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Article

Potential Antagonistic Effects of Acrylamide Mitigation during Coffee Roasting on Furfuryl Alcohol and 5-Hydroxymethylfurfural

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Abstract: The three heat-induced coffee contaminants acrylamide, furfuryl alcohol (FA) and 5-hydroxymethylfurfural (HMF) were analyzed in a collective of commercial samples as well as in *Coffea arabica* seeds roasted under controlled conditions from very light Scandinavian style to very dark Neapolitan style profiles. Regarding acrylamide, average contents in commercial samples were lower than in a previous study in 2002 (196 compared to 303 µg/kg). The roasting experiment confirmed the inverse relationship between roasting degree and acrylamide content, i.e. the lighter the coffee the higher the acrylamide content. However, FA and HMF were inversely related to acrylamide and found in higher contents in darker roasts. Therefore, mitigation measures must consider all contaminants and not be focused isolatedly on acrylamide, specifically since FA and HMF are contained in much higher contents with lower margins of exposure compared to acrylamide.

Keywords: coffee; acrylamide; furfuryl alcohol; 5-hydroxymethylfurfural; risk assessment

1. Introduction

Acrylamide is a heat-induced contaminant with frequent occurrence in foods and beverages [1-4]. It has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans [5]. The EFSA suggested that its margin of exposure indicates a concern for neoplastic effects based on animal evidence [6]. Coffee is an important topic in reduction of acrylamide, because its consumption may lead to 20-30% of total daily intake [7].

Following the first findings of acrylamide in foods and research into its formation mechanism [8,9], it was quickly discovered that coffee behaves differently from all other foods. While typically the acrylamide content rises with color or browning degree due to its origin as Maillard reaction product, for coffee its content decreases from light to very dark roasts [10]. The maximum of acrylamide is formed very early in the roast and then decreases until the desired roasting degree is reached. Experimental studies have shown that the final acrylamide content purely depends on the roasting degree but not on the profile by which this degree is achieved (i.e. neither very slow nor very quick roasting methods have any influence) [10]. Currently, literature offers only speculation into the breakdown product of acrylamide during roasting or the reaction leading to its degradation [11].

Acrylamide is a product formed during coffee roasting by the Maillard reaction, a major pathway comprising the reaction between asparagine and reducing sugars [12,13]. The formation

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capacity is limited by the amount of asparagine [14], which is the reason for higher acrylamide contents found in *Coffea canephora* ("robusta") coffee due to its higher asparagine content.

Mitigation options may start with agronomy (e.g. species and variety selection, fertilization etc.) and roasting, but also have included strategies during processing such as asparaginase addition or lactic acid bacteria, none of which left the feasibility stage [15]. Careful removal of defective coffee beans is recommended, because these contain significantly higher amounts of asparagine (>2 fold), which is a major precursor of acrylamide formation [7,16]. Storage of coffee may lead to considerable reduction, but the final brew preparation is believed to have little influence due to the excellent water-solubility of acrylamide [15]. Some authors suggested that the variation detected in commercial samples may predominantly reflect differences in storage time [17]. Supercritical fluid extraction can be applied to reduce acrylamide by up to 79% [18]. Vacuum processing was suggested as a measure to reduce acrylamide in medium roasted coffee by 50% [19].

From all these factors, roasting was the predominant focus of previous research, and consistent findings hint that an increased roasting degree leads to a decrease in acrylamide formation [10,14,20-24].

Following several years of voluntary industry action with minimization concept [25], mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food were recently implemented in an EU regulation [26]. The producers need to identify the critical roast conditions to ensure minimal acrylamide formation. They also need to ensure that the level of acrylamide in coffee is lower than the benchmark level of 400 µg/kg.

2. Materials and Methods

2.1. Analytical methodology

The analysis of acrylamide was conducted according to the standard method EN 16618:2015 using liquid chromatography in combination with tandem mass spectrometry (LC/MS/MS). In deviation to this standard, samples were defatted with a mixture of isohexane and butyl methyl ether. Furthermore solid-phase extraction (SPE) was only used for clean-up, not for concentrating the acrylamide [11]. With this method, a limit of detection (LOD) of 10 µg/kg, and a limit of quantification (LOQ) of 30 µg/kg can be achieved. A repeatability relative standard deviation (RSDr) of 6% was determined within our laboratory. The method was applied successfully in several proficiency tests.

Analysis of furfuryl alcohol (FA) and 5-hydroxymethylfurfural (HMF) was accomplished using nuclear magnetic resonance (NMR) spectroscopy as previously described [27].

2.2. Samples and roasting experiments

Samples were obtained from official sampling for food control purposes in the German federal state Baden-Württemberg from all stages of trade, mainly supermarkets and artisanal roasters. For roasting experiments, directly imported single estate terrace coffees were supplied by Amarella Trading (Mannheim, Germany). Coffee beans were roasted using an FZ-94 [2.4 kg] Laboratory Roaster (CoffeeTech, Tel Aviv, Israel). The roast profiles were recorded and controlled using Artisan v1.5.0 (https://artisan-scope.org).

2.3. Risk assessment methodology

Risk assessment was conducted using the margin of exposure (MOE) methodology according to the method for comparative risk assessment previously published for alcoholic beverages [3].

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3. Results

3.1. Results of roasting experiments

The same green coffee (*Coffea arabica*) was subjected to roasting using 6 different profiles, namely coffee roasting (quick and slow drying), espresso roasting (quick and slow drying) as well as Scandinavian roasting (very light roasting) and Neapolitan roasting (very black roasting). The roasting profiles are shown in Figure 1.

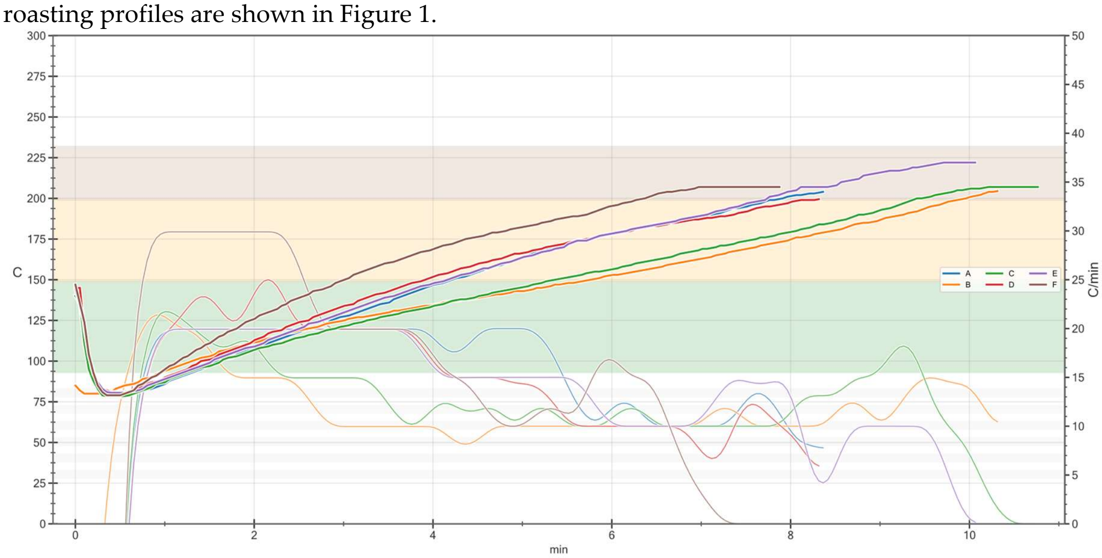

Figure 1. Profiles of experimental coffee roasting (A. Coffee quick drying; B. Coffee slow drying; C. Espresso slow drying; D. Scandinavian coffee; E. Espresso Neapolitan; F. Espresso quick drying)

Some numerical descriptors of the roasting profiles are provided in Table 1 as well as the analytical results for the samples. The roasting profile had a significant influence on the contents of the process contaminants. Drop temperature as well as area under the curve (AUC) are inversely related to acrylamide content, while the contents of furfuryl alcohol and HMF are positively correlated to both roasting parameters. The charge temperature was not correlated to any parameter. There is an inverse linear statistically significant relationship between acrylamide and furfuryl alcohol ($p=0.027$) and between acrylamide and HMF ($p=0.007$), while furfuryl alcohol and HMF are linearly correlated in a positive fashion ($p=0.024$).

Table 1. Indicators of roasting and analytical results of roasted coffee.

Profile	Charge [1] (°C)	Drop [2] (min)	Drop [2] (°C)	AUC [3]	Acrylamide (µg/kg)	Furfuryl alcohol (mg/kg)	HMF (mg/kg)
Scandinavian coffee	145	08:22	200	555	470	70	40
Coffee quick drying	140	08:24	204	566	200	124	74
Coffee slow drying	85	10:21	205	673	210	128	62
Espresso quick drying	147	07:55	203	625	170	170	66
Espresso slow drying	140	10:48	207	762	150	173	78
Neapolitan espresso	145	10:06	222	796	130	223	84

[1] Temperature at charge of roaster. [2] Drop=end of roast. [3] Area under the curve (indicator how much total energy the beans have received during roasting).

The full results of analysis of 78 samples are provided in Annex A, Table S1. The results are summarized in Table 2. From the sub-group of samples analyzed for both contaminants, acrylamide and furfuryl alcohol, an inverse linear relationship was detected, which was on the margin of

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statistical significance (p=0.057). However, no correlation between HMF and acrylamide was detected, while HMF and furfuryl alcohol were positively correlated (p=0.007).

The comparison of results in Table 2 shows that the acrylamide content in roasted coffee and in instant coffee has been considerably lowered over the years. None of the samples has exceeded the EU benchmark levels.

Table 2. Comparison of analysis results from 2002 with current results from 2015-2018 (summary from Annex A, Table S1).

Category according to EU Regulation 2017/2158	Year of analysis	Number of samples	Average [µg/kg]	Median [µg/kg]	90th percentile [µg/kg]
Roast coffee	2002 (data from [11])	5	303	313	461
Roast coffee	2015	4	118	130	138
Roast coffee	2018	19	196	160	314
Instant (soluble coffee)	2013	6	642	686	831
Instant (soluble coffee)	2015	7	483	356	805
Instant (soluble coffee)	2016	5	379	269	664
Instant (soluble coffee)	2018	18	461	450	772
Coffee substitutes exclusively from cereals	2013-2016	5	401	436	575
Coffee substitutes from a mixture of cereals and chicory	2012-2018	13	555	591	801

Finally, the results of comparative risk assessment using the margin of exposure methodology are shown in Table 3. The risk assessment uses survey data from the literature due the restricted, non-representative sampling in the current study. Another contaminant, furan, for which excellent literature data is available, has been included as well.

While the contents of acrylamide and furan are much lower than the ones of furfuryl alcohol and HMF (ppb instead of ppm range), the toxicity thresholds of both compounds are also much lower with acrylamide being the compound with effects at the lowest concentrations of all 4 compounds. Nevertheless, due to the higher exposure, HMF and furfuryl alcohol have the lowest margins of exposure, and hence the highest risk. Three of the compounds, acrylamide, furfuryl alcohol and HMF, have MOEs below the threshold of 10,000 and may therefore pose a risk to the consumer. Furan falls below this threshold only in worst-case scenarios (P95 exposure) and can be seen as compound with least risk.

Table 3. Risk assessment of several roasting contaminants in coffee

Contaminant	Average/P95 content in roasted coffee	Average/P95 exposure for drinking 1 cup of coffee [1]	Toxicological threshold [2]	Average/P95 margin of Exposure (MOE) [3]
Acrylamide	249 / 543 µg/kg [6]	0.05 / 0.10 µg/kg bw/day	0.18 mg/kg bw/day (BDML10) [28]	3,815 / 1,749
Furfuryl alcohol	251 / 392 mg/kg [29]	0.05 / 0.07 mg/kg bw/day	53 mg/kg bw/day (NOEL) [30]	1,114 / 713
HMF	689 / 1688 mg/kg [31]	0.13 / 0.32 mg/kg bw/day	79 mg/kg bw/day	605 / 247

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			(BMDL10) [32]	
Furan	38 / 107 µg/L [33]	0.12 / 0.14 µg/kg bw/day [33]	1.28 mg/kg bw/day (BMDL10) [34]	42,134 / 3,113 [33]

[1] Calculated assuming 14 g of coffee powder per 0.2 L cup (according to ISO 6668 [35]) and assuming 100% extraction yield, except for furan for which data from brewed beverage analyses were available. Average bodyweight 73.9 kg [36]. The data for furan were probabilistically calculated and taken from [33]. All other values were own calculations using point estimates.

[2] NOEL: no-observed effect level; BMDL10: benchmark dose lower confidence limit for 10% response

[3] MOE = Toxicological threshold / exposure. The higher the MOE, the lower the risk. A MOE > 10,000 is typically interpreted as low risk for genotoxic carcinogens.

4. Discussion

Roasting properties of coffee are basically dependent on the amount of heat transferred into the coffee beans during roasting and on the roasting time [17]. A good indicator for the achieved heat transfer rate is the area under the curve of the roasting profile. These values show a negative correlation with acrylamide during our roasting experiment, confirming the inverse relationship of roasting energy and acrylamide [10,14,20-24].

Interestingly, despite the early findings that acrylamide in coffee decreases with roasting degree, there is still considerable misinformation about this topic. Some small artisanal coffee roasters even advertise on their webpages that their "mild" process with only up to 200°C would result in lower acrylamide contents. The contrary being clearly the case, however.

Compared to results from our institutes published in 2002 (average acrylamide content in coffee: 303 µg/kg, median 313 µg/kg; 90% percentile 461 µg/kg) [11], the contents found by this study were lower. In Germany, the minimization of acrylamide has been most advanced of all EU member states [25]. Manufacturers should therefore not be challenged, even if the current benchmark level would become the new legal maximum limit [25]. Our results confirm this opinion, since none of our official samples was found to be in exceedance of the benchmark level.

Some authors have questioned the influence of species, e.g. Mojksja and Gielecinska [22], who found no significant difference in acrylamide content between Arabica and Robusta coffee. Our restricted results of two pure *C. canephora* coffees (260-270 µg/kg) are actually above the average of all coffee samples (196 µg/kg), which is consistent with the majority of literature [14,24,37], but comparison in our case is restricted due to the fact that the species is unknown in most of the analyzed commercial samples. It may be speculated that the influence is due to the lower quality of commercial *C. canephora* coffee with a higher degree of defective beans.

There are only few studies available on the correlation of other contaminants with acrylamide. Kocadagli et al. [38] studied the kinetics of both acrylamide formation and HMF formation and found similar tendencies, meaning both acrylamide and HMF are reduced by more intense roasts. This is in contrast to our results, which detected this behavior only for acrylamide but not for HMF. An explanation may the different methodology in Kocadagli et al. [38], which did not apply a commercial coffee roaster but only an oven at 220°C for 5-60 min. We therefore believe that our results may have a higher relevance for commercial coffee roasting. Nevertheless, there remains some uncertainty in HMF exposure from coffee. For example, the survey reported by Arribas-Lorenzo [31] from Spain found higher levels than the more restricted sample in our study.

For other heat-induced contaminants besides acrylamide, no action has been typically taken to reduce levels and there are also no EU benchmark or maximum levels for contaminants besides acrylamide. Therefore, focus and research activity in the past have been mainly aimed at acrylamide. The Codex Code of Practice to reduce acrylamide in foods currently does not provide guidance for coffee because to date "no commercial measures for reducing acrylamide in coffee are currently available" [15,39]. While this opinion is probably outdated, as various measures have been pointed out (see introduction), our findings suggest that indeed no measures should be implemented that solely focus on acrylamide. Using a holistic risk assessment approach, all major heat-induced

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contaminants in coffee need to be modelled prior to pointing out any measure. Otherwise it could well mean that the benefit gained by reduction of acrylamide might be outweighed by the risk of other contaminants such as furfuryl alcohol that are concomitantly increased by the measure. As other authors have pointed out [7,20], holistic risk-benefit analysis would be most preferable as the mitigation of acrylamide might not only lead to increased formation of other contaminants such as furfuryl alcohol [40], but also leads to reduced contents in beneficial compounds in coffee such as antioxidants.

Compared to other life-style factors such as tobacco smoking or alcohol drinking, the cancer risk from coffee (if any exists) appears to be rather low. According to IARC, epidemiological studies even suggest a lack of carcinogenicity of drinking coffee for cancer of the liver [41,42], which is the major target organ of the heat-induced contaminants.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table S1. Full analytical results of samples measured between 2012-2018 for acrylamide, furfuryl alcohol and HMF.

Sample ID	Sample description	Category according to EU Regulation 2017/2158	Year	AA (µg/kg)	FA (mg/kg)	HMF (mg/kg)
12119400	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2012	803	-	-
12119400-1	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2012	792	-	-
12119400-2	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2012	806	-	-
12119400-3	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2012	759	-	-
130122855	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2013	664	-	-
130123291	100% soluble coffee	Instant (soluble) coffee	2013	866	-	-
130123334	Coffee, soluble	Instant (soluble) coffee	2013	495	-	-
130124497	Coffee substitute, soluble	Coffee substitutes exclusively from cereals	2013	436	-	-
130124499	Coffee, soluble	Instant (soluble)	2013	744	-	-

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		coffee				
130127835	100% soluble coffee	Instant (soluble) coffee	2013	796	-	-
130128813	Coffee, soluble	Instant (soluble) coffee	2013	325	-	-
130128818	100% soluble coffee	Instant (soluble) coffee	2013	628	-	-
130130022	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2013	591	-	-
130132127	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2013	214	-	-
130132150	Coffee substitute, soluble	Coffee substitutes exclusively from cereals	2013	619	-	-
130237309	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2013	387	-	-
150231135	Coffee, soluble	Instant (soluble) coffee	2015	1135	-	-
150231200	Coffee, soluble	Instant (soluble) coffee	2015	199	-	-
150231825	Coffee substitute, soluble	Coffee substitutes exclusively from cereals	2015	508	-	-
150231835	Turkish coffee	Roast coffee	2015	127	-	-
150309974	Coffee	Roast coffee	2015	70	-	-
150309977	Coffee, decaffeinated	Instant (soluble) coffee	2015	335	-	-
150334870	Espresso Italiano	Roast coffee	2015	132	-	-
150334875	Coffee, soluble	Instant (soluble) coffee	2015	356	-	-
150334880	Coffee, soluble	Instant (soluble) coffee	2015	320	-	-
150337674	Coffee	Roast coffee	2015	141	-	-
150337675	Coffee, decaffeinated	Instant (soluble) coffee	2015	585	-	-
150337676	Coffee	Instant (soluble) coffee	2015	452	-	-
160450717	Malt coffee	Coffee substitutes exclusively from cereals	2016	370	-	-
160451307	Coffee, soluble	Instant (soluble) coffee	2016	223	-	-
160451426	Coffee, soluble	Instant (soluble) coffee	2016	273	-	-
160451967	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2016	361	-	-
160452173	100% soluble coffee, 100% Arabica	Instant (soluble) coffee	2016	269	-	-

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160452684	Coffee substitute, soluble	Coffee substitutes exclusively from cereals	2016	74	-	-
160454844	Coffee, soluble	Instant (soluble) coffee	2016	206	-	-
160472527	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2016	407	-	-
160472529	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2016	431	-	-
160509176	Coffee, soluble	Instant (soluble) coffee	2016	925	-	-
160509194	Espresso	Roast coffee	2016	447		
160574673	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2016	347	-	-
180352602	Coffee substitute, soluble	Coffee substitutes from a mixture of cereals and chicory	2018	650	-	-
180352683	Coffee	Roast coffee	2018	160	108	46
180365240	Costa Rica Arabica coffee	Roast coffee	2018	170	108	46
180379248	India Monsooned	Roast coffee	2018	150	74	34
180398113	coffee	Roast coffee	2018	210	156	52
180409591	Arabica-Robusta-mixture, coffee	Roast coffee	2018	130	133	48
180420281	coffee, organic	Roast coffee	2018	110	122	57
180420519	coffee	Roast coffee	2018	95	104	46
180433746	coffee	Roast coffee	2018	310	76	53
180439193	coffee, Ethiopia	Roast coffee	2018	150		
180444177	coffee	Roast coffee	2018	170	121	42
180447473	coffee	Roast coffee	2018	110	116	57
180453665	Coffee sumatra	Roast coffee	2018	120	119	52
180468077	coffee	Roast coffee	2018	130	122	51
180478363	Coffee substitute, soluble	Instant (soluble) coffee	2018	730		
180481476	Coffee substitute, soluble	Instant (soluble) coffee	2018	110	64	40
180486743	Coffee substitute, soluble	Instant (soluble) coffee	2018	670	-	-
180486745	Coffee substitute, soluble	Instant (soluble) coffee	2018	460	-	-
180489109	Coffee substitute, soluble	Instant (soluble) coffee	2018	420	-	-
180489247	Coffee substitute, soluble	Instant (soluble) coffee	2018	440	-	-
180492032	100% soluble coffee, 100% Arabica	Instant (soluble) coffee	2018	870	-	-
180492048	Coffee, soluble	Instant (soluble) coffee	2018	660	-	-
180494672	Coffee, soluble	Instant (soluble)	2018	910	-	-

		coffee				
180504580	Coffee, soluble	Instant (soluble) coffee	2018	420	-	-
180520043	100% soluble coffee, 100% Arabica	Instant (soluble) coffee	2018	690	-	-
180533580	Pure Canephora coffee	Roast coffee	2018	260	42	40
180533581	Pure Canephora coffee	Roast coffee	2018	270	37	49
180533582	Arabica coffee	Roast coffee	2018	270	78	46
180533583	Arabica coffee	Roast coffee	2018	460	55	43
180533584	Arabica coffee	Roast coffee	2018	330	60	35
180539910	Coffee, soluble	Instant (soluble) coffee	2018	600	-	-
180552699	100% Organic Arabica coffee	Roast coffee	2018	120	-	-

AA: Acrylamide; FA: Furfuryl alcohol; HMF: 5-Hydroxymethylfurfural; "-": parameter not analyzed in that sample

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