °C was very slow (**Figure 7**). After 10 min less than 10 µg/kg was determined, and even after 18 min only ~ 25 µg/kg was found. Sugars were consumed in a similar manner as compared to that in almonds: After 10 min over 80% of the reducing sugars was degraded, while 70% of the free asparagine and almost 80% of the total free amino acids were still present. Sucrose was not significantly depleted during the whole roasting process.

After 18 min 50% of the free amino acid pool was still found, while only about 10% of the reducing sugars were left. Aspartic acid and citrulline were the most stable amino acids, while glutamine was again very unstable (not detectable after 8 min), and still 40% of the initial content of free asparagine was found after 18 min. Amino acids obviously reacted much more slowly than reducing sugars, as was also observed in potatoes, almonds, and cereals (*14*, *19*, *29*).

Overall, the following conclusions can be drawn: (i) Almonds form much more acrylamide during roasting than hazelnuts, which is explained by the far larger content of free asparagine in almonds. (ii) Beside the concentration of free asparagine, the roasting temperature is the main controlling factor for acrylamide formation in almonds. (iii) By selecting almonds low in free asparagine and by reducing the roasting temperature a significant reduction of the acrylamide content can be achieved. However, as development of flavor and color is also dependent on chemical composition and roasting conditions further research is needed to find the optimal combination of raw material and process conditions in order to obtain a product both with low acrylamide content and favorable sensory properties.

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LITERATURE CITED

(1) Friedman, M. Chemistry, biochemistry, and safety of acrylamide. A review. *J. Agric. Food Chem.* **2003**, *51*, 4504−4526.

(2) IARC. Acrylamide. In *Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals*; International Agency for Research on Cancer, Ed.; IARC: Lyon, 1994; pp 389−433.
(3) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.
(4) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R. H.; Gonde, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Muller, D.; Tallmadge, D.; O'Brien, J.; Thompson, S.; Silvani, D.; Whitmore, T. A review of acrylamide: An industry perspective on research, analysis, formation and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323−347.
(5) Stadler, R. H.; Scholz, G. Acrylamide: An update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control. *Nutr. Rev.* **2004**, *62*, 449−467.
(6) Acrylamide levels in food should be reduced because of public health concern says UN expert committee. *WHO/FAO Press Release*, March 2, 2005.
(7) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.
(8) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M. C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.
(9) Yaylayan, V. A.; Wnorowski, A.; Locas, C. P. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753−1757.
(10) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782−4787.
(11) Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550−5558.
(12) Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W. Acrylamide in foods: Occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802−808.
(13) Boon, P. E.; de Mul, A.; van der Voet, H.; van Donkersgoed, G.; Brette, M.; van Klaveren, J. D. Calculations of dietary exposure to acrylamide. *Mutat. Res.* **2005**, *580*, 143−155.
(14) Amrein, T. M.; Schönbächler, B.; Rohner, F.; Lukac, H.; Schneider, H.; Keiser, A.; Escher, F.; Amadò, R. Potential for acrylamide formation in potatoes: data from the 2003 harvest. *Eur. Food Res. Technol.* **2004**, *219*, 572−578.
(15) Grob, K.; Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Imhof, D.; Amrein, T. M.; Pfefferle, A.; Bazzocco, D. French fries with less than 100 μg/kg acrylamide. A collaboration between cooks and analysts. *Eur. Food Res. Technol.* **2003**, *217*, 185−194.
(16) Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R. Acrylamide in gingerbread: Critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **2004**, *52*, 4282−4288.
(17) Biedermann, M.; Grob, K. Model studies on acrylamide formation in potato, wheat flour and corn starch; ways to reduce acrylamide contents in bakery ware. *Mitt. Lebensmittelunter. Hyg.* **2003**, *94*, 406−422.
(18) Vass, M.; Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R. Ways to reduce the acrylamide formation in cracker products. *Czech J. Food Sci.* **2004**, *22*, 19−21.
(19) Amrein, T. M.; Andres, L.; Schönbächler, B.; Conde-Petit, B.; Escher, F.; Amadò, R. Acrylamide in almond products. *Eur. Food Res. Technol.* **2005**, *221*, 14−18.
(20) Weisshaar, R. Acrylamid in Backwaren—Ergebnisse von Modellversuchen. *Dtsch. Lebensm.—Rundsch. (in German)* **2004**, *100*, 92−97.
(21) Seron, L. H.; Poveda, E. G.; Moya, M. S. P.; Carratalá, M. L. M.; Berenguer-Navarro, V.; Grané-Teruel, N. Characterisation of 19 almond cultivars on the basis of their free amino acids composition. *Food Chem.* **1998**, *61*, 455−459.
(22) Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Grob, K.; Egli, P.; Mändli, H. Two GC-MS methods for the analysis of acrylamide in foods. *Mitt. Lebensmittelunter. Hyg.* **2002**, *93*, 638−652.
(23) Soler, L.; Cañellas, J.; Sauracalixto, F. Changes in carbohydrate and protein content and composition of developing almond seeds. *J. Agric. Food Chem.* **1989**, *37*, 1400−1404.
(24) Pearson, T. C. Spectral properties and effect of drying temperature on almonds with concealed damage. *Food Sci. Technol.* **1999**, *32*, 67−72.
(25) Özdemir, M.; Seyhan, F. G.; Bakan, A. K.; Ilter, S.; Özay, G.; Devres, O. Analysis of internal browning of roasted hazelnuts. *Food Chem.* **2001**, *73*, 191−196.
(26) Ruggeri, S.; Cappelloni, M.; Gambelli, L.; Carnovale, E. Chemical composition and nutritive value of nuts grown in Italy. *Ital. J. Food Sci.* **1998**, *10*, 243−252.
(27) Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W.; Hayward, S.; Sahagian, M.; Ramesh, M.; Leclerc, Y. Acrylamide in French fries: Influence of free amino acids and sugars. *J. Agric. Food Chem.* **2004**, *52*, 3801−3806.
(28) Bråthen, E.; Knutsen, S. H. Effect of temperature and time on the formation of acrylamide in starch-based and cereal model systems, flat breads and bread. *Food Chem.* **2005**, *92*, 693−700.
(29) Elmore, J. S.; Koutsidis, G.; Dodson, A. T.; Mottram, D. S.; Wedzicha, B. L. Measurement of acrylamide and its precursors in potato, wheat, and rye model systems. *J. Agric. Food Chem.* **2005**, *53*, 1286−1293.
(30) Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Grob, K. Methods for determining the potential of acrylamide formation and its elimination in raw materials for food preparation, such as potatoes. *Mitt. Lebensmittelunter. Hyg.* **2002**, *93*, 653−667.
(31) Stadler, R. H.; Verzegnassi, L.; Varga, N.; Grigorov, M.; Studer, A.; Riediker, S.; Schilter, B. Formation of vinylogous compounds in model Maillard reaction systems. *Chem. Res. Toxicol.* **2003**, *16*, 1242−1250.
(32) Sohn, M.; Ho, C. T. Ammonia generation during thermal degradation of amino acids. *J. Agric. Food Chem.* **1995**, *43*, 3001−3003.
(33) Hollnagel, A.; Kroh, L. W. Formation of alpha-dicarbonyl fragments from mono- and disaccharides under caramelization and Maillard reaction conditions. *Eur. Food Res. Technol.* **1998**, *207*, 50−54.
(34) Hoenicke, K.; Gatermann, R. Studies on the stability of acrylamide in food during storage. *J. AOAC Int.* **2005**, *88*, 268−273.
(35) Alasalvar, C.; Shahidi, F.; Liyanapathirana, C. M.; Ohshima, T. Turkish tombul hazelnut (*Corylus avellana* L.). 1. Compositional characteristics. *J. Agric. Food Chem.* **2003**, *51*, 3790−3796.

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