European Food Research and Technology (2018) 244:1381–1388
https://doi.org/10.1007/s00217-018-3051-2

ORIGINAL PAPER

Acrylamide in cocoa: a survey of acrylamide levels in cocoa and cocoa products sourced from the German market

Marion Raters[1] · Reinhard Matissek[1]

Received: 7 December 2017 / Revised: 30 January 2018 / Accepted: 3 February 2018 / Published online: 19 February 2018

Abstract
The aim of this project was to conduct a comprehensive monitoring of acrylamide in cocoa and chocolate products. Within the scope of this research work, we examined 94 semi-finished cocoa products (cocoa beans, nibs, powder, masses, and cocoa butter) and 269 cocoa and chocolate products (milk chocolates, chocolates, chocolates with additional ingredients, white chocolates, and cocoa-containing drink powders) for their respective acrylamide content levels. The range of acrylamide content levels determined came to < LOQ–490 µg kg^{-1} (LOQ, limit of quantification < 30 µg kg^{-1}). Furthermore, our examinations showed that semi-finished cocoa products had higher acrylamide levels (mean value 190 µg kg^{-1}), in the mean, than did cocoa and chocolate products (mean value 50 µg kg^{-1}). In addition, it can be stated that all examined cocoa samples proved to have acrylamide levels distinctly below the general German signal value of 1000 µg kg^{-1}. An individual German signal value or EU indicative value/benchmark for acrylamide content levels in cocoa and chocolate products is currently not available.

Keywords Acrylamide · Liquid chromatography mass spectrometry (LC-MS/MS) · Cocoa · Cocoa products · Chocolates

Introduction

Cocoa and chocolate products count among the most frequently consumed confectionery in Germany. In 2016, Germany's per-capita consumption of chocolate goods came to 9.55 kg, with the consumption of cocoa-containing food preparations reaching 0.46 kg. This corresponds to a total per-capita consumption of confectionery of 30.55 kg [1]. In addition to the positive components that can be found in cocoa and chocolate products, such as cocoa flavanols [2], cocoa may also contain undesirable substances, however. Hence, in the interests of preventive consumer health protection, it makes sense to analyse cocoa and chocolate products for the occurrence of process contaminants such as acrylamide.

Acrylamide is an α,β-unsaturated carbonyl compound which is used as the monomer of the synthetic polyacrylamide [3]. Acrylamide was first detected in various heat-treated, carbohydrate-rich foodstuffs in 2002. Acrylamide is formed during the roasting process at $T > 100$ °C [4, 5]. It is mainly formed via the Maillard reaction process from the amino acid asparagine and carbonyl compounds such as the reducing sugars glucose and fructose [6–8].

From a toxicological point of view, the intake of high acrylamide levels is definitely to be seen as a relevant issue: acrylamide was classed as a category 2A substance, "probably carcinogenic", by the IARC (International Agency for Research on Cancer). The metabolisation of acrylamide also produces glycidamide, the latter likewise being toxicologically relevant [4, 9]. Hence acrylamide is deemed to be the forerunner of a previously unknown and completely new group of toxicological significant substances, the so-called "foodborne toxicants". After a reassessment conducted in 2013 by Germany's Federal Institute for Risk Assessment (BfR), the BfR came to the conclusion that a correlation between carcinogenesis and the intake of acrylamide can neither be assumed nor ruled out [10]. In 2015, EFSA published its first full risk assessment of acrylamide in food. Experts from EFSA's Panel on Contaminants in the Food Chain (CONTAM) reconfirmed previous evaluations that acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups. Evidence from

✉ Marion Raters
marion.raters@lci-koeln.de

1 Food Chemistry Institute of the Association of the German Confectionery Industry, Adamsstraße 52-54, 51063 Cologne, Germany

animal studies shows that acrylamide and its metabolite glycidamide are genotoxic and carcinogenic [11].

In the interests of preventive consumer health protection, already shortly after the discovery of acrylamide as a "foodborne toxicant", the German Federal Office for Consumer Protection and Food Safety (BVL) introduced so-called "Signalwerte" (signal values) for various product groups such as potato crisps and fine bakery wares and these values have been continually reduced [12]. Following the good example set by Germany, the EU introduced so-called "indicative values" for certain product groups in 2011 [13]. In the meantime, the European commission has adopted a flexible concept for regulating and minimising acrylamide levels in foodstuffs. This involves getting the European specialist associations to take up "best practice" guidelines in a so called "code of practice" and increasing the binding nature of the code for the industry stakeholders. In addition, the earlier "indicative values" are to serve as reference values for the successful implementation of minimisation measures (in future known as "benchmarks") within the scope of the regulatory proposal. On 11th December 2017, the EU regulation No 2017/2158 of 20th November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food entered into force, it shall apply from 11th April 2018 [14]. The European benchmarks releases the signal values. Only for food categories with no specific benchmarks in the EU regulation No 2017/2158 the German signal values keep their validity in the German market. No specific indicative values, and to date no benchmarks, have been set for cocoa and chocolate products.

So far hardly any comprehensive studies of acrylamide in cocoa and chocolate products have been conducted. However, the formation of acrylamide can occur in these foodstuffs through the cocoa bean roasting process (at temperatures of 100–160 °C). Within the scope of a corresponding monitoring study conducted in 2015, KÖPPEN et al. examined 140 samples (chocolates with various local content levels and cocoa powders) sourced from German retail markets by means of HPLCMS/MS in combination with stable isotope dilution analysis (SIDA). The determined acrylamide levels fell within a range of 9–1747 μg kg^{-1} [15]. KÖPPEN et al. additionally recommended that the dynamic minimisation concept of the German Federal Office for Consumer Protection and Food Safety (BVL) should also be applied to cocoa and chocolate products.

In the EU database for acrylamide which is evaluated within the scope of the EFSA opinion in 2015 a total of 44 samples called "other products based on cocoa" were mentioned, with a mean acrylamide level of 104 μg/kg [11].

Within the scope of an internal, unpublished study conducted by the Association of the German Confectionery Industry (BDSI) over the period 2003 to 2005, semi-finished cocoa products such as cocoa and chocolate products were likewise examined to determine their acrylamide content levels. In this context, no acrylamide was detected in fresh cocoa beans. Acrylamide content levels of between ≤ 30 and 700 μg kg^{-1} were determined in cocoa masses from 11 different places of origin. The mean content levels of the 23 examined chocolate samples came to 139 μg kg^{-1} in the dark chocolates ($N = 18$) and 67 μg kg^{-1} in the milk chocolates ($N = 5$), respectively [16].

In this context, the aim of this study was to conduct a comprehensive monitoring of acrylamide in semi-finished cocoa products as well as in cocoa and chocolate products from the German retail market intended for direct end-consumer.

Materials and methods

Solvents and reagents

Acrylamide, > 99% (CAS registry number 79-06-1), was supplied by Sigma (Taufkirchen, Germany) and deuterium labelled acrylamide-*d3* (99%) by LGC Standards GmbH (Wesel, Germany). All other chemicals and solvents were analysis-grade or HPLC-grade and were obtained from Merck (Darmstadt, Germany).

Standard preparation

Stock solutions of acrylamide standard and internal standard acrylamide-*d3*, 1 mg ml^{-1}, were prepared in acetonitrile/water (50/50, v/v) and stored at 4 °C. Working standard solutions, for spiking samples as well as for the standard curve, were obtained by dilutions using acetonitrile/water (50/50, v/v).

Sample extraction

Analytical procedure was done according to the LCI-internal validated method [17]. The samples were homogenised and 20 ml of water and 400 μl of internal standard acrylamide*d3* (5 μg ml^{-1}) were added to 2 g of the homogenised sample. The samples were extracted by ultrasonic treatment (15 min, 60 °C) and 20 ml of acetonitrile was added. Clean-up of the extracts was performed using 500 μl of Carrez I and II (Potassium ferrocyanide $K_4Fe(CN)_6 \times 3H_2O$, 150 g L^{-1}) and Carrez II (Zinc sulphate $ZnSO_4 \times 7\ H_2O$, 300 g L^{-1}), respectively, and the samples were then centrifuged (4500 rpm for 10 min, 4 °C). Before injection into the LC–MS/MS system, the supernatant was passed through a syringe filter (0.45 μm, Roth Rotilabo Nylon).

LC-MS/MS analysis

Mass spectrometry measurements were performed using a HPLC-system 1260 Infinity™ (Agilent Technologies, Germany) coupled with a Triple Quad™ 4500 mass spectrometer (Sciex, Germany). The results were processed using an Analyst version 1.6.1 data system (Sciex). Analytical separation was achieved using a Lichrospher 100 CN 5 µm (250 × 4 mm) with a guard column 5 µm (Merck, Darmstadt, Germany). The elution mode was isocratic, using a mixture of acetonitrile and water (0.5:99.5, v/v) containing 0.1% (v/v) of concentrated formic acid as LC eluent. The flow rate was 0.25 ml min^{-1} and the injection volume was 20 µl. Acrylamide was identified by multi-reaction monitoring (MRM) in positive electrospray ionisation mode (ESI+). Three different fragment ion transitions were monitored for both acrylamide (m/z 72→72, m/z 72→52 and 72→44) and the internal standard (m/z 75→75, m/z 75→58 and 75→44), $t_R = 11.6$ min. The electrospray source had the following settings (with nitrogen): capillary voltage 3 kV; cone voltage 40 V; source temperature 450 °C.

Quantification of acrylamide was performed using the ratio of the peak area of the quantifying ion for acrylamide (m/z 72→52) to the peak area of the quantifying ions for the deuterated internal standard acrylamide*d3* (m/z 75→58).

Method validation

Prior to analysis of acrylamide, the LCMS/MS method was validated to ensure the quality of the data. The linearity of the calibration curve was checked by a series of standard solutions of acrylamide ranged from 1 to 500 µg L^{-1} at seven different concentrations. The recoveries and the relative standard deviation were studied by the prepared samples spiked with different concentrations of acrylamide (spiking levels 50–1000 µg kg^{-1}). The method showed a good linearity in concentrations ranging from 1 to 500 µg mL^{-1} with a correlation coefficient of 0.997. Limit of detection (LOD) and the limit of quantification (LOQ) was found to be 10 and 30 mg kg^{-1}, respectively. The recoveries ranged from 91 to 97%, and the repeatability relative standard deviation (RSD_r) was 7%.

Quality control

The laboratory participated in various proficiency tests organised by the Food Analysis Performance Assessment Scheme (FAPAS) of the Central Science Laboratory (CSL) York (UK) for biscuits, Test No. 3067 (FAPAS, 2016), and for vegetable crisps, Test No. 3073 (FAPAS, 2017), with *z* scores of 1.2 and 1.3, respectively.

Sampling

The examined samples of cocoa and chocolate products possessed different cocoa content levels. The samples were taken first from various laboratory purchases sourced from German retailers (samples were drawn from October 2015 to December 2016) and second several samples were provided by industrial companies operating in the cocoa processing sector. The composition of the sample pool is shown in Table 1.

Results and discussions

Cocoa and cocoa products

The monitoring project analysed a total of 363 samples for acrylamide and statistically evaluated the corresponding findings. Our study included 94 semi-finished cocoa products (cocoa beans, nibs, powder, masses, and cocoa butter) and 269 cocoa and chocolate products (milk chocolates, chocolates, chocolates with additional ingredients, white chocolates, and cocoa-containing drink powders) sourced from the German retail market. The statistical results summaries of the, respectively, examined product categories are presented in Tables 2 and 3. The distribution of the quantified acrylamide content levels is presented in the pie charts in Fig. 1 and 2. A boxplot revealing the distribution of acrylamide levels among the individual product categories of our research work is presented in ascending order of medians in Fig. 3.

The statistical evaluation of our monitoring of the semi-finished cocoa products shows that acrylamide was detectable in 97% of the 94 samples examined. The content levels determined showed a maximum value of 490 µg kg^{-1} (in a

Table 1 Composition of sample pool

	N
Cocoa beans, unroasted	3
Cocoa powder	25
Cocoa nibs, roasted	13
Cocoa mass	50
Cocoa butter	3
Milk chocolates/-couverture	67
Chocolates/-couverture	93
Chocolates with additional ingredients[a]	93
Cocoa-containing drink powders	9
White chocolates	7
Total	363

[a]e.g. puffed rice, almonds, hazelnuts, dairy-based fillings, biscuits, raisins

Table 2 Statistical data of acrylamide monitoring in semi-finished cocoa products

Product	N	N > LOD (%)	Acrylamide (μg/kg)			
			Range	Mean	Median	90th percentile
Cocoa beans, unroasted	3	3 (100)	50–80 ± 6	70 ± 5	70 ± 5	80 ± 6
Cocoa powder	25	25 (100)	40–440 ± 31	180 ± 6	200 ± 14	330 ± 23
Cocoa nibs, roasted	13	13 (100)	50–380 ± 27	200 ± 14	200 ± 14	370 ± 26
Cocoa mass	50	50 (100)	40–490 ± 34	210 ± 15	150 ± 11	400 ± 28
Cocoa butter	3	0 (0)	≤ 30[a]	≤ 30[a]	≤ 30[a]	≤ 30[a]
Total	94	91 (97)	≤ 30[a]–490 ± 34	190 ± 6	160 ± 6	380 ± 6

± RSD_r

[a]LOD ≤ 30 mg/kg

Table 3 Statistical data of acrylamide monitoring in chocolates and finished cocoa-containing products

Product	Total cocoa dry weight (%)[b]	N	N > LOD (%)	Acrylamide (μg/kg)			
				Range	Mean	Median	90th percentile
Milk chocolates/milk chocolate couvertures	25	67	20 (30)	≤ 30[a]–90 ± 6	≤ 30[a]	≤ 30[a]	60 ± 4
Chocolates/chocolate couvertures	35/35	93	87 (94)	≤ 30[a]–400 ± 28	90 ± 6	80 ± 6	160 ± 11
Chocolates with additional ingredients		93	46 (49)	≤ 30[a]–200 ± 14	40 ± 4	≤ 30[a]	80 ± 6
Cocoa-containing drink powders		9	0 (0)	≤ 30[a]	≤ 30[a]	≤ 30[a]	≤ 30[a]
White chocolates		7	0 (0)	≤ 30[a]	≤ 30[a]	≤ 30[a]	≤ 30[a]
Total		269	153 (57)	≤ 30[a]–400 ± 28	50 ± 4	40 ± 3	110 ± 8

± RSD_r

[a]LOD ≤ 30 mg/kg

[b]According to the German Cocoa Regulation (KakaoVO, 2003)

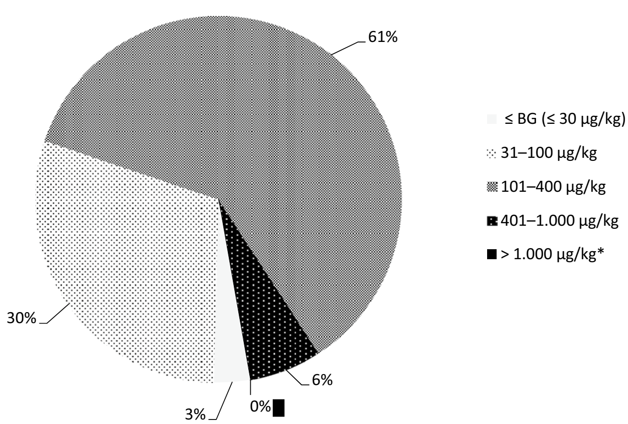

Fig. 1 Distribution of acrylamide in semi-finished cocoa products. *German signal value

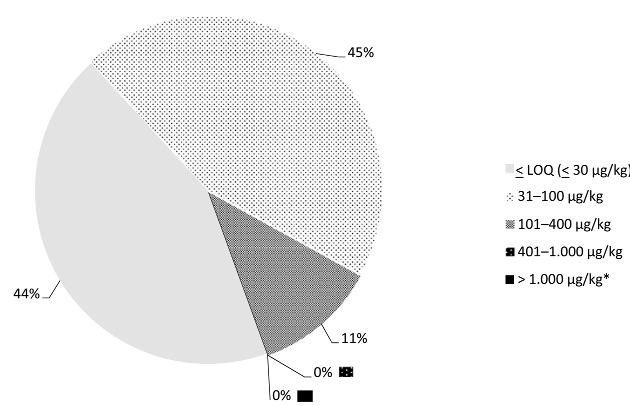

Fig. 2 Distribution of acrylamide in chocolates and finished cocoa-containing products. *German signal value

cocoa mass stemming from the Côte d'Ivoire), an overall mean of 190 μg kg^{-1}, and a total median of 160 μg kg^{-1}. The majority of analysed semi-finished cocoa products (61%) showed acrylamide content levels of between 101 and 400 μg kg^{-1} (cf. Fig. 1).

A comparison of individual groups of semi-finished cocoa products listed in Table 1 shows that cocoa butter was free of acrylamide but cocoa masses, by contrast, had the highest acrylamide content levels (mean 210 μg kg^{-1}). In comparing the unroasted cocoa beans (raw, mean 70 μg kg^{-1}) with the roasted raw cocoa products (nibs 200 μg kg^{-1}, powders 180 μg kg^{-1}, and masses 210 μg kg^{-1}, mean values, respectively) it was shown that the roasted semi-finished cocoa products—as anticipated—had the higher acrylamide content levels due to their heat treatment. A further plausible aspect is that, compared to the cocoa nibs (mean 200 μg kg^{-1}), the acrylamide content levels detected in the

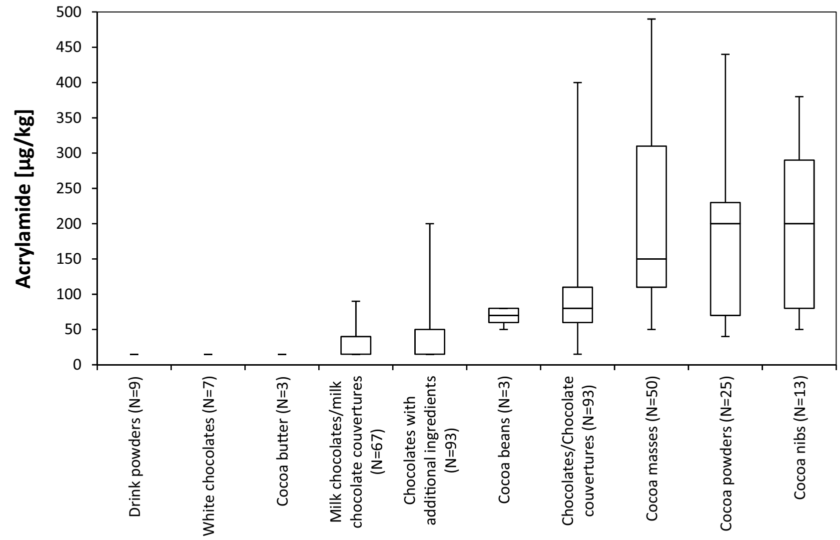

Fig. 3 Distribution of acrylamide content levels in different product categories. Statistical data from different series indicated as *N* showing the 25–75% percentile, whiskers indicating minimum and maximum levels observed

cocoa masses showed a mean of 210 µg kg^{-1}. Cocoa masses are finely-ground cocoa nibs whose manufacture involves no heat treatment of any kind.

Evaluation of the acrylamide content levels in end-consumer cocoa and chocolate products revealed that 57% of the examined samples contained acrylamide levels above the limit of quantification. The range of acrylamide content levels determined came to between < LOQ and 400 µg kg^{-1}. The mean acrylamide level was determined at 50 µg kg^{-1} and the median at 40 µg kg^{-1}. The majority (89%) of the examined samples showed levels below 100 µg kg^{-1} (cf. Fig. 2). A comparison of the individual product groups covered by our monitoring project showed clearly that chocolates—due to possessing the biggest overall cocoa content level of at least 35% (corresponding to the German Cocoa Regulation [18])—had the highest acrylamide content levels (mean 90 µg kg^{-1}). However, variations in the acrylamide content levels were not only detected between, but also within, the individual cocoa and chocolate product groups (cf. Fig. 3).

Chocolates with additional ingredients

The 93 samples of chocolates with additional ingredients examined within this project partly contained large amounts of additional foodstuffs, such as dairy-based fillings, hazelnuts, almonds, puffed rice, raisins, biscuits, or fruits. Figure 4 presents the acrylamide content levels detected in chocolates with additional ingredients, sorted by ingredients and shown in ascending order of each group's respective acrylamide levels.

An evaluation of the individual ingredients showed that all chocolates containing dairy-based fillings ($N=6$) were free of acrylamide. Similarly, only low acrylamide levels were detected in products containing fruits ($N=4$, all ≤ the mean of 40 µg kg^{-1} for the entire product group). However, chocolates containing fine bakery wares, cereal products such as puffed rice, nuts or nougat, or chocolates with liquid alcoholic fillings showed higher acrylamide concentration levels (cf. Fig. 5). Moreover, the highest contaminations with acrylamide were detected in chocolates containing almonds or almonds covered in chocolate/cocoa powder. This is attributable to the comparatively high asparagine content level of 980–6410 mg kg^{-1} in almonds [19].

In conclusion, however, it can be noted that over a range of ≤ LOQ–490 µg kg^{-1} all acrylamide content levels detected in the cocoa samples examined in this project (semi-finished cocoa products and cocoa and chocolate products, $N=363$) lay well below the general German signal value of 1000 µg kg^{-1}. An individual German signal value or EU indicative value/benchmark for acrylamide content levels in cocoa and chocolate products is currently not available.

Correlations

Based on the overall cocoa content levels stated by the manufacturers, the Pearson correlation was calculated to establish whether a correlation existed between the quantified acrylamide content levels and the overall cocoa content levels stated by the manufactures. Figure 5 shows a correlation between the acrylamide content levels of the product groups milk chocolates/milk chocolate couvertures and chocolate/chocolate couvertures in relation to the respective cocoa content of those samples where information on the total cocoa content level was available ($N=139$).

Calculation of the Pearson correlation coefficient between the known cocoa content levels and the quantified acrylamide levels produced a value of 0.59 ($N=139$). This makes it clear that although there is indeed a correlation between

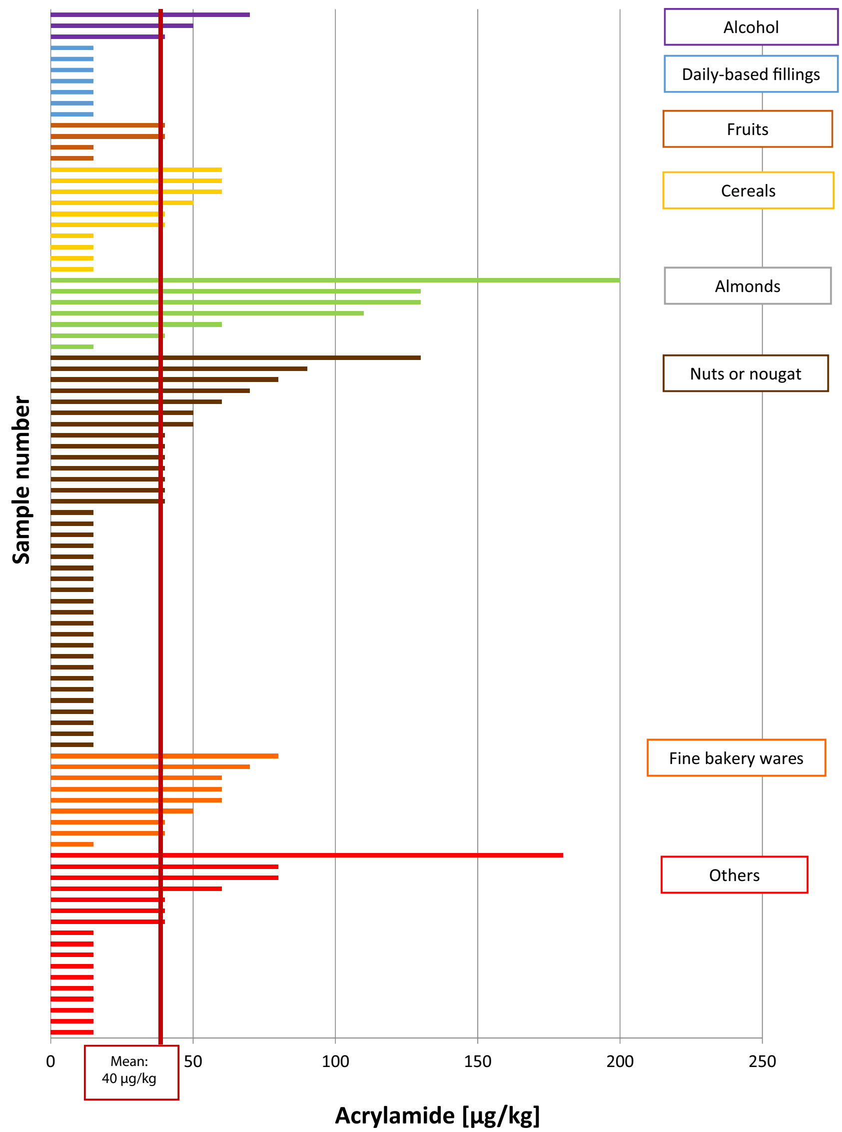

Fig. 4 Acrylamide content levels in chocolates with additional ingredients, $N = 93$ (shown in ascending order of each group's respective acrylamide levels)

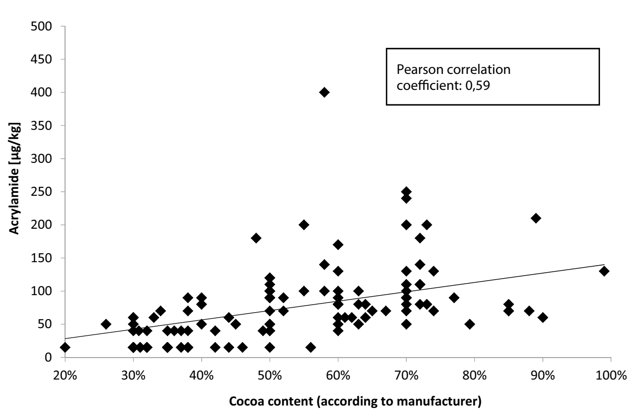

Fig. 5 Pearson correlation coefficient between cocoa content and acrylamide content ($N = 139$)

the cocoa content level and the determined acrylamide concentration, this is not a significant one. It can thus be deduced that, in addition to total cocoa content, there are additional factors which also have an impact on the formation of acrylamide and its concentration level. Possible influential factors include the composition of the cocoa beans, such as their asparagine and reducing sugars content, the well-known partially very strong origin-related variation during fermentation and drying, and the differing roasting conditions [20].

Comparison with reference data

In the monitoring study conducted by KÖPPEN et al. in 2015 on a total of 140 cocoa products sourced from the German retail trade, acrylamide was detected in all the examined samples. The quantified acrylamide levels ranged between 9 and 1747 µg kg^{-1}. Milk chocolates and dark/fine dark chocolates were analysed as having means of (90.2 ± 62.2) and (384.9 ± 131.7) µg kg^{-1}, respectively. Two samples (de-oiled cocoa powders) exceeded the general German signal value of 1000 µg kg^{-1} [15].

Comparing the results of this project with the results of KÖPPEN et al., it finally can be concluded that our monitoring established significantly lower acrylamide levels in all of the categories included in our research work. For example, the mean of the chocolates/chocolate couvertures examined by KÖPPEN et al. at 385 µg kg^{-1} is higher by a factor of 4.3 than the mean of 90 µg kg^{-1} determined in this monitoring project. A possible explanation for this deviation could be that the monitoring conducted by KÖPPEN et al. included many chocolates with a total cocoa content level of 80 to 99%. The chocolates examined within our project largely contained total cocoa content levels of between 50 and 70%, according to information provided by the manufacturers. Only 17 of 93 samples had a higher total cocoa content level.

The acrylamide levels detected in this study for cocoa powder (mean: 180 ± 6 µg/kg, n = 25) are well comparable with the few data mentioned in the EFSA scientific opinion of acrylamide in food (2015) [11]. In this database, a mean acrylamide level of 178 µg/kg in cocoa powder samples (n = 13) was indicated. The other acrylamide levels in this database of the product category called "chocolate and chocolate based confectionary" (mean 73 µg/kg, n = 31) are hardly comparable with our results because of the unclear specification.

Conclusions

Within the scope of a comprehensive acrylamide monitoring of a total of 363 cocoa products, we detected a broad range of acrylamide concentrations (≤ 30–490 µg kg^{-1}). Our research shows that cocoa and chocolate products from the German retail market intended for direct end-consumer consumption contain only relatively low acrylamide levels compared with other foods such as roasted coffee and coffee substitutes, and fried potato products.

Since, based on current knowledge, no meaningful intervention parameters/control points exist for reducing acrylamide levels within the cocoa production process and, furthermore, acrylamide levels detected in end-consumer cocoa and chocolate products are low compared to other foodstuffs, the inclusion of cocoa and chocolate products in the dynamic minimisation concept is, in our view, not reasonable.

Acknowledgements We thank the German Cocoa and Chocolate Foundation (Stiftung der Deutschen Kakao- und Schokoladenwirtschaft) for its financial sponsorship of this project.

Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. BDSI (2017) Süßwarentaschenbuch 2016/2017—Struktur und Entwicklungstendenzen der Süßwarenindustrie der Bundesrepublik Deutschland—Eine wirtschaftliche Analyse. https://www.bdsi.de/fileadmin/redaktion/onlineshop/Inhaltsverzeichnis_BDSI-S%C3%BC%C3%9Fwarentaschenbuch__2017.pdf
2. EFSA (2012) Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13 (5) of Regulation (EC) No 1924/2006. EFSA J 10(7):2809
3. Matissek R, Baltes W (2016) Lebensmittelchemie, 8. Aufl. Springer, Berlin
4. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 50(17):4998–5006
5. Raters M, Matissek R (2012) The big bang acrylamid—10 jahre acrylamid—rückblick und status quo. DEUT LEBENSM-RUNDSCH 108:184–189
6. Mottram DS, Wedzicha BL, Dodson AT (2002) Acrylamide is formed in the Maillard reaction. Nature 419(6906):448–449
7. Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert M, Riedikers S (2002) Acrylamide from maillard reaction products. Nature 419(6906):449–450
8. Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK et al (2003) Acrylamide formation mechanism in heated foods. J Agric Food Chem 51(16):4782–4787
9. Granvogl M, Koehler P, Latzer L, Schieberle P (2008) Development of a stable isotope dilution assay for the quantitation of glycidamide and its application to foods and model systems. J Agric Food Chem 56:6087–6092
10. BFR (2013) Acrylamid in Lebensmitteln, Stellungnahme Nr. 043/2011 des BfR vom 29. Juni 2011, ergänzt am 21. Januar 2013. http://www.bfr.bund.de/cm/343/acrylamid-in-lebensmitteln.pdf. Accessed 07 Dez 2017
11. EFSA (2015) Scientific opinion on acrylamide in food. EFSA J 13(6):4104
12. BVL (2015) Einheitliches europäisches Niveau für die Überwachung der Acrylamid. http://www.bvl.bund.de/DE/01_Lebensmittel/02_UnerwuenschteStoffeOrganismen/04_Acrylamid/03_EU_Richtwerte/richtwerte_node.html. Accessed 07 Dez 2017
13. EU Commission (2013) Recommendation 2013/647 of 8 November 2013 on investigations into the levels of acrylamide in food. Off J Eur Union No L 301:15 **(from 12.11.2013)**
14. EU Commission (2017) Commission regulation 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Off J Eur Union No L 304:24 **(from 21.11.2017)**
15. Köppen R, Rasenko T, Koch M (2015) Überblick über die Acrylamidgehalte von Kakao und Schokolade. Bundesanstalt für Materialforschung und -prüfung (BAM) Berlin. DEUT LEBENSM-RUNDSCH 111:261–267
16. BDSI (2005) Kompendium des Koordinierungskreises Acrylamid der Fachsparte Schokolade, Schokoladenerzeugnisse und Kakao und der Fachsparte Zuckerwaren im BDSI, Stand: Mai (**unpublished**)

17. Matissek R, Raters M, Friedman M, Mottram D (2005) Analysis of acrylamid in Food. Chemistry and safety of acrylamid in food. Adv Exp Med Biol 561:293–302
18. EU Commission (2000) Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption. Official Journal of the European Communities EG No.197:19
19. Seron LH, Poveda EG, Moya MSP, Carratala MLM, Bernguer-Navarro V, Grane TN (1994) Characterisation of 19 almond cultivars on the basis of their free amino acid composition. Food Chem 61:451–455
20. Żyżelewicz D, Oracz J, Krysiak W, Budryn G, Nebesny E (2017) Effects of various roasting conditions on acrylamide, acrolein, and polycyclic aromatic hydrocarbons content in cocoa bean and the derived chocolates. Dry Technol 35(3):363–374