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Characterisation of new sources of acrylamide in food marketed in Belgium

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ABSTRACT

This study provides occurrence data for acrylamide in various foodstuffs, including those covered by Recommendation (EU) 2019/1888, from 210 samples purchased on the Belgian market. Detection frequencies exceeded 84% in potato-based products other than fries, vegetable crisps, black olives, cocoa powders, coffee substitutes and cereals and snacks. Large variations in acrylamide levels were found in cereals and snacks, with no correlation between cereal type or processing. Snacks containing chia did not show higher acrylamide levels than other cereal-based snacks.
Maximum levels found were 4389 and 3063 µg kg^{−1} in coffee substitutes and vegetable crisps, respectively. Potato-based products contained 2 to 27 times less acrylamide when prepared in oven, compared to deep fryer processing. Artificially oxidised "Californian-style" black olives contained five times more acrylamide than "Greek-style" olives. In bread, pastries, nuts, oilseeds, dried fruits and confectionaries, detection frequencies varied from 27 to 69% and the average acrylamide content was <30 μ g kg⁻¹.

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Introduction

Acrylamide is a well-known process contaminant in carbohydrate-rich foods prepared at temperatures higher than 120°C and low moisture content (Mottram et al. [2002;](#page-12-0) Stadler et al. [2002](#page-13-0)). It has been classified as probably carcinogenic (group 2A) by the International Agency for Research on Cancer (IARC) due to its carcinogenic effect on rodents (IARC [2010\)](#page-12-1). Since its discovery in food by the University of Stockholm in 2002, intensive research has focused on understanding its formation mechanisms. Mottram et al. ([2002](#page-12-0)) concluded that acrylamide is formed in food rich in amino acids such as asparagine and reducing sugars when heated at a temperature higher than 120°C as a product of the Maillard reaction. Later, other pathways and precursors leading to acrylamide formation have been highlighted by Keramat et al. ([2011](#page-12-2)).

Therefore, Recommendation 2010/307/EU of the European Commission ([2010](#page-12-3)), advised European Union (EU) Member States to monitor acrylamide levels in targeted foods known to contain high acrylamide levels and/or contribute significantly to the human dietary intake. Hence, the impact of the process and the selection of raw material on acrylamide generation have been widely studied in food such as bread, potato fries and coffee (EFSA [2015\)](#page-12-4). For instance, Biedermann et al.

Based on European monitoring data from 2010 to 2013, in 2015 the European Food Safety Agency (EFSA) panel for the Contaminants in the Food Chain estimated that the margin of exposure (MOE), compared to a BMDL (benchmark dose lower level) of 0.17 mg/kg bw/day was below 10.000. Hence, EFSA concluded that the presence of acrylamide in food might be a concern for human health regarding the carcinogenic effect (EFSA [2015\)](#page-12-4). Since then, Regulation (EU) 2017/2158 established mitigation measures for various foods to

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[^{\(2002\)}](#page-11-0) demonstrated the importance of water content in acrylamide generation. They observed that acrylamide content in wet potatoes cooked at 120°C was $\lt 20$ µg kg⁻¹, but higher than 10 000 µg kg⁻¹ for dry potato powder. Lantz et al. [\(2006](#page-12-5)) concluded that time and degree of roasting were the main factors influencing acrylamide levels in coffee. Even more, prolonging the roasting process could lead to a decrease in acrylamide. For instance, an average of 374 μ g kg⁻¹ of acrylamide was observed for light-roasting coffee but only $187 \,\mu g$ kg⁻¹ for dark-roasting coffee (EFSA [2015](#page-12-4), [2022\)](#page-12-6). Acrylamide is also often found in bread, but at a lower level (42 μ g kg⁻¹ on average) since acrylamide is particularly located in the crust, which level is lowered when considering the sample as a whole (Surdyk et al. [2004;](#page-13-1) EFSA [2015](#page-12-4)).

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reduce acrylamide levels as much as possible in the final product. Examples are the selection of raw materials with low asparagine and/or reduced sugar content and optimisation of the storage condition to minimise the production of these precursors. Advice is also given regarding the cooking process. For example, for french fries the frying temperature has to be kept between 160°C and 175°C and oven cooking has to be performed between 180°C and 220°C.

Furthermore, this Regulation also assigns benchmark levels (BMLs) for specific food categories that the food sector should be able to achieve when respecting the mitigation measures. However, it was recognised that the number of data available on the presence of acrylamide in the foodstuffs covered by Regulation (EU) 2017/2158 is insufficient (European Commission [2019](#page-12-7)). In addition, Regulation (EU) 2017/2158 does not include all food categories that may contain significant quantities of acrylamide and which could potentially contribute to the total dietary exposure of acrylamide. As acrylamide tends to be formed through precursors such as asparagine and reducing sugars, it can virtually concern all cereal-based and tuber food undergoing heating. For instance, recent research highlights the wide variation of acrylamide levels (57 to 1660 µg kg-1) in corn products such as tortilla crisps, crackers and popcorn (Žilić et al. [2022\)](#page-13-2). Root vegetable crisps other than potato-based have also been characterised by a high acrylamide content (Breitling-Utzmann and Wendler [2019](#page-11-1); Nguyen et al. [2022](#page-12-8); MacDonald et al. [2023\)](#page-12-9). Furthermore, Becalski et al. ([2011\)](#page-11-2) reported that other specific food products that are not cereal-based can generate substantial amounts of acrylamide, even when the heating temperature is set at 100°C, which is lower than the often quoted acrylamide formation temperature of 120°C. Their study showed that acrylamide levels up to 332 μ g kg⁻¹ can be achieved during the drying process of fruits such as prunes. These authors concluded that acrylamide in prunes and prune juice very likely originates from sugars and asparagine, which is present in considerable amounts in the raw material. Breitling-Utzmann et al. [\(2023](#page-11-3)) and Nguyen et al. [\(2022](#page-12-8)) showed that high acrylamide levels could be found in black olives, especially in "Californian" style black olives undergoing an artificial oxidised process (50–1100 μg kg⁻¹). In this process, olives are artificially coloured by air oxidation and the use of the colour fixative agent E579 (ferrous gluconate), followed by a sterilisation step. The sterilisation process involved temperatures ranging from 110°C to 121°C for up to 50 minutes, exceeding the temperature threshold for acrylamide formation (Casado and Montaño [2008\)](#page-11-4).

Recommendation (EU) 2019/1888 about monitoring of acrylamide in certain foodstuffs includes potatobased products other than fries, potato-based composite dishes, black olives, various types of pastries and cerealbased crackers and snacks, nuts, oilseeds, dried fruits and some confectionaries (European Commission [2019\)](#page-12-7). In parallel, EFSA also issued a call on 25 October 2019 to collect data at the European level on the acrylamide contents of foodstuffs based on chia seeds or chia flour and which have undergone a cooking process. This call echoes an EFSA scientific opinion of March 2019 (EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA Panel) et al. [2019\)](#page-12-10) stating that partial substitution of wheat flour with chia flour and using chia seed in bread and cookies may lead to higher levels of acrylamide in the final product. The lack of such essential occurrence data in these less-studied foods precludes a precise estimation of acrylamide exposure for the consumers and the associated risk for the population.

All together, the spectrum of food products addressed by Recommendation (EU) 2019/1888 is very large and creates a challenge for selecting the adequate analytical method for all these food categories. As reviewed by Keramat et al. ([2011](#page-12-2)), most analytical methods use water as an extraction medium. However, pure water tends to form an emulsion in case of cereal-based matrices that may clog filters and instruments. This phenomenon can be avoided by adding an organic solvent such as n-propanol or Carrez I and II solutions. The QuEChERS method involves an extraction with a water and acetonitrile (ACN) mixture in the presence of NaCl, as proposed by Anastassiades et al. [\(2003](#page-11-5)). De Paola et al. [\(2017\)](#page-11-6) successfully applied the QuEChERS method to dried-fruits and edible seeds for acrylamide extraction. A key advantage of QuEChERS lies in the use of ACN, which mitigates emulsion formation, while the presence of NaCl facilitates the transfer of acrylamide into the ACN phase. Furthermore, QuEChERS is a modular method, as a common extraction is used for various food products before using a specific mix of dispersive-solid phase extraction (d-SPE) sorbents for clean-up (Anastassiades et al. [2006](#page-11-7)). This modularity potentially reduces the developmental complexity and makes analysis more straightforward, which is essential to face the variety of food targeted by Recommendation (EU) 2019/1888.

The present study aims to fill the data gaps for specific food categories targeted by Recommendation (EU) 2019/ 1888. First, two analytical methods have been developed and validated to address the diversity of the food categories. The first method was dedicated to coffee substitutes and cocoa powder for beverages, while the second method was a modular QuEChERS-based method applied to all remaining food categories. Then, occurrence data were collected by analysing 210 food samples targeted by the Recommendation (EU) 2019/1888 and representative of the Belgian food diet. Particular attention was paid to include food items containing chia (flour or seeds) to verify the hypothesis from the Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies [\(2009](#page-13-3)). Furthermore, acrylamide distribution in food products was investigated to determine if a specific type of cereal, vegetable or process was responsible for the higher acrylamide content.

Materials and methods

Reagents and chemicals

Acrylamide and deuterated acrylamide (acrylamide-d₃) standards were obtained from Ehrenstorfer GmbH (Augsburg, Germany). Hexane (HPLC grade), ACN (HPLC-S grade), methanol (HPLC grade) and ethyl acetate (Pesti S) were obtained from Biosolve (Valkenwaard, The Nederlands), formic acid 98–100% from Merck (Darmstadt, Germany) and dichloromethane (HyperSolve HPLCTM) from BDH chemicals (England). Distilled water (<18 M Ω resistivity) was produced by a Milli-Q system (Millipore Corp., Bedford, M, USA). Potassium hexacyanoferrate(II) trihydrate $(K_4[Fe(CN)_6]$ x 3.H₂O), zinc sulphate heptahydrate $(ZnSO₄ x 7.H₂O)$, magnesium sulphate and sodium chloride were purchased from Merck N.V/S.A. (Overijse, Belgium). Bulk PSA (40 µm), bulk C18 $(40-70 \,\mu m)$ and SPE accuCAT 200 mg cartridges were obtained from Agilent Technologies (Machelen, Belgium).

Standard stock solutions of acrylamide and acrylamide-d₃ were prepared at 1 mg mL⁻¹ in water/ methanol (50/50 v/v). Working solutions at 10 μg mL⁻¹ and 1 μg mL⁻¹ were made from stock solutions in water/methanol (50/50 v/v). All solutions were stored at -20° C. The stability of acrylamide-d₃ is regularly checked with a mass spectrometer during the monitoring program. The applied method has been subject to proficiency tests over the years, showing good results [\(Table 2\)](#page-3-0).

Sampling strategy

The sampling plan was based on specific food products targeted by Recommendation (EU) 2019/1888 (European Commission [2019](#page-12-7)). Moreover, other food products containing chia were added to the sampling plan due to the ESFA Scientific opinion on the safety of

Table 1. MS/MS parameters for the analysis of acrylamide and acrylamide- d_3 .

Compound	Precursor ion Product ion (m/z)	(m/z)	Cone voltage (V)	Collision energy (eV)
Acrylamide	72.1	55.0 (O*)	20	10
	72.1	72.1 (q^{**})	20	
	72.1	44.0	20	10
Acrylamide-d ₃	75.0	58.0 (Q)	20	10
$*$ \cap quantifier $**$ g qualifier				

Q quantifier, **q qualifier.

chia seeds (*Salvia hispanica* L.) as a novel food for extended use under Regulation (EU) 2015/2283 (European Parliament and Council [2015](#page-12-11); EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA Panel) et al. [2019](#page-12-10)). The number of samples per category of food products was based on their availability in the Belgian market and the consumption habits of the Belgian population according to the National Food Consumption Survey (De Ridder et al. [2016](#page-11-8); Bel et al. [2016\)](#page-11-9). The brand selection has been made based on the Belgian market share data from the Euromonitor database [\(2017](#page-12-12)). Specific stores, such as bakeries, organic stores, and restaurants, were also included to reflect Belgians' consumption habits as closely as possible. Moreover, particular care has been taken to include at least one product bearing an organic label in each food category. A total of 210 samples were purchased from Belgian retailers in 2019 and 2020. The selected food products were cereals and snacks ($n = 64$ [11 with chia seeds]), potato-based products other than fries (*n* = 43), pastries (*n* = 32), bread (*n* = 30 [8 with chia seeds]), grilled nuts and oilseeds (*n* = 16), dried fruits and confectionaries $(n = 11)$, vegetable crisps $(n = 8)$, coffee substitutes $(n=7)$, black olives $(n=5)$, cocoa powders for beverage $(n = 5)$. Fifty-four (25%) samples were labelled as organic (Table S1, [Table 3](#page-4-0)).

Table 2. Results obtained for acrylamide in proficiency tests and in blind samples.

Test material	Assigned and fortification value $(\mu g kg-1)$	Measured value ± MU $(\mu q \ kq-1)$	z-score $(\sigma =$ 22%)
Proficiency test:	673	$657 + 98.6$	-0.1
EURL-PAH 2017 potato chips			
EURL-PC PT-2018-01 Bread	37.4	34 ± 5.1	-0.4
EURL-PC PT-2019-03 coffee	244	242 ± 48.4	0.0
EURL-PC PT-2020-05 cereal-	37	50.4 ± 7.56	0.6
based baby food			
Blind samples:			
Fried potato products	75	74 ± 11.1	
Pastries	150	$164 + 24.6$	
Black olives	300	318 ± 47.7	
Nuts and oilseeds (roasted)	125	$136 + 20.4$	
Dried fruits and confectionaries	250	254 ± 38.1	
Cocoa powder	150	142 ± 28.4	

MU: measurement uncertainty.

n: number of samples. The mean is calculated on the lower-bound (LB) approach, i.e. LOD and LOQ were fixed at 7 and 20 μg kg⁻¹, respectively. Results below LOQ were replaced by 0. *13 samples were cooked twice using two distinct methods (i.e. oven and deep fryer).

Sample preparation and homogenization

Samples were analysed as consumed, in line with Regulation (EU) 2017/2158 (European Commission [2017](#page-12-13)). Samples bought as "ready-to-eat" were directly homogenised. Samples requiring a cooking process (e.g. croquettes, sweet potato fries, etc.) were prepared according to the manufacturer's instructions described on the packaging. This approach ensured that the different cooking modes were evenly represented across all samples. Moreover, 20 samples were cooked following both oven and fryer instructions to assess the effect of the cooking process. Samples were homogenised with a mill (IKA M20, IKA®-Werke GmbH & Co. KG, Staufen, Germany) in accordance with Regulations (EU) 2017/2158 and 333/2007 (European Commission [2007](#page-12-14), [2017\)](#page-12-13).

Determination of acrylamide

The methodology for analysing acrylamide in a broad range of samples was based on the QuEChERS extraction (Anastassiades et al. [2003](#page-11-5)), which is common to all food categories, except for "coffee substitutes" and "chocolate powder for beverages" for which Carrez solutions I and II were added prior to the extraction step. Subsequently, a customised clean-up procedure was implemented for each food category based on d-SPE with C-18 and PSA sorbents.

Extraction and clean-up

First, 25 µL acrylamide-d₃ at 1 µg mL⁻¹ is added to 1.0 g of the homogenised sample, followed by a QuEChERS extraction, except for coffee and cocoa products, by adding 10 mL of Milli-Q water, 10 mL of ACN, 0.5 g of NaCl and $4 g$ of MgSO₄ to the sample before shaking for 1 min by hand, followed by a centrifugation step. A volume of 4 mL of supernatant were cleaned up on d-SPE, consisting of a specific amount of PSA and C18, depending on the sample's nature. Fried potato products, root tuber and vegetable-based products (such as vegetable crisps), black olives, oil seeds, nuts and food high in sugar (such as nougat, caramel or dried grapes) needed 150 mg PSA, while cereal-based products (such as rice and maize crackers), pastries and pancakes needed 300 mg PSA and 300 mg C18. The obtained supernatant was evaporated to 500 µl under a nitrogen stream and the volume was adjusted at 2 mL with Milli-Q water. Finally, 500 µl of this solution was filtered on a 0.2 µm PVDF autofiltrating vial prior to LC-MS/MS analysis.

For coffee substitutes and cocoa products, an extraction was performed with 10 mL of Milli-Q water and 1 mL of Carrez I and II solutions and shaking with a vortex for 10 min. After centrifugation, 6 mL of supernatant was taken for a liquid-liquid extraction with 13 mL of ethyl acetate. The upper layer of ethyl acetate was transferred into a new Falcon tube and the liquid-liquid extraction was repeated with 13 mL of fresh ethyl acetate. Both upper phases were combined and 1 mL of Milli-Q water was added before concentrating the solution to 1 mL under a nitrogen stream. Finally, the concentrated extract (1 mL) was loaded on a multimode SPE cartridge in a pass-through mode and $500 \mu l$ of purified extract was collected. The eluate was filtered on a 0.2 µm PVDF auto-filtrating vial before injection on the LC-MS/MS.

UPLC-MS/MS analysis

LC-MS analyses were performed using an Ultra Performance Liquid Chromatography system (Acquity Ultra Performance IKA®) coupled to a Xevo TQ mass spectrometer (Waters, Milford, MA, USA) controlled by LC[®] v4.2 software. Five microlitres of the samples were injected into a Hypercarb column $(100 \times 2, 1 \text{ mm}, 5 \text{ µm})$. The column temperature was set at 45°C. The mobile phase was composed of Milli-Q water, 0.5% methanol and 0.1% formic acid. Chromatographic separation was performed at a flow rate of 0.4 mL min⁻¹ with an isocratic elution mode. [Table 1](#page-3-1) summarises the optimised MS/MS parameters for monitoring acrylamide and acrylamide-d₃. Two transitions were monitored, one for quantification and the other for the confirmation. Capillary voltage for electrospray was 3.5 kV, source temperature 150°C, desolvation temperature 550°C, cone gas flow 80 L h⁻¹ and desolvation gas flow 800 $L h^{-1}$.

Method validation

The protocol followed to validate the method is described in supplementary information Section S1. Briefly, both methods used have been validated for linearity, matrix effect, recovery, trueness, limit of detection (LOD) and limit of quantification (LOQ). Validation criteria were based on Regulation (EU) 2017/2158 and the SANTE document (European Commission [2017,](#page-12-13) [2021\)](#page-12-15).

Quality assurance and control

The sensitivity and stability of the LC-MS/MS system have been controlled by injecting a calibration point at LOQ level (20 μ g kg⁻¹) at the beginning and the end of each chromatographic run. A S/N > 10 for both acrylamide transitions and for both injections ensured that there was no sensitivity loss. Additionally, the concentrations of each calibration level were back-calculated using the regression equations of the calibration curve and with a 1/x weighing factor. A deviation lower than ±20% compared to the theoretical concentration was required to ensure accurate quantification. A procedural blank was analysed for each batch of samples to ensure absence of acrylamide contamination, either at the level of the extractions or at the LC-MS/MS system. Extraction efficiencies were evaluated for each analysis batch by checking the recovery of a control sample of the same nature as the analysed samples, fortified at 250 μ g kg⁻¹.

Results & discussion

Method validation

All foods targeted by Recommendation (EU) 2019/1888 were first sorted into categories according to their main constituents (i.e. sugar, water, or fat content) and their nature (i.e. cereal or vegetable-based food). Ten categories were validated: cereal-based baby food, vegetable and tuber-based crisps, fried potato products, black olives, pancakes, pastries, oil seeds and nuts, dried fruits and confectionaries, cocoa powder for beverages, coffee substitutes (Tables S2, S3). An overview of the detailed validation results is provided in supplementary information Section S2. No significant matrix effects were observed for all validated food categories (Table S2). Hence, the ME is not taken into account for the determination of the recovery. All recoveries ranged from 91.7 to 106%, matching the EU criteria (70–110%). Precision in repeatability and reproducibility conditions were below 15%, matching EU criteria fixed at 14.5% and 22%, respectively (European Commission [2017;](#page-12-13) Table S3). For all matrices LOD and LOQ have been set at 7 and 20 µg kg−1, respectively. MUs have been calculated from the bias and RSD_{RW} data from control charts initially generated with validation data. Thereafter MU was fixed at 15% for all food categories, except for coffee substitutes and cocoa powder for beverages (20%).

Finally, the method has been successfully evaluated by participating in 4 proficiency tests organised by the European Reference Laboratory for processing contaminants. In all cases, acceptable *z*-scores $(\langle \pm 2 \rangle)$ were obtained. Regarding blind samples, a good match was obtained between the fortification level and the measured values, including the measurement uncertainty for the blind samples [\(Table 2\)](#page-3-0).

Occurrence data

The validated methods have been applied to analyse 210 samples distributed in 10 different food categories. Thirteen potato-based products have been analysed twice, to assess if the cooking mode (oven and deep fryer cooking) would have an impact on the final acrylamide content. Eventually, 223 analyses have been conducted. Results of acrylamide occurrence in each food category are presented in [Table 3,](#page-4-0) while all individual results are shown in the supplementary information (Table S1). Acrylamide has been detected in 61% of the 223 analyses performed, with levels ranging from \lt LOD to 4389 µg kg⁻¹ in a coffee substitute sample [\(Table 3](#page-4-0)). Globally, vegetable crisps, potato-based products other than fries (deep fryer cooked) and black olives reached a relatively high acrylamide level. In

contrast, cocoa powder for beverages showed a wide variation in acrylamide content. Similarly, cereals and snacks showed a wide variation of acrylamide content without exceeding the BML for related products, except for one sample of honey-roasted muesli (Table S1). A wide variation was observed in the acrylamide content in coffee substitutes, depending on the substitutes' nature (EFSA [2015](#page-12-4)). Breads, pastries, dried fruits and confectionaries were characterised by the lowest detection frequency of acrylamide [\(Table 3\)](#page-4-0).

Food categories with relatively high levels

Vegetable crisps

Vegetable crisps were one of the most contaminated food categories, with a detection frequency of 100%. No BML exists for vegetable crisps in Regulation (EU) 2017/2158. Potato crips are the closest regulated food category, with a BML set at 750 μ g kg⁻¹. Compared to this level, three out of eight samples exceeded this level, i.e. two crisps samples containing beetroot, carrot and sweet potato (1554 and 2183 µg kg^{-1}) and one crisp based on manioc with a maximum acrylamide content of 3063 μ g kg⁻¹ [\(Table 3](#page-4-0), S1). The type of vegetables used in the crisps may be the source of the acrylamide. However, three other samples with similar compositions contained less than 250 µg kg^{-1} of acrylamide. These results are in agreement with studies of MacDonald et al. [\(2023](#page-12-9)) who found acrylamide levels ranging from 58.6 to 2634 µg kg^{-1} , Oellig et al. ([2022](#page-12-16)) who reported 77.3 to 3090 μ g kg⁻¹ and with findings of 123 to 3606 μg kg⁻¹ from Nguyen et al. [\(2022\)](#page-12-8). Higher levels may be due to the selection of raw materials and/ or the cooking process. Breitling-Utzmann and Wendler ([2019](#page-11-1)) already demonstrated the strong link between precursor content (asparagine, reducing sugars) in the raw material, temperature, cooking time and final acrylamide levels for some vegetables (beetroot, sweet potatoes and carrots) and the higher acrylamide content generated in sweet potatoes for a similar process.

The sample with an initial acrylamide content of 2183 μg kg⁻¹ was collected again, though with a different batch number, and its individual vegetable types (beetroots, carrots and sweet potatoes) were sorted out and analysed individually to identify the source of this high acrylamide level. The highest acrylamide contents were found in beetroot and carrot crisps (1117 and 1020 μg kg⁻¹, respectively), followed by sweet potato (548 μ g kg⁻¹). These results do not confirm the observations on the higher acrylamide content generated in sweet potatoes of Breitling-Utzmaan and Wendler

[\(2019](#page-11-10)). However, these variations can result from the intra-variability of precursors inside a vegetable type depending on the season, geographical area and storage conditions (Breitling-Utzmann and Wendler [2019](#page-11-1)). Nevertheless, some producers succeeded in keeping the acrylamide content largely below the BMDL for potato crisps, set at 750 µg kg^{-1} by the European Commission ([2017\)](#page-12-13). Since vegetable crisps have grown in popularity, it might be interesting to see if applying the mitigation measures fixed for potato-based crisps could effectively reduce their acrylamide content as well. Moreover, Annex I of Regulation (EU) 2017/2158 already fixed mitigation measures for potato-based crisps, such as the selection of variety showing fewer precursors content, stock and transportation conditions and cooking processes to limit acrylamide formation at the maximum (European Commission [2017](#page-12-13)). The same information for vegetable crisps could be investigated, evaluated and subsequently implemented.

Coffee substitutes

Coffee and cereal and/or chicory coffee substitutes are included in Regulation (EU) 2017/2158 with a BML ranging from 400 to 4000 µg kg^{-1} ([Table 3\)](#page-4-0), depending on their type (EFSA [2015](#page-12-4)). Moreover, Recommendation (EU) 2019/1888 extends the monitoring to coffee substitutes not based on cereal or chicory as well. Seven samples were purchased on the market, including web shops. These are detailed in Table S1. Acrylamide was detected in 86% of the samples and ranged from < LOD to 4389 μ g kg⁻¹, the highest level detected in any sample in this study ([Table 3](#page-4-0)). Acrylamide was not detected in the sample mainly composed of fruits and tea leaves, while the 5 plant and/or nuts-based samples contained acrylamide levels in the same order of magnitude as regular roasted coffee (ca. 250 µg kg^{-1} ; EFSA [2015](#page-12-4)). Higher acrylamide content was found in coffee containing roasted chicory roots, as expected for this type of coffee and the highest level (4389 μ g kg⁻¹) was found in a sample of roots and rhizome (Table S1). As rhizomes and roots contain more sugar than the other parts of the plant, this may explain the high level of acrylamide (Mojska and Gielecińska [2013](#page-12-17)). The coffee substitutes reported in the literature were always based on cereals or chicory but not on other alternatives. Claeys et al. [\(2016](#page-11-11)) reported acrylamide levels of up to 5400 μ g kg⁻¹ for 84 coffee substitutes on the Belgian market between 2002 and 2013, while the levels reported in the EFSA opinion (2015) on acrylamide were 510 μg kg⁻¹ for malt, barley and wheat coffee and 2942 μ g kg⁻¹ for chicory coffee. It should be noted that the EU recommendation requested data for coffee substitutes, not based on

cereals and chicory (European Commission [2019\)](#page-12-7). However, the presence of acrylamide in this product should not be a concern for the general Belgian population as the availability of such products on the Belgian market is very limited.

Potato-based products

French fries were among the first foods deeply studied, as they showed relatively high acrylamide levels. However, data is lacking for fried potato-based products other than fries. For instance, the EFSA opinion of 2015 was based on 1598 data on French fries but only 96 data for other potato fried products. Sweet potato fries have also gained in popularity in recent years. Hence, Recommendation (EU) 2019/1888 targeted these food products to gather missing data.

Therefore, potato balls, potato cubes, duchesse potatoes, croquettes, rosti and sweet potato fries were purchased and regrouped into the potato-based products category. This food category was divided depending on the cooking mode (oven or deep-fried cooking). The results show that deep-frying generates a higher level of acrylamide (up to 25 times), when compared to oven cooking, with a maximum acrylamide content of 1503 µg kg^{-1} for a potato cube sample ([Table 3](#page-4-0), Table S1). Interestingly, when a golden colour was obtained at the end of the preparation, the acrylamide content was lower than the BML for "Other potato products from potato dough" fixed at 750 μg kg^{-1} . This colour is also mentioned in Regulation (EU) 2017/2158 as a criterion for achieving acceptable acrylamide levels in fried food (European Commission [2017](#page-12-13)). However, information about stopping cooking when a golden colour is reached was mentioned only on 50% of the labels. A brown colour was obtained for all samples with a level higher than 750 μg kg^{-1} , despite the strict application of cooking instructions. More indications about the final golden colour could reduce the acrylamide levels.

Additionally, 13 samples were prepared both with oven and deep fryer to investigate the impact of the cooking mode on the acrylamide content, both following the manufacturer instructions. The results confirmed that deep-frying led systematically to higher acrylamide levels [\(Table 4](#page-7-0)). The acrylamide content for seven of the 13 deep-fried samples was below the BML of 750 μ g kg⁻¹. For all oven-cooked samples, the acrylamide content was below the BML, even below the LOQ for 3 samples and was not even detected in a potato ball sample [\(Table 4\)](#page-7-0). [Figure 1](#page-8-0) shows the comparison between 4 fried potato dough-based subcategories, but the high dispersion of results for potato

LOD: 7 µg kg⁻¹; LOQ: 20 µg kg⁻¹; N.A.: not applicable.

balls and potato cubes does not allow to draw any conclusion.

A specific sub-category was "sweet potato fries," a novel food category with increasing popularity, where relatively high levels of acrylamide were found (Table S1). Currently, no BML applies to this product in Regulation (EU) 2017/2158, but the most closely related category is potato fries, with a BML of 500 μ g kg⁻¹. All samples based on sweet potato fries exceeded this level (Table S1). These results show that including sweet potato fries in Regulation (EU) 2017/2158 might be relevant, including mitigation measures.

Black olives

The acrylamide content in black olives ranged from 31.9 to 575 μg kg⁻¹, showing a wide variation ([Table 3,](#page-4-0) S1). Samples were divided into two sub-categories: natural black olives, also called "Greek-style" and artificially coloured (oxidised) black olives, known as "Californianstyle." The "Californian-style" olives are artificially coloured by air oxidation and the use of the colour fixative agent E579 (ferrous gluconate), followed by a sterilisation step. The sterilisation process involves temperatures ranging from 110°C to 121°C for up to 50 minutes, exceeding the temperature threshold for acrylamide formation. This additional step in the production of "Californian-style" olives could possibly explain why the average acrylamide content $(503 \mu g kg^{-1})$ is 5 times higher than that of the "Greek-style category" (63.9 µg kg⁻¹) [\(Table 1](#page-3-1)). The E579 agent does not contribute to acrylamide formation (Casado and Montaño [2008;](#page-11-4) Charoenprasert and Mitchell [2014\)](#page-11-12) but may indicate the use of the Californian-style process and the presence of a sterilisation step. Several authors have reported that this sterilisation process primarily

Figure 1. Acrylamide content in the four fried potato dough-based sub-categories. Boxes indicate 25th, 50th and 75th percentiles, whiskers represent minimum and maximum, the cross represents the average, and the horizontal line represents the median. Values <loq have been replaced by 0. Statistical outliers are represented by a dot.

contributes to acrylamide formation in black olives (Amrein et al. [2007;](#page-11-13) Casado and Montaño [2008](#page-11-4); Charoenprasert and Mitchell [2014](#page-11-12); Brenes-Álvarez et al. [2024](#page-11-14)). Breitling-Utzmann et al. [\(2023\)](#page-11-3) studied the link between acrylamide in oxidised black olives and their packaging. They found that, on average, olives packed in cans and glass jars contained more acrylamide than those packed in plastic boxes. They reported that this result may be due to the fact that plastic boxes cannot undergo intensive sterilisation as cans and glass jars. A study by Martín-Vertedor et al. ([2020](#page-12-18)) demonstrated that the ripeness stage of olives significantly impacts acrylamide content in table olives, with differences of up to 39% depending on ripeness. Consequently, ripeness could be an important parameter to consider in processing and in future mitigation measures. Interestingly, Duedahl-Olesen [\(2019\)](#page-12-19) conducted a study on the effects of home cooking on black olives, and observed an extremely high acrylamide formation (up to 5270 μ g kg⁻¹) for "Californian-style" black olives. Since "Californian-style" olives are widely consumed in Mediterranean dishes (pasta/pizzas) or appetisers, it may be relevant to regulate this kind of olives and suggest mitigation measures to reduce the acrylamide exposure associated with the sterilisation process.

Food categories with variable levels

Cocoa powder

Acrylamide was detected in the 5 cocoa samples, of which the cocoa content ranged from 13 to 100% [\(Table 3](#page-4-0)). The acrylamide levels ranged from <LOQ to 141 µg kg−1, without correlation between acrylamide level and cocoa content. The mean acrylamide content was 58.0 μ g kg⁻¹, which is approximately three times lower than the mean level (178 μ g kg⁻¹) reported by EFSA ([2015\)](#page-12-4). It is also between 5 and 6 times lower than the mean levels reported in a UK study (364 and 274 μg kg^{-1} in 2020 and 2021, respectively), for which the exact composition of the samples was not available (MacDonald et al. [2023](#page-12-9)). A recent study conducted by Kruszewski and Obiedziński [\(2020\)](#page-12-20) made similar observations and studied the origin of the discrepancies. They concluded that it might be explained by the variety and geographical origin of cocoa beans, the form of roasted cocoa beans (whole beans or nibs) and the roasting conditions (temperature, relative humidity and roasting time).

Cereals and snacks

"Cereals and snacks" are generally consumed as snacks or appetisers ([Table 3\)](#page-4-0). Acrylamide has been detected in 84% of the 64 samples, ranging from <LOD to 353μ g kg⁻¹, showing wide variability ([Table 1](#page-3-1), [Figure 2\)](#page-9-0). Maize and rice crackers and maize, wheat and manioc snacks have been further subdivided according to the cereal type and the production process involved (extrusion, puffing) to assess if there was a difference in acrylamide content depending on the cereal type or the heating process involved. For instance, crackers are usually extruded and snack samples were puffed maize or rice grains. [Figure 2](#page-9-0) illustrates the wide variation of acrylamide levels observed among cereals and snack products. Manioc data is not shown, as only 2 samples were analysed, wherein no acrylamide was detected (Table S1). Samples composed of a mix of cereals are not shown either. These results are similar to those found in a recent UK retail monitoring study where acrylamide content varied from 50 to 474 μ g kg⁻¹ (MacDonald et al. [2023\)](#page-12-9).

In the present study, all acrylamide levels were below the BML for "cereal crisps other than potato crisps," which is the most closely regulated food category with a BML set at 400 μg kg⁻¹ (European Commission [2017\)](#page-12-13). However, it is very common for parents to give rice and maize crackers or extruded corn/wheat corn as snacks to young children. For "baby foods, processed cereal-based foods for infants and young children excluding biscuits and rusks" and "biscuits and rusks for young children," the BMLs are specifically set at 40 and 150 μ g kg⁻¹, respectively. In this case, one rice waffle sample dedicated to young children with an acrylamide content of 41.5 µg kg^{-1} , exceeded the BML.

Nevertheless, it is essential to understand if the variation comes from the raw materials and/or the production process. A review by Žilić et al. [\(2022\)](#page-13-2) concluded that acrylamide precursors in corn-based products strongly depended on genetic factors and environmental and storage conditions. Hence, it might be relevant to regulate this food category and apply mitigation measures based on selecting the cereal varieties and optimising the storage conditions.

In the "honey roasted muesli" category, the acrylamide content was below 60 µg kg^{-1} for 10 out of 11 samples, except for one outlier at 353 μ g kg⁻¹. This suggests that apparently honey does not increase acrylamide content when compared to other products in the cereals and snacks category. The elevated acrylamide level of the outlier may also originate from other sources, such as additional ingredients or the cooking process (Table S1).

The acrylamide content in the 11 products containing chia seeds varied between <LOD to 190 μ g kg⁻¹ (Table S1, [Table 3](#page-4-0), [Figure 2](#page-9-0)) with an average of 43.3 μ g kg⁻¹. No link could be made between acrylamide

Figure 2. Acrylamide content in cereals and snacks food sub-categories. Boxes indicate 25th, 50th and 75th percentiles, whiskers represent minimum and maximum, the cross represents the average, and the horizontal line represents the median. Values <loq have been replaced by 0. Statistical outliers are represented by a dot. Rice and maize crackers were extruded and maize and wheat snacks were puffed.

content and percentage of chia seeds used in the recipes for these samples. The levels found in this study were lower than those in previous studies, since Das and Srivastav [\(2012](#page-11-15)) found 100 to 600 µg kg^{-1} for biscuits and crackers (without chia seeds), and Croft et al. ([2004](#page-11-16)) reported 118 to 573 µg kg−1 in biscuits. The EFSA opinion (2015) mentions acrylamide contents of 201 to 297 μ g kg⁻¹ for cookies, but does not specify the presence or absence of chia seeds. However, recent studies by Mesías et al. [\(2023](#page-12-21)) and Hölzle et al. [\(2025\)](#page-12-22) demonstrated that acrylamide was found in chia seeds undergoing a thermal process. Notably, roasting ground chia seeds produced up to 9 times more acrylamide than roasting whole seeds. In the present study, only whole chia seeds were included in the samples. A recent study by Schouten et al. ([2024\)](#page-12-23) studied the effect of adding 10% nuts, dried fruits, dried seeds and black olives to biscuits on acrylamide content. They concluded that, although this addition increased acrylamide levels, accurately predicting the final content based on individually cooked ingredients was challenging. This highlights the importance of understanding how ingredients interact during the cooking process. In the present study, chia seeds were present in limited proportion in comparison with these studies (<6% on average; Table S1), which may further mitigate acrylamide formation.

Food categories with low levels

Bread and pastries

Bread and pastries exhibited detection frequencies of 33% and 53%, respectively, as presented in [Table 3.](#page-4-0) The quantified levels ranged from values below LOD to 211 μ g kg⁻¹ for bread and from below LOD to 32.5 μ g kg⁻¹ for pastries. The low detection frequencies in these categories were expected, as acrylamide is mainly produced in the crust, which represent a small surface-to -volume ratio and a small percentage of the total sample. Consequently, the acrylamide level is lower in the entire food product. The obtained results are similar to results previously reported by Croft et al. [\(2004](#page-11-16)) (i.e. <25 μ g kg⁻¹ for croissants and from 25 to 50 μ g kg⁻¹ for bread) but lower compared to the data included in the EFSA opinion (2015) on acrylamide (i.e. 61 to 71 μ g kg⁻¹ for cakes and pastries) and the UK monitoring of 2020–2021 (92 μ g kg⁻¹ for pastries and 63 μ g kg⁻¹ for bread in average; MacDonald et al. [2023\)](#page-12-9). Nevertheless, two bread samples contained a higher acrylamide content, i.e. a rusk sample (211 µg kg^{-1}) and a bread containing olives (98 μ g kg⁻¹). The larger surface-to-volume ratio can explain the higher acrylamide level in rusk. These results agree with the study of MacDonald et al. [\(2023\)](#page-12-9), who obtained 194 μ g kg⁻¹ on average for similar products. As illustrated above, the presence of olives in bread may explain higher acrylamide levels, since maximum acrylamide levels in olives were up to 575 μ g kg⁻¹ [\(Table 3](#page-4-0)). No correlation was identified between acrylamide levels in bread and the inclusion of chia seeds or flour. Galluzzo et al. ([2021](#page-12-24)) conducted an extensive investigation into the influence of chia flour on the acrylamide content of wheat bread. Their findings indicate that substituting wheat flour with chia flour does not lead to an increase in acrylamide content.

Dried fruits and confectionaries

Among 11 samples, only one nougat and one fudge sample contained 25.4 and 59.6 µg kg⁻¹ acrylamide, respectively. No acrylamide was detected in dried fruits. These results are similar to those of the Canadian acrylamide monitoring programme of 2012 and the UK national survey of 2020–2021 (Bureau of Chemical Safety [2012](#page-11-17); MacDonald et al. [2023](#page-12-9)). However, the UK study reported a high acrylamide level in one dried apricot sample $(454 \,\text{µg} \, \text{kg}^{-1})$. Amrein et al. ([2007\)](#page-11-13) found a variable but quantifiable $(>10 \mu g kg^{-1})$ acrylamide content in dried fruits, ranging from 19 to 146 μg kg⁻¹. De Paola et al. [\(2017\)](#page-11-6) confirmed those results with an acrylamide content in dried prunes ranging from 14.7 to 124 μ g kg⁻¹.

Nuts and oilseeds (roasted)

The detection frequency for acrylamide in the 16 samples of nuts and oilseeds (roasted) was 69%. Acrylamide content ranged from <LOD to 155 μg kg⁻¹ with an average of 29.5 μg kg⁻¹ [\(Table 3\)](#page-4-0). The highest level (considered an outlier) was found in a sample of roasted oilseed (Table S1). These results are in accordance with the results of De Paola et al. ([2017](#page-11-6)) and the UK monitoring of 2020–2021 (MacDonald et al. [2023\)](#page-12-9) since low acrylamide levels (below 100 μ g kg⁻¹) were found for most samples. In their studies, the highest results were found in sunflower seeds, although low levels were detected in other sunflower seeds. Other studies reported acrylamide levels that varied widely from <LOD for almonds, hazelnuts, pine nuts and walnuts and <LOQ for pistachios up to 1000 μ g kg⁻¹ in roasted almonds (Amrein et al. [2005](#page-11-18); Žilić [2016](#page-13-4); De Paola et al. [2017\)](#page-11-6). Jägerstad and Skog [\(2005](#page-12-25)) reported an acrylamide level of 66 µg kg^{-1} in sunflower seeds, while a mean value of $93 \mu g kg^{-1}$ was reported in the EFSA opinion ([2015\)](#page-12-4) on acrylamide for roasted nuts and seeds.

Conclusion

This survey of foods on the Belgian market provides valuable additional data on acrylamide in all food categories targeted by Recommendation (EU) 2019/ 1888. Occurrence data revealed relatively elevated acrylamide levels in vegetable crisps and "Californian" style black olives, coffee substitutes (without chicory) and deep-fried potato-based products. Adapted mitigation measures on variety selection, storage conditions of the raw materials and the production process could also be implemented for these food categories. In contrast, dried fruits, confectionaries, bread and pastries exhibited low detection frequencies of acrylamide. Lastly, this study did not confirm the hypothesis that chia flour or seeds may generate substantial amounts of acrylamide compared with traditional wheat bread.

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