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Acrylamide in other foods

Determination of acrylamide levels in some food categories and estimation of the exposure for the Belgian population

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EXECUTIVE SUMMARY

Summary

Acrylamide is a chemical substance that has been classified as potentially carcinogenic (group 2A) by the International Agency for Research on Cancer (IARC) due to its carcinogenic effect on rodents. This classification was approved during the WHO consultation in 2002 and EFSA confirmed in 2015 that acrylamide is a carcinogenic and genotoxic substance. Acrylamide is naturally formed in food during high temperature transformation processes (above 120 ° C) such as cooking, grilling, frying or roasting. It is formed from sugar and amino acids in a chemical reaction known as the Maillard reaction that is responsible for the browning of food and the corresponding characteristic taste.

Acrylamide can be produced in a wide range of foods, mainly those based on potatoes or cereals, such as crisps, fries, bread, cookies, but also in coffee, cocoa, etc... It is formed both during industrial and domestic cooking processes. Many factors influence the final levels of acrylamide in food, i.e. the transformation processes (e.g. temperature and cooking time), parameters intrinsic to the ingredients (i.e. pH, water activity, the use of salts or specific ingredients, but the main contributor is the presence of the initial precursors asparagine and reducing sugars. The concentration of these precursors in food depend not only on the type of cereals (wheat, rye) or on the potato variety, but also on their geographical origin and their storage conditions.

The influence of each of these parameters on the formation of acrylamide was extensively and the conclusions were compiled in a toolbox also called mitigation measures, giving the keys to reduce as much as possible the acrylamide content in the final product. An overview of these mitigation measures is also available in Annexes I and II of Regulation (EU) 2017/2158. Furthermore, this Regulation also assigns benchmark levels for certain food categories that the food sector should be able to achieve when respecting the mitigation measures. However, not all food categories are included in this Regulation and other types of food may contain significant quantities of acrylamide. To remedy this shortcoming, the European Commission published Recommendation (EU) 2019/1888 concerning the monitoring of the presence of acrylamide in certain other foodstuffs. In parallel, EFSA also issued a call on the 25th of October 2019 to collect data on a European scale on the levels of acrylamide in foodstuffs based on chia seeds or chia flour and which have undergone a cooking process. This call echoes a EFSA scientific opinion of March 2019 stating that partial substitution of wheat flour with chia seed flour can lead to higher levels of acrylamide in the final product. This study is also part of the discussions on Novel Food Regulation (EU) 2015/2283. Therefore, this research project was issued in order to collect missing data on acrylamide contamination for different food categories, as targeted by Recommendation (EU) 2019/1888 and the EFSA call. A secondary objective was the determination of the influence of the cooking method used (oven versus fryer) on the production of acrylamide specifically for potato-based foods. Next, the impact of domestic cooking practices of the Belgian population were investigated. Afterwards, the concentration data were combined with the consumption data of the most recent National Food Consumption Survey (FCS2014) and the risk of the Belgian consumer related to exposure of acrylamide was investigated using the Margin of Exposure approach. The main conclusions are summarized in the following paragraphs.

The food categories included in Recommendation (EU) 2019/1888 and the EFSA call of 2019, were investigated. In total, 217 samples were purchased on the Belgian market and the acrylamide content

was determined. To this end, sensitive analytical methods have been developed and validated, allowing accurate quantification. After extraction of acrylamide from the food and subsequent clean-up, the extracts were analyzed by liquid chromatography in combination with triple quadrupole tandem mass spectrometry (LC-MS/MS).

Next, the validated methods were applied for the analysis of the samples. For some food categories, very low amounts of acrylamide have been found or acrylamide was not even detected. This was particularly the case for the samples included in the categories “bread” (e.g. milk bread, hamburger bun, tortilla, etc.) and “pastries” (e.g. viennoiseries, donuts, churros, and pancakes). These results could be explained on the one hand by the effect of the mitigation measures that were already in place in 2017 (Regulation (EU) 2017/2158). On the other hand, in most cases, only a thin layer of the outer part of the food contains the acrylamide (i.e. crust) and this diluted in the total volume of the food. This reasoning can also explain the low concentration of acrylamide that was found in puff pastry (e.g. zakouskis), dried fruits and nougats and caramels.

Certain food categories were characterized by a high variability in the acrylamide concentrations. Indeed, samples based on cereals such as rice and corn waffles contained acrylamide varying from below the detection limit to 259 µg kg^{-1} while even a concentration up to 337 µg kg^{-1} was quantified in crackers based on wheat and corn. It is quite difficult to explain this variability as different factors need to be considered. For example, the transformation processes can vary from one producer to another and it has already been demonstration that a transformation processes involving extrusion can enhance the acrylamide formation. In addition, the variety of cereals and their geographic origin can also have an important role. The same is true for the category including nuts and oilseeds where concentrations ranged from below the detection limit up to 155 µg kg^{-1} have been determined.

Coffee substitutes based on plant and/or nuts were also included in the study. Strong variations were found ranging from below the detection limit up to more than 4000 µg kg^{-1}. It has to be noted that these concentrations were measured in the dry product and not in the prepared coffee-like drink. The variability can be influenced by the origin of the plant, but also on the part of the plant that was used, e.g. roots tend to produce much more acrylamide than the leaves. When comparing the results with the benchmark level for coffee substitutes included in Regulation (EU) 2017/2158 (i.e. 4000 µg kg^{-1}), the highest result found in this category was slightly higher (i.e. 4389 µg kg^{-1}). However this kind of product is not often consumed by the Belgian population.

Very high concentration of acrylamide were also found in other food categories, for example acrylamide concentrations up to 3063 µg kg^{-1} were found in Indeed, vegetable-based crisps (sweet potato, parsnip, carrot, beetroot, cassava). Although no benchmark level is available for this food category, 3 of these samples had to be notified to the Federal Agency for Safety of the Food Chain (FASFC) because of the potential health risk of this concentration (> 1500 µg kg^{-1}).

Analysis of black olives has shown that high level of acrylamide in certain samples (up to 575 µg kg^{-1}) are strongly correlated with the processing method known as "Californina-style". During this process, a sterilization step reaching 121 °C (i.e. above the threshold for acrylamide formation) is executed for several minutes. Although this conclusion is drawn from the analysis of only 5 samples, it seems to be confirmed by scientific literature.

The last food category that was included in this study were the fried potato products. The acrylamide concentration varied from below the detection limit up to 1503 µg kg^{-1}. This can originate from many different sources (e.g. potato variety, storage conditions, initial concentration of the precursors, etc.). Also the shape of the food can be important. Potato balls and potato cubes have a higher surface-volume ratio compared to the classical croquettes, leading to higher concentrations of acrylamide. This is confirmed in this study, where the highest concentrations were found in potato balls. Not only fried potato products were included in this study, but also some potato-based dishes were analysed. These results have shown that food prepared using a microwave. Overall the acrylamide concentration was very low in these products, but this is most probably related to the dilution effect. Indeed, these products were ready-to-eat meals that also contained meat and vegetables.

For some of the potato based dishes, more than one preparation instruction was present on the packaging. Therefore, the impact of the type of the preparation process on acrylamide formation could be investigated. To this end, 11 samples (e.g. röstis, duchess apple, potato balls) were prepared by frying and in the oven and the instructions on the packaging were meticulously followed. The results have demonstrated that preparation by frying results in an acrylamide formation that is 5 to 25 times higher compared to the preparation using the oven. Up till now, no benchmark level was available for these types of food. However, when comparing the results with the benchmark level of the most closely related category (i.e. French fries - 750 µg kg^{-1}), more than 50% of the samples prepared by frying exceeded this level, while all the acrylamide levels were well below this benchmark level when prepared in the oven.

This illustrates the importance of the cooking habits of the consumer on the acrylamide formation. Therefore, this was further investigated. Fourteen participants were recruited and they were asked to prepare, according to their own habits, samples of croquettes, sweet potato fries and hamburger buns. At the same time, they were asked to complete a questionnaires related to their cooking habits in general (e.g. following of the instructions on the packaging, criteria for when the preparation is stopped,, etc.). Each food item was also prepared in the laboratory according to the specified instructions. All acrylamide levels in the food prepared by the participants were lower compared to the reference preparation in the laboratory. It was also very interesting to see that more than half of the participants not respect the cooking instructions indicated on the packaging, but they use the criteria ‘golden yellow colour of the food’ to stop the preparation process.. Furthermore, this study has demonstrated that the acrylamide contents correlate perfectly with the final colour of the food. Furthermore by respecting this colour criterion, acceptable concentrations of acrylamide were reached. In addition, this golden yellow colour is also recommended in Regulation (EU) 2017/2158.

Afterwards, the analytical results (i.e. mean and highest concentration in every food category) were used for a dietary intake assessment of the Belgian population for acrylamide by combining them with the results of the latest National Food Consumption Survey in a lower and upper bound scenario. Overall, it can be concluded that the exposure to acrylamide is lower compared to the intake assessment reported by EFSA. This originates from the food items analysed in the framework of this study. Indeed, the selection was in accordance with Recommendation (EU) 2019/1888 that intends to fill the data gap of occurrence data related to the presence of acrylamide in food. Given the fact that most of the highest contributors to acrylamide exposure are already included in Commission Regulation (EU) 2017/2158 and these foods were not included in this study, the exposure levels determined in this study were lower compared to previous dietary intake studies. Next, the major contributors were determined. For all age groups, the intake assessment originates mostly from grain-

and grain-based products, followed by coffee, cocoa, tea and infusion for children and adolescents, while the adults are also exposed via composite dishes.

In order to assess the risks of dietary exposure to acrylamide for the Belgian population, exposure data were combined with hazard-related information. As acrylamide is potentially genotoxic, no Tolerably Daily Intake (TDI) could have been established. Therefore, the risk characterization was performed using the Margin of Exposure approach (MOE). Two reference points were considered as critical. (i) Non-neoplastic effects for which a $BMDL_{10}$ value of 0.43 mg kg^{-1} bw day^{-1} was calculated for the most relevant and sensitive endpoint for neurotoxicity, i.e. the incidence of peripheral nerve (sciatic) axonal degeneration observed in F344 rats exposed to acrylamide in drinking water for two years (ii) Neoplastic effects for which a $BMDL_{10}$ of 0.17 mg kg^{-1} bw day^{-1} was calculated i.e. the lowest $BMDL_{10}$ from data of incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F1 mice exposed to acrylamide for two years. For both reference points, the MOE was calculated using the exposure data of the mean and maximum occurrence levels. For neurotoxic effects, all MOEs were well above 100, so it can be concluded that exposure to acrylamide is not of concern regarding neurotoxic effects. However, for the neoplastic effects, almost all MOEs were substantially lower than 10 000, it should be concluded that although the available human studies have not yet demonstrated acrylamide to be a human carcinogen, the MOEs based on the current levels of dietary exposure to acrylamide indicate a concern with respect to neoplastic effects.

The results obtained in this study are completely in accordance with the conclusions of EFSA.

Résumé

L'acrylamide est une substance chimique qui a été classifiée comme probablement carcinogène (groupe 2A) par l'agence internationale de recherche sur le cancer (IARC) du à son effet carcinogène sur les rongeurs. Cette classification a été approuvée lors de la consultation de la WHO en 2002 et l'EFSA a confirmé en 2015 que l'acrylamide est une substance carcinogénique et génotoxique. Ce composé se forme naturellement dans les aliments lors de processus de transformation à haute température (supérieure à 120 °C) tel que la cuisson, le grillage, la friture, mais également la torréfaction. L'acrylamide se forme à partir de sucre et d'acides aminés lors d'une réaction chimique connue sous le nom de réaction de Maillard. C'est cette dernière qui est responsable du brunissement des aliments et du goût caractéristique qui s'en suit.

L'acrylamide peut être produit dans une vaste gamme d'aliment, principalement ceux à base de pomme de terre ou de céréales, tels que les chips, les frites, le pain, les biscuits, le café, le cacao, etc. Celui-ci est formé aussi bien lors des processus de cuisson industrielle que domestique. De nombreux facteurs influencent les teneurs finales en acrylamide dans la nourriture. Les paramètres des processus de transformation comme la température et le temps de cuisson jouent un grand rôle, mais les teneurs finales en acrylamide ne dépendent pas uniquement de ceux-ci. En effet, les teneurs finales sont également fortement influencées par une vaste gamme de paramètres intrinsèques aux ingrédients utilisés. Citons par exemple, le pH des aliments, l'activité de l'eau, l'utilisation de sels ou d'agents de levages spécifiques. L'élément le plus contributeur à la formation de l'acrylamide est la présence des précurseurs initiaux nécessaires à sa formation : l'asparagine et les sucres réducteurs. Les teneurs de ces précurseurs dépendant non seulement du type de céréale (blé, seigle) ou de la variété de pomme

de terre utilisée par exemple, mais aussi de leur origine géographique et de leur condition de stockage avant transformation.

La formation de l'acrylamide dépend donc de très nombreux paramètres. Leur influence sur les teneurs finales en acrylamide ont été étudiées de manière exhaustive et les conclusions ont été compilées dans une sorte de boite à outils encore appelée mesures d'atténuation donnant les clés pour réduire au maximum la teneur en acrylamide dans le produit final. Ces mesures d'atténuation sont également disponibles dans les annexes I et II du règlement Européen (UE) 2017/2158 du 20 novembre 2017. Ce règlement attribue également des teneurs de références (benchmark levels) pour certaines catégories d'aliments que le secteur alimentaire est capable d'atteindre en respectant ces bonnes pratiques. Cependant, ce document ne reprend pas toutes les catégories d'aliments susceptibles de contenir des quantités non-négligeables d'acrylamide et qui pourraient potentiellement contribuer de façon importante à l'exposition alimentaire totale en acrylamide. Pour pallier à ce manque, la commission européenne a publié la recommandation (UE) 2019/1888 du 7 novembre 2019 concernant le suivi de la présence d'acrylamide dans certaines autres denrées alimentaires. En parallèle l'EFSA a également émis un appel le 25 octobre 2019 visant à la collecte de données à l'échelle Européenne sur les teneurs en acrylamide de denrées alimentaires à base de graines ou de farine de chia et ayant subits un processus de cuisson. Cet appel fait écho à une opinion scientifique de l'EFSA de mars 2019 faisant état du fait que la substitution partielle de la farine de blé par de la farine de graine de chia peut conduire à des niveaux plus important d'acrylamide dans le produit final. Cette étude s'insère également dans le cadre des discussions du Règlement (UE) Novel food 2015/2283. C'est autour de cette recommandation et de cette opinion européenne que s'est articulé ce projet d'étude. L'objectif premier de ce projet visait à la collecte de données encore manquantes de la contamination en acrylamide pour différentes catégories d'aliments, telles que ciblées par la recommandation (UE) 2019/1888 et l'appel de l'EFSA. Ensuite plus spécifiquement pour les aliments à base de pomme de terre, déterminer l'influence du mode de cuisson utilisée (four/friteuse) sur la production d'acrylamide afin de déterminer si une des méthodes peut être à l'origine d'un risque plus important pour la santé du consommateur. Finalement, comme il est important de prendre en compte les cuissons domestiques des produits à base de pomme de terre pouvant représenter une contribution non négligeable à l'exposition totale à l'acrylamide, une étude a été réalisée afin de déterminer si les habitudes de cuisson des belges peuvent être à l'origine d'un risque ou non pour leur santé. Les données de concentrations ont été ensuite combinées avec les données de consommation de la dernière enquête nationale de consommation et les risques pour la population belge liés à une exposition à l'acrylamide ont été évalué sur base de la marge d'exposition. Les paragraphes suivants résument les conclusions importantes qui peuvent être tirées de ce projet d'étude.

Dans le cadre de la collecte de données visée par la recommandation (UE) 2019/1888 et l'appel de l'EFSA de 2019, pas moins de 217 échantillons ont été achetés en vue d'en déterminer leur teneur en acrylamide. A cette fin, des méthodes analytiques permettant une quantification précise et permettant d'atteindre des niveaux de quantification suffisamment bas ont été développées et validées. Les mesures ont été effectuées par chromatographie liquide suivie d'une détection par spectrométrie de masse en tandem. Après validation, ces méthodes ont été utilisées par la suite pour l'analyse de tous les échantillons inclus dans ce projet.

Pour beaucoup de catégories d'aliments, des quantités très faibles d'acrylamide ont été retrouvées voir dans certains cas l'acrylamide n'a pas été détectée. Ce fut le cas notamment de tous les aliments pouvant rentrer dans la catégorie « pain » (ex : pain au lait, bain pour hamburger, tortilla, etc…), mais

aussi toutes les pâtisseries (viennoiseries, donuts, beignets, croustillons, churros, crêpes et pancake). L'explication à ces faibles quantités d'acrylamide retrouvées peut s'expliquer d'une part par les mesures d'atténuation misent en place dans le règlement (UE) 2017/2158 devant être respectées par le secteur agroalimentaire. D'autre part, par le fait que c'est surtout une fine couche de la partie extérieure de ces aliments qui est fortement cuite. Etant donné que cette couche ne représente qu'une très faible surface par rapport au volume total de l'aliment, l'acrylamide se retrouve en quelque sorte dilué dans la masse. Ce raisonnement par extension peut aussi s'appliquer à d'autres aliments produits à base de pâte feuilletée comme les zakouskis (petit-four) par exemple. Le même constat peut être dressé pour les fruits secs où toutes les teneurs en acrylamide étaient inférieures à la limite de détection (LOD). Pour les nougats et les caramels, plus de 50 % des échantillons étaient inférieurs à la limite de quantification (LOQ) ou à la LOD, la concentration maximale retrouvée était relativement faible : 59.6 µg kg^{-1}.

Certaines catégories d'aliments sont caractérisées par des teneurs très variables pour des échantillons de même nature, même si les concentrations retrouvées n'étaient pas anormales. Ce fut le cas pour les échantillons à base de céréales comme les galettes de riz et de maïs avec des valeurs s'étalant de <LOD à 259 µg kg^{-1} ou encore les crackers à base de blé et de maïs (de <LOD à 337 µg kg^{-1}). Il est assez difficile d'expliquer cette variabilité et ceci ne fait pas partie des objectifs de cette étude. Cependant, il peut être précisé que les origines peuvent être multiples. Les processus de transformations peuvent varier d'un producteur à l'autre. Les procédés de transformation impliquant un processus d'extrusion sont également reconnus comme produisant plus d'acrylamide. De plus, comme expliqué précédemment, la variété de céréale peut jouer un grand rôle, de même que leur origine géographique. Il en va de même pour la catégorie fruits à coque et graines d'oléagineuse où des concentrations s'étalant de <LOD à 155 µg kg^{-1} ont été observées.

Les substituts de café autres que ceux à base de chicorée et de céréales ont également été analysés. De fortes variations ont été retrouvées allant de concentrations <LOD jusqu'à des teneurs de plus de 4000 µg kg^{-1}. Ces teneurs variables peuvent dépendre de l'origine de la plante, mais également de la partie de la plante qui a été utilisée. Les racines ont tendances à produire beaucoup plus d'acrylamide que les feuilles par exemple. En prenant en compte la teneur la plus élevée (4389 µg kg^{-1}), on peut se rendre compte qu'elle est légèrement supérieure à la valeur de référence la plus grande pour les substituts de café (4000 µg kg^{-1}). Ce type de produit est cependant très faiblement consommé en Belgique et est peu vendu sur le sol belge.

D'autres aliments ont été remarqués par la forte teneur en acrylamide pouvant être atteinte. Ce fut le cas des chips à base de légumes (patate douce, panais, carotte, betterave, manioc). Des valeurs pouvant atteindre 3063 µg kg^{-1} ont été retrouvées. Bien qu'aucune teneur de référence ne soit disponible pour cette catégorie d'aliment, 3 de ces échantillons ont dû être notifiés à l'AFSCA en raison du risque que les teneurs (> 1500 µg kg^{-1}) pouvaient présenter pour la santé du consommateur.

L'analyse d'olives noires a mis en évidence que les hautes teneurs en acrylamide dans certains échantillons (jusqu'à 575 µg kg^{-1}) sont fortement corrélées à une méthode de processus de transformation connue sous le nom de « Californina-style ». Lors de ce processus, une étape de stérilisation avant la mise sur le marché est utilisée. Durant ce procédé, des températures de 121 °C (température de formation de l'acrylamide) peuvent être atteintes pendant plusieurs minutes. Bien que cette conclusion soit tirée d'une analyse de 5 échantillons, ceci semble être confirmé par la littérature scientifique..

Des quantités fortement variables d'acrylamide ont également pu être mises en évidence pour les produits à base de pommes de terre à frire, allant de concentration <LOD jusqu'à 1503 µg kg^{-1}. Il n'est jamais facile d'expliquer cette variabilité, mais il peut être supposé à l'instar des céréales que cela dépend des variétés de pomme de terre utilisées ou du stockage de ces dernières et donc, des proportions en précurseurs initiaux. La forme de l'aliment peut aussi jouer un rôle. Les pommes cubes ou pommes noisettes ont un rapport surface volume plus faible que les croquettes, ce qui peut faire augmenter les teneurs en acrylamide. Les aliments cuits au four à micro-onde ont par contre montrés des teneurs en acrylamide très faibles. Ceci peut s'expliquer par le fait que certains de ces produits étaient des plat préparés contenant également de la viande et des légumes ne produisant que très peu d'acrylamide. Les produits à base de pomme de terre constituant ces plats sont donc en quelques sortent dilués dans l'échantillon, de même que l'acrylamide produit, sachant que le plat est analysé dans sa globalité.

Finalement, une étude plus exhaustive sur l'impact du mode de cuisson (four/friteuse) a été réalisée sur des produits à base de pomme de terre (röstis, pomme duchesse, pommes noisettes). A l'issu de l'étude, il a été démontré qu'en suivant scrupuleusement les indications de préparation, que la cuisson à la friteuse pouvait produire de 5 à 25 fois plus d'acrylamide que la cuisson au four. Bien qu'aucune teneur de référence ne peut être utilisée pour les aliments analysés durant cette étude, en se basant sur la valeur pour la catégorie la plus proche (750 µg kg^{-1}) plus de 50 % des échantillons cuits à la friteuse ont dépassé ce niveau, contrairement à la cuisson au four où tous les niveaux d'acrylamide était largement en deçà de cette valeur de référence.

Ces résultats ont d'autant plus suscités l'intérêt de regarder du côté des consommateurs belges et de leurs habitudes de cuisson afin d'en déduire l'impact sur la quantité d'acrylamide produit. A cette fin, quatorze participants ont été recrutés et il leur a été demandé de cuire selon leur pratique habituelle, des échantillons de croquettes, de frites de patate douce et de pains hamburger selon leur mode de cuisson préférentiel (friteuse, four, toaster). En parallèle, il leur a été demandé de remplir des questionnaires relatifs à leurs habitudes de cuisson de manière générale (respects des consignes sur l'emballage, critères pour stopper la cuisson, etc…). Fort heureusement, des quantités d'acrylamide largement en deçà des valeurs de références ont été observées à l'issue des cuissons par les candidats. D'autres informations très intéressantes ont été mises en évidence grâce au croisement des informations fournies par les questionnaires et les données analytiques. Ainsi, plus de la moitié des candidats disent ne pas respecter les consignes de cuisson fournies sur les emballages des aliments, en particulier les temps de cuisson estimant que celles-ci ne permettent pas d'obtenir les qualités organoleptiques attendues. En les interrogeant sur quels étaient les critères pour stopper la cuisson, la majorité des candidats ont répondus que l'obtention d'une couleur « jaune dorée » était leur critère principal. Ceci est très positif car il a été montré durant cette étude que d'une part, les teneurs en acrylamide était parfaitement corrélées à la coloration finale de l'aliment et que d'autre part, en respectant ce critère de couleur, des concentrations acceptables d'acrylamide étaient atteintes. De plus, cette couleur jaune dorée est également celle préconisée dans le règlement (UE) 2017/2158.

Afin d'évaluer les risques d'exposition alimentaire à l'acrylamide pour la population belge, les données d'exposition ont été combinées avec des informations relatives aux dangers que peut présenter l'acrylamide. L'acrylamide étant potentiellement génotoxique, aucune dose journalière admissible (DJA) n'aurait pu être établie. Par conséquent, la caractérisation des risques a été effectuée à l'aide de l'approche de la marge d'exposition (ME). Deux points de référence ont été considérés comme

critiques. (i) L'effet non néoplasique pour lequel une $BMDL_{10}$ de 0,43 mg kg-1 pc jour-1 a été calculé comme critère le plus pertinent et le plus sensible pour la neurotoxicité, c'est-à-dire l'incidence de la dégénérescence axonale du nerf périphérique (sciatique) observée chez les rats F344 exposés à l'acrylamide via l'eau potable pendant deux ans. (ii) Les effets néoplasiques pour lesquels une $BMDL_{10}$ de 0,17 mg kg-1 pc jour-1 a été calculé, i.e. $BMDL_{10}$ la plus basse des données sur l'incidence des adénomes et adénocarcinomes des glandes hardériennes chez des souris mâles B6C3F1 exposées à l'acrylamide pendant deux ans. Pour les deux valeurs de référence, la MOE a été calculée à l'aide des données d'exposition des niveaux d'occurrence moyens et maximaux. Pour les effets neurotoxiques, toutes les ME étaient bien au-dessus de 100, Il peut donc être conclu que l'exposition à l'acrylamide n'est pas préoccupante en ce qui concerne les effets neurotoxiques. Cependant, pour les effets néoplasiques, presque toutes les ME étaient sensiblement inférieures à 10 000. Il convient de conclure que bien que les études disponibles sur l'homme n'aient pas encore démontré que l'acrylamide est cancérogène pour l'homme, les ME basées sur les niveaux actuels d'exposition alimentaire à l'acrylamide indiquent une préoccupation concernant les effets néoplasiques.
Les résultats obtenus dans le cadre de cette étude sont entièrement conformes aux conclusions de l'EFSA.

Samenvatting

Acrylamide is een chemische stof die door het Internationaal Agentschap voor Kankeronderzoek (IARC) is geclassificeerd als potentieel kankerverwekkend (groep 2A) vanwege het kankerverwekkende effect op knaagdieren. Deze classificatie werd goedgekeurd tijdens de WHO-raadpleging in 2002 en EFSA bevestigde in 2015 dat acrylamide een kankerverwekkende en genotoxische stof is. Deze verbinding wordt van nature gevormd in voeding tijdens transformatieprocessen bij hoge temperatuur (boven 120 °C) zoals koken, grillen, frituren of braden. Acrylamide wordt gevormd uit suiker en aminozuren via een chemische reactie die bekend staat als de Maillard-reactie. Deze reactie is verantwoordelijk voor de bruine kleur van bereide levensmiddelen en de overeenkomstige karakteristieke smaak.

Acrylamide kan worden gevormd in een zeer veel verschillende levensmiddelen, voornamelijk die op basis van aardappelen of granen, zoals chips, friet, brood, koekjes, maar ook in koffie, cacao, etc. Het wordt zowel tijdens industriële als huishoudelijke kookprocessen gevormd. De uiteindelijke hoeveelheid wordt beïnvloed door veel factoren zoals de parameters van de transformatieprocessen (vb temperatuur en kooktijd), maar ook paramaters die inherent zijn aan de gebruikte ingrediënten (vb pH van het levensmiddel, water activiteit, het gebruik van zouten of specifieke ingrediënten). De belangrijkste parameter voor de vorming van acrylamide is echter de aanwezigheid van de eerste voorlopers die nodig zijn voor de vorming ervan, nl. asparagine en reducerende suikers. Hun aanwezigheid wordt niet alleen bepaald door het type graan (tarwe, rogge) of van de gebruikte aardappelsoort, maar ook door hun geografische oorsprong en de condities waaronder ze worden bewaard. De invloed van elk van deze parameters op de vorming van acrylamide werd uitgebreid onderzocht en de conclusies werden verzameld in een 'toolbox' van mitigatie-maatregelen die ervoor zorgen dat het acrylamidegehalte in het eindproduct zoveel mogelijk wordt verminderd. Een overzicht van deze maatregelen is ook opgenomen in de bijlagen I en II van Verordening (EU) 2017/2158. Bovendien wordt in deze verordening ook 'referentieniveaus' toegekend voor bepaalde voedingscategorieën. Niet alle voedingscategorieën zijn echter in deze verordening opgenomen en andere levensmiddelen kunnen ook aanzienlijke hoeveelheden acrylamide bevatten. Om deze tekortkoming te verhelpen, heeft de Europese Commissie Aanbeveling (EU) 2019/1888 gepubliceerd over de opvolging van de aanwezigheid van acrylamide in bepaalde andere levensmiddelen.

Tegelijkertijd heeft EFSA op 25 oktober 2019 ook een oproep gedaan om op Europese niveau gegevens te verzamelen over de acrylamidegehaltes in levensmiddelen op basis van chiazaad of chiameel en die werden bereid. Deze oproep weerspiegelt een wetenschappelijk advies van EFSA van maart 2019 waarin staat dat gedeeltelijke vervanging van tarwebloem door chiazaadmeel kan leiden tot hogere acrylamidegehaltes in het eindproduct. Deze studie maakt ook deel uit van de discussies in het kader van de Novel Food Regulation (EU) 2015/2283. Vervolgens, werd een onderzoeksproject opgezet om ontbrekende gegevens over acrylamidegehaltes in verschillende voedingscategorieën te verzamelen, zoals beoogd door Aanbeveling (EU) 2019/1888 en de EFSA-oproep. Een tweede objectief was het bepalen van de invloed van de gebruikte bereidingsmethode (oven versus frituren) op de vorming van acrylamide, en dan specifiek voor aardappelproducten. Nadien werd de impact van de binnenlandse kookpraktijken van de Belgische bevolking onderzocht. Vervolgens, werden de concentratiegegevens gecombineerd met de gegevens van de meest recente Nationale voedingsconsumptie enquête (VCP2014) en werd het risico geëvalueerd op basis van de *Margin of Exposure* (MOE). Een samenvatting van de belangrijkste conclusies is weergegeven in de volgende paragrafen.

De verschillende voedingscategorieën die zijn opgenomen in Aanbeveling (EU) 2019/1888 en de EFSA-oproep van 2019, werden onderzocht. In totaal werden 217 stalen aangekocht op de Belgische markt en werd het acrylamidegehalte bepaald. Er werden gevoelige analytische methoden ontwikkeld en gevalideerd, waardoor nauwkeurige kwantificering mogelijk was. Na extractie van acrylamide uit het levensmiddel en een verder opzuivering, werd het extract geanalyseerd met vloeistofchromatografie in combinatie met tandem-massaspectrometrie (LC-MS/MS). Vervolgens werden de gevalideerde methoden toegepast voor de analyse van de stalen. Voor sommige voedingscategorieën werden zeer lage hoeveelheden acrylamide teruggevonden of werd acrylamide niet gedetecteerd. Dit was met name het geval voor de stalen in de categorieën "brood" (bijv. melkbrood, hamburgerbroodje, tortilla, enz.) en "Gebak" (bijv. Viennoiseries, donuts, churros en pannenkoeken). Deze resultaten kunnen enerzijds worden verklaard door het effect van de mitigerende maatregelen die al in 2017 van kracht waren (Verordening (EU) 2017/2158). Anderzijds, is acrylamide in de meeste stalen enkel aanwezig in een dunne laag aan de buitenzijde van het staal (de korst) en dit wordt verdund in het totale volume van het staal. Deze redenering kan ook de lage concentratie acrylamide verklaren die werd aangetroffen in bladerdeeg-gerechten (bijv. Zakouskis), gedroogd fruit, nougat en karamel.

Bepaalde voedingscategorieën werden gekenmerkt door een grote variabiliteit in de acrylamideconcentraties. Inderdaad, levensmiddelen op basis van granen zoals rijst- en maiswafels bevatten acrylamide variërend van niet-gedetecteerd tot 259 µg kg^{-1} en in crackers op basis van tarwe en maïs werd zelfs een concentratie van 337 µg kg^{-1} gekwantificeerd. Deze variabliteit is moeilijk te verklaren omdat er met verschillende factoren rekening moet worden gehouden. De transformatieprocessen kunnen bijvoorbeeld van producent tot producent verschillen en het is al aangetoond dat transformatieprocessen met extrusie de vorming van acrylamide kunnen verhogen. Daarnaast kan de verscheidenheid aan granen en hun geografische oorsprong ook een belangrijke rol spelen. Hetzelfde geldt voor de categorie van noten en oliehoudende zaden, waarbij concentraties variërend van niet-gedetecteerd tot 155 µg kg^{-1} werden bepaald.

Koffiesurrogaten op basis van plant en/of noten werden ook geanalyseerd in het kader van dit onderzoeksproject. Ook hier werden grote variaties in het acrylamidegehalte geobserveerd van niet-gedetecteerd tot meer dan 4000 µg kg^{-1}. Opgemerkt moet worden dat deze concentraties werden gemeten in het droge product en niet in de bereide drank. De variabiliteit kan worden beïnvloed door de oorsprong van de plant, maar ook door het deel van de plant dat werd gebruikt, b.v. wortels

produceren veel meer acrylamide dan de bladeren. Bij het vergelijken van de resultaten met het referentieniveau voor koffiesurrogaten opgenomen in Verordening (EU) 2017/2158 (4000 µg kg^{-1}), dient opgemerkt te worden dat het hoogste resultaat (4389 µg kg^{-1}) hoger is dan deze waarde. Dit soort producten wordt echter niet vaak geconsumeerd door de Belgische bevolking.
Een zeer hoog acrylamide-gehalte werd ook gevonden in andere voedingscategorieën. Zo werd een concentratie van 3063 µg kg^{-1} gevonden in groentechips (zoete aardappel, pastinaak, wortel, rode biet, cassave). Hoewel er geen referentieniveau beschikbaar is voor deze voedingscategorie, moesten 3 van deze monsters worden genotificeerd bij het Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV) vanwege het potentiële gezondheidsrisico van deze concentratie (> 1500 µg kg^{-1}).

Bij de analyse van zwarte olijven, werd ook een hoog acrylamidegehalte gekwantificeerd in bepaalde monsters (tot 575 µg kg^{-1}). Dit is sterk gecorreleerd met de verwerkingsmethode die bekend staat als "Californina-style". Tijdens dit proces wordt gedurende enkele minuten een sterilisatiestap uitgevoerd bij 121 ° C (d.w.z. boven de drempel voor acrylamidevorming). Hoewel deze conclusie gebaseerd is op slechts 5 stalen, lijkt deze te worden bevestigd door wetenschappelijke literatuur.

De laatste voedingscategorie die werd onderzocht is de gefrituurde aardappelproducten. Het acrylamidegehalte varieerde van niet-gedetecteerd tot 1503 µg kg^{-1}. Dit kan veroorzaakt worden door veel verschillende factoren zoals de aardappelvariëteit, bewaarcondities, initiële concentratie van de voorlopers, enz. Ook de vorm van het levensmiddel kan belangrijk zijn. Aardappelbolletjes en aardappelblokjes hebben een hogere oppervlakte-volumeverhouding vergeleken met de klassieke kroketten, wat leidt tot hogere concentraties acrylamide. Dit wordt bevestigd in dit onderzoek, waar de hoogste concentratie werd gedetecteerd in aardappelbolletjes. Niet alleen gefrituurde aardappelproducten werden onderzocht, maar ook enkele andere aardappelgerechten. De resultaten hebben aangetoond dat voeding dat werd bereid met een magnetron, over het algemeen minder acrylamide bevat. Dit heeft waarschijnlijk te maken met verdunningseffect. Deze stakel waren namelijk kant-en-klare gerechten die ook nog vlees en groenten bevatten. De fractie aardappel in het totale volume van het gerecht is dus eerder beperkt.

Bij sommige aardappelgerechten werden meerdere bereidingsinstructies vermeld op de verpakking. Hierdoor kon de impact van het bereidingsproces op de vorming van acrylamide worden onderzocht. Elf stalen (röstis, pomme duchesse en aardappelballetjes) werden bereid door frituren en in de oven waarbij de instructies nauwgezet werden opgevolgd. Op basis van de resultaten kan worden geconcludeerd dat bereiding door frituren leidt tot een verhoogde acrylamide concentratie. Er werd namelijk 5 tot 25 keer meer acrylamide gevormd door frituren in vergelijking met een bereiding in de oven. Tot op heden, werd nog geen referentieniveau vastgelegd voor deze levensmiddelen. Bij vergelijking van de resultaten met het referentieniveau van de meest verwante categorie (dwz frietjes - 750 µg kg^{-1}), werd dit niveau echter overschreden in meer dan 50% van de levensmiddelen bereid door frituren, terwijl alle acrylamidegehaltes lager waren dan dit referentieniveau wanneer het levensmiddel werd bereid in de oven.

Dit illustreert het belang van de kookgewoonten van de consument op de vorming van acrylamide. Daarom werd dit verder onderzocht. Veertien deelnemers werden geselecteerd en er werd gevraagd om, volgens eigen gewoonten, kroketten, zoete frietjes en hamburgerbroodjes te bereiden. Tegelijkertijd werd hen gevraagd een vragenlijst in te vullen met betrekking tot hun kookgewoonten in het algemeen (bijv. het volgen van de instructies op de verpakking, criteria voor wanneer de

bereiding wordt gestopt, enz.). Elk levensmiddel werd ook in het laboratorium bereid volgens de gespecificeerde instructies. Alle acrylamidegehaltes in het door de deelnemers bereide gerecht waren lager in vergelijking met het referentiegerecht bereid in het laboratorium. Uit de enquête bleek ook dat meer dan de helft van de deelnemers de kookinstructies op de verpakking niet respecteert, maar dat ze de criteria 'goudgele kleur van het voedsel' gebruiken om het bereidingsproces te stoppen. Verder heeft deze studie aangetoond dat het acrylamidegehalte perfect correleert met de uiteindelijke kleur van het bereide levensmiddel. Door dit kleurcriterium te respecteren, werden bovendien aanvaardbare concentraties acrylamide gevormd. Bovendien wordt deze goudgele kleur ook aanbevolen in Verordening (EU) 2017/2158.

Daarna werden de analytische resultaten (d.w.z. gemiddelde en hoogste concentratie in elke voedingscategorie) gebruikt voor een evaluatie van de inname van acrylamide door de Belgische bevolking door ze te combineren met de resultaten van de laatste Nationale voedselconsumptiepeiling (VCP2014) in een onder- en bovengrensscenario. In het algemeen, kan worden geconcludeerd dat de blootstelling aan acrylamide lager is dan de door EFSA gerapporteerde inname. Dit kan verklaard worden op basis van de geselecteerde voedingscategorieën. De selectie was inderdaad in overeenstemming met Aanbeveling (EU) 2019/1888, die bedoeld is om de lacune in beschikbare gegevens over acrylamidegehaltes in levensmiddelen te vervolledigen. Aangezien de belangrijkste voeding categorieën voor de blootstelling aan acrylamide al werden opgenomen in Verordening (EU) 2017/2158, en deze niet werden opgenomen in het project, zijn de berekende innames lager in vergelijking met de EFSA-studie. Vervolgens werd onderzocht welke voedingscategorieën verantwoordelijk zijn voor de inname. Voor alle leeftijdsgroepen is de inname voornamelijk afkomstig van granen en graan-gebaseerde producten, gevolgd door koffie, cacao, thee en infusie voor kinderen en adolescenten, terwijl de volwassenen ook worden blootgesteld via bereide gerechten.

Uiteindelijk werden de risico's van blootstelling aan acrylamide via de voeding voor de Belgische bevolking geëvalueerd. Hiervoor werden de innameberekeningen gecombineerd met gevaar-karakterisatie. Aangezien acrylamide potentieel genotoxisch is, kan er geen Tolerable Daily Intake (TDI) worden vastgelegd. Daarom werd de risicokarakterisering uitgevoerd met behulp van de Marge of Exposure-benadering (MOE). Twee referentiepunten werden als kritiek beschouwd. (i) Niet-neoplastische effecten waarvoor een $BMDL1_0$-waarde van 0,43 mg kg^{-1} lg dag^{-1} werd berekend voor het meest relevante en gevoelige eindpunt voor neurotoxiciteit, dwz de incidentie van perifere zenuw (ischias) axonale degeneratie waargenomen bij F344-ratten blootgesteld aan acrylamide in drinkwater gedurende twee jaar en (ii) neoplastische effecten waarvoor een $BMDL_{10}$ van 0,17 mg kg^{-1} lg dag^{-1} werd berekend, dwz de laagste $BMDL_{10}$ uit gegevens over de incidentie van Harderiaanse klieradenomen en adenocarcinomen bij mannelijke B6C3F1-muizen blootgesteld aan acrylamide gedurende twee jaar. Voor beide referentiepunten werd de MOE berekend met behulp van de blootstellingsgegevens van de gemiddelde en maximale concentratieniveaus. Voor neurotoxische effecten waren alle MOE's ruim boven de 100, dus kan worden geconcludeerd dat blootstelling aan acrylamide geen reden tot bezorgdheid is met betrekking tot neurotoxische effecten. Voor de neoplastische effecten waren echter bijna alle MOE's aanzienlijk lager dan 10.000, maar er moet worden geconcludeerd dat hoewel de beschikbare studies bij mensen nog niet hebben aangetoond dat acrylamide kankerverwekkend is voor de mens, de MOE's gebaseerd op de huidige niveaus van blootstelling van de voeding aan acrylamide duiden op bezorgdheid met betrekking tot neoplastische effecten.

De resultaten van dit onderzoek zijn volledig in overeenstemming met de conclusies van EFSA.

INTRODUCTION AND AIMS OF THE PROJECT

Introduction

Acrylamide (AA) is a chemical substance that has been classified by the International Agency for Research on Cancer (IARC) as potential carcinogen to humans (Group 2A) based on its carcinogenicity in rodents. This classification was endorsed by the WHO Consultation in 2002 and EFSA has confirmed in 2015 that AA is a carcinogenic and genotoxic substance. Due to the genotoxicity aspect, EFSA has established a Benchmark Dose Lower Confidence Limit (BMDL10) for tumors at 0.17 milligrams per kilogram body weight per day (mg kg^{-1} body weight (bw) day^{-1}) and for other effects a BMDL10 of 0.43 mg kg^{-1} bw day^{-1} [1].

AA has probably always been present in cooked foods, however, its presence in foods was first reported by the Swedish National Food Administration (SNFA) in 2002. Since a lot of research has studied the various aspects of AA formation, it is currently well characterised that AA formation in food comes from multiple routes (**Figure 1**) linked to the Maillard reactions, with the asparagine pathway as the most prominent route.

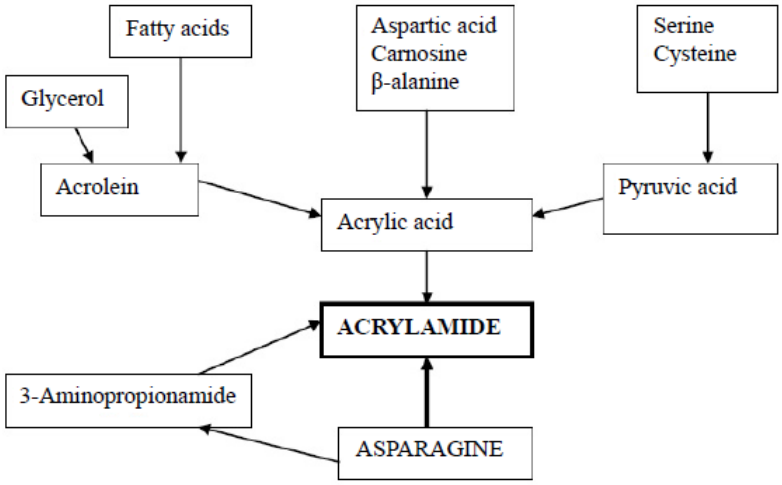

Figure 1. AA formation mechanism. Adapted from Eriksson (2005) [2].

AA formation from asparagine requires the presence of a reactive carbonyl substance such as reducing sugars (glucose, fructose and maltose) which are the starting point of multiple reaction cascades leading to AA as can be seen in **Figure 2**.

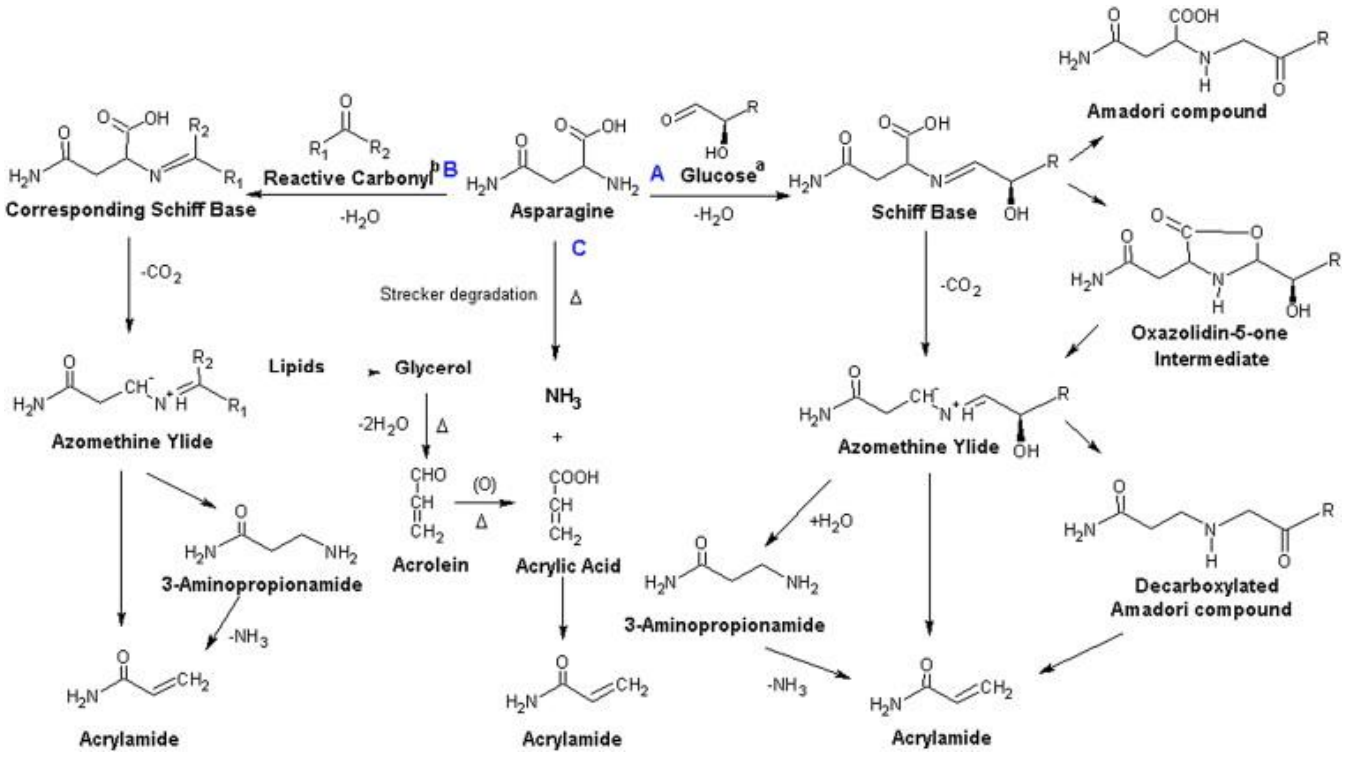

Figure 2. Mechanisms for the formation of AA based on asparagine. From Cheng et *al.* 2013 [3].

These different formation pathways illustrate the complexity of AA formation in food. In addition, the rate and scale of AA formation depends on many key factors that are described below.

Asparagine content :

The concentration of the major precursor asparagine in the raw materials impacts the magnitude of AA formation. Asparagine is an unusual amino acid, produced by different plant species during normal physiological processes (e.g. seed germination), but can be induced by environmental stresses such as exposure to toxic metals, pathogen attack, drought and nutrient deprivation. Sulphur deprivation has a drastic effect on free asparagine concentration in wheat and barley grain and this is exacerbated when nitrogen supply is increased [4].

Baking temperature and water content :

Cooking temperature is the second most influential parameter as boiling and steaming do not typically influence AA production [4]. This indicates that higher temperatures and/or low moisture conditions are needed for AA formation. In this respect, elevated baking temperature (higher than 120 °C) and/or extended baking time increase AA levels in the product, while water content affects the chemical route and the mechanism pathway of AA formation. For example, fructose is more reactive than glucose for AA formation during heating due to its lower melting point (126 °C, compared to glucose (157 °C). In addition, when the moisture content decreases during the baking process, sugars initially dissolved in water begin to crystallize. After crystallization, melting is required to change their state to liquid, in order to make them chemically reactive. Hence, reducing sugar, having a lower melting point, is expected to form AA earlier during heating.

Influence of pH :

Another influential parameter of AA formation is the pH. When acids are added to the preparation, the rate of AA formation is drastically reduced. However, when citric acid is used, excessive hydrolysis

of sucrose takes place which increases the concentration of reducing sugars, that in return favors AA formation.

Presence of cations :

Addition of mono and divalent cations (Na^+ and Ca^{2+} or Mg^{2+}) to products before cooking, showed a remarkable effect on AA reduction. It has been shown that treatment of potato slices with Ca^{2+}, reduces AA formation during heating. Cations would prevent the formation of Schiff base, which is the key intermediate leading to AA. Very recently, results from Komprda et *al.* (2017) [5] demonstrated that replacing the leavening agent ammonium hydrogen carbonate (NH_4HCO_3) with baking powder ($NaHCO_3$) in conjunction with calcium cation and acidifying agent (citric acid) decreased AA content by 47-86 % in gingerbread without impacting the sensory quality.

Hence, AA concentration in foods ultimately depends on both compositional and process variables which need to be controlled in order to decrease its levels. The Confederation of the Food and Drink Industries of the EU summarized the research progress on the reduction of AA and listed the parameters that influence the final level of AA, creating the "CIAA Toolbox" in 2005 and has been continuously updated with scientific developments. The latest version of the toolbox was published in 2013 with mitigation tools only for products with higher risk of AA formation based on the CODEX code of practice for reduction of AA in foods (CAC/RCP 67-2009). However, with the Regulation (EU) 2017/2158, the mitigation measures relevant to food businesses are now set out in the Annexes I and II of the legislation. Currently, indicative AA values are set in the EU Recommendation (EU) 2013/647, and exceedances should trigger an investigation to resolve and understand the problem. These values will be replaced by benchmark levels which are performance indicators used to verify the effectiveness of the mitigation measures and when the benchmark level is exceeded the producer shall review the mitigation measures applied and adjust processes and controls with the aim to achieve levels of AA as low as reasonably achievable below the benchmark levels. The Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of AA in food was implemented on April,11 2018. An overview of the (indicative) benchmark levels is given in **Table 1**.

Table 1. Benchmark levels from the Regulation (EU) 2017/2158 for certain food categories

Food categories	Benchmark level [µg kg^{-1}]
French fries (ready-to-eat)	500
Potato crisps from fresh potatoes and from potato dough Potato-based crackers Other potato products from potato dough	750
Soft bread	
a) Wheat based bread	50
b) Soft bread other than wheat based bread	100
Breakfast cereals (excl. Porridge)	
- *Bran products and whole grain cereals, gun puffed grain*	300
- *Wheat and rye base products (1)*	300
- *Maize, oat, spelt, barley and rice based products (1)*	150
Biscuits and wafers	350
Crackers with the exception of potato based crackers	400

Crispbread	350
Ginger bread	800
Products similar to the other products in this category	300
Roast coffee	400
Instant (soluble) coffee	850
Baby foods, processed cereal based foods for infants and young children excluding biscuits and rusks (3)	40
Biscuits and rusks for infants and young children (3)	150

(1) Non-whole grain and/or non-bran based cereals. The cereal present in the largest quantity determines the category.

(2) the benchmark level to be applied to coffee substitutes from a mixture of cereals and chicory takes into account the relative proportion of these ingredients in the final product.

(3) As defined in Regulation (EU) No 609/2013.

Situation of the problem

Although this Regulation entered into force on April 11, 2018, it is recognized that the number of data available on the presence of AA in the foodstuffs covered by the Regulation (EU) 2017/2158 is insufficient [6]. In addition, it does not include all the food categories that may contain significant quantities of AA and which could potentially contribute to the total dietary exposure to AA. To address this shortcoming, the European Commission published Recommendation (EU) 2019/1888 [6] concerning the monitoring of the presence of acrylamide in certain foodstuffs. The foodstuffs included in this Recommendation are listed in **Table 2**.

In parallel, EFSA also issued a call on 25 October 2019 [7] to collect data at European level on the AA contents of foodstuffs based on chia seeds or chai flour and which have undergone a cooking process. This call echoes an EFSA scientific opinion of March 2019 [8] stating that partial substitution of wheat flour with chia flour and the use of chia seed in bread and cookies may lead to higher levels of AA in the final product. This study is also relevant for the ongoing discussions on Novel Food Regulation (EU) 2015/2283.

Table 2. List of food for monitoring of the presence of acrylamide as presented in the Recommendation (EU) 2019/1888

Food
Potato products
- ***Rösti*** - ***Croquettes, pommes duchesse, pommes noisettes, …*** - ***Potato casserole (and vegetable casserole)*** - ***Potato and meat meal/cheese meal***
Bakery products
- ***Rolls (hamburger rolls, whole wheat rolls, milk rolls, …)*** - ***Pita bread, Mexican tortillas*** - ***Croissant*** - ***Doughnuts***

- ***Specialty bread (pumpernickel, ciabatta with olives, onion bread, …)***
- ***Pancakes***
- ***Crisp cookies from thin strip of dough and deep fried***
- ***Churros***

Cereal products

- ***Rice and maize crackers***
- ***Cereal snacks (such as extruded maize and/or wheat products)***
- ***Honey roasted muesli***

Other

- ***Vegetables crisps/fries***
- ***Roasted nuts and oilseeds***
- ***Dried fruits***
- ***Roasted coca beans and derived cocoa products***
- ***Olives in brine***
- ***Coffee substitutes not based on chicory or cereals***
- ***Fudge, caramel, nougats, …***

Aim of the project

The primary aim of this project is to collect missing data on AA content for various food categories targeted by Recommendation (EU) 2019/1888 and the EFSA call [6, 7]. The second aim is the identification of potential food categories with abnormally high or unexpected AA concentrations. These data will be combined with the National food consumption survey of 2014 performed by Sciensano (FCS 2014) [9]. This will allow to further complete the existing exposure assessment of the Belgian population to AA. Consequently, a more profound risk assessment for the different sub-populations according to their age and diet will be carried out. Hence, the competent authorities will receive updated, allowing them to implement appropriate measures.

Two additional studies have also been carried out in parallel. The first study evaluated the impact of different cooking methods on the production of AA in food. For this, the same sample has been cooked using two different cooking modes (fryer and oven) before determining the AA content. The purpose was to identify the impact of the cooking method on the production of AA. Another study investigated the Belgian cooking habits at home. Participants have been recruited and were asked to cook food (e.g. croquettes, sweet potato fries, breads) according to their own cooking practices in order to evaluate the impact of the Belgian cooking habits on the AA content and the corresponding potential health risks.

PROJECT OUTLINE AND PLANNING

The project has been divided in 5 working packages. Brief description and planning of these working packages are given in the Gant chart below. Exhaustive details are given in the section Materials & Methods.

	October	November		December	January	February	March	April	May
WP1 Sampling									
T1.1 *Sampling Plan development*	▮	▮							
T1.2 *Consultation with the client and validation of the sampling*	▮	▮							
T1.3 *Collection, preparation, grinding & storage of samples*		▮	▮	▮					
WP2 Sample analysis			▮	▮	▮	▮			
WP3 Exposure assessment						▮	▮		
WP4 Coordination & communication									
T4.1 *Data processing, creation of database tables and writing of reports*		▮	▮	▮	▮	▮	▮	▮	▮
T4.2 *Organisation & consultation with the steering comitee*	▮	▮	▮	▮	▮	▮	▮	▮	▮
WP5 Table with results in SSD2 format			▮	▮	▮	▮	▮	▮	▮

Key events

Project Approval: 07th of October 2020

Kick-off meeting: 29th of October 2020

Intermediate report submission: 09th of December 2020

Intermediate meeting: 10th of December 2020

Meeting on the management of WP3: 02nd of February 2020

Final report submission: 31st of May 2020

Final meeting: 10th of June 2020

MATERIALS & METHODS

Sampling & Sample management

1 FOOD CATEGORY SELECTION

The food category's choice has been based on Recommendation (EU) 2019/1888 on the monitoring of the presence of AA in certain foods [6] that are not covered by the Regulation (EU) 2017/2158, suspected to contain AA and for which a few or no data are available. Recently, an EFSA opinion, published in 2019, mentioned that certain food categories containing chia seeds (biscuits) may lead to substantial increase in AA content. The same phenomenon has been observed with food for which wheat flour has been partially substituted with chia flour and baked at 190°C [8]. Hence, food commodities containing chia seed have been added to the sampling plan (e.g. cookies, breads, crisps). Finally, sweet potato based products have been added as it seems that this kind of potato can produce higher level of AA during heating process compared to traditional potatoes.

2 NUMBER OF SAMPLES PER FOOD CATEGORY

Number of samples per food category was defined based on the National food consumption survey (FCS2014) [9]. A higher number of samples was attributed to food the most frequently eaten compared to others. Finally, the availability of the on the Belgian market was also taken into account as a parameter to modulate the quantity of samples per food category.
After presenting the first results for the food categories maize waffles, rice waffles and crackers, the FPS Health, Food Chain Safety and Environment noted that this kind of food is frequently given to children and benchmark levels are not the same for this food category depending if they are consumed by adults or children [10]. That's why, it has been proposed to analyze a sample of the same food category specifically attended to children. Hence, 2 samples of waffles and crackers for children have been added to the sampling plan in order to check if there were significant difference regarding their AA content. As the sampling phase has been done during the period of Christmas, Federal Agency of Safety of the Food Chain (FASFC) proposed to add some puff pastries also known as "petits-four" or "zakouskis". Accordingly, 3 samples of puff pastries have been added to the sampling plan.

Additional samples were planned in the project in order to carry out a comparative study between the different cooking modes (fryer versus oven). To this end, 13 potato-based samples have been baked with 2 different cooking modes. The purpose was to check whether there is a significant difference in terms of AA production according to the cooking method considered.

3 SELECTION OF BRANDS AND PURCHASING OF THE SAMPLES

The sampling was carried out by favoring the representativeness of the food products available in Belgium and according to the habits of consumers. Based on data from the Euromonitor report [11], brands the most frequently purchased by the Belgian population has been selected. In this database, all distributor brands are mixed together in the same category. To this category in particular, brands have been selected depending on the market shares in Belgium. The selected supermarkets were Colruyt, Delhaize and Carrefour, representing 72.1% of the market for non-private brands. Specific stores, such as bakeries, organic stores (Bio-planet, Bepositive and Bio bon), restaurants, chip shops were also included to reflect as much as possible Belgian consumption habits while respecting the

requirements of Rcommendation (EU) 2010/307 [12]. Particular care has been taken to include products bearing the organic label for each food category.

4 GRINDING, ENCODING & STORAGE OF SAMPLES

Once samples were bought, all relevant information was encoded in an Excel file. Among others, date of purchase, name, brand, store of purchasing, food category, bio food, cooking step necessary, specific ingredient relevant regarding the presence of AA were captured. Then, pictures of the package were taken containing the abovementioned information and other relevant information for the project or for the competent authority such as, ingredients, barcodes, batch number, cooking instruction and bio label (if available). This information simplifies the coding of results in SSD2 format and the reporting of samples to competent authorities in case of abnormal AA content that may be a risk for the consumer. Next, the samples were grinded and homogenized with a blender according to their size. For small food items, the whole sample was grinded. For larger samples, the samples were first homogenized and only half of the sample was grinded. Finally, samples were labelled and stored in a cold room at -20 °C until their analysis. Samples will be stored in a cold room during 6 months after the end of the project. All pictures of the samples are included in annex 1.

5 COOKING OF SAMPLES

As mentioned in Recommendation (EU) 2010/307 and (EU) 2013/647 [12, 13], food samples should be analyzed as ready to eat. In the case of samples requiring a cooking process (e.g. croquettes, fries, etc…) samples will be prepared according the manufacturer's instruction present on the packing label [12]. In the case of different cooking modes were indicated (e.g. oven, fryer, microwave, toaster), one cooking mode has been chosen in order to obtain a representativeness of the different cooking modes at the level of each sample group. If a cooking method is preferably indicated on the label, this cooking method will be chosen.

In this project, three different cooking modes have been used: fryer, oven and microwave. For the frying process, a professional fryer has been used. The temperature was set on 175 °C and controlled regularly with an adapted probe. For the oven and the microwave cooking, as no professional equipment was available, recent domestic oven and microwave have been used on which the correct temperature (°C) or the adequate power (W) were reachable. Afterwards, pictures of cooked samples have been taken before grinding. All pictures and cooking protocols (time, temperature, power, cooking mode) used for each sample are given in annex 2.

6 STUDY OF CONSUMERS COOKING HABITS :

A preliminary study on the cooking habits of the Belgian population has also been conducted. The purpose was to have an idea of the AA content generation during the food preparation by consumers according their cooking habits. Indeed, as mentioned by an EFSA opinion on AA, certain home cooking practices for fried potato products can increase total exposure to AA up to 80% [1]. Based on the Belgian consumption and level of AA usually found, croquettes have been chosen as representative for potato base products as it is the most consumed food in this category after fries [9]. Sweet potato fries have also been added to this preliminary study due to the higher amount of AA that can be produced in comparison with classical fries and due to the increase of its availability on the market and fast-food since recent years. Twelves croquettes, 8 sweet potatoes fries cooked by consumers themselves, recruited among the staff of Sciensano. Four buns have also been cooked by participants with a toaster

in order to check the AA level before and after this heating processing. For each food group included in this study, a same batch of product has been given to all participants in order to avoid bias due to inter-batch variability that may lead to differences in AA content.

Participant recruitment:
Emails containing an invitation to cook different kind of samples (croquettes, sweet potato fries and/or buns) have been send to different services of Sciensano. It has to be noted that the services were selected on the basis that their personnel were not aware of the existence or purpose of this study to avoid any bias related to the cooking behavior. Indeed, similar studies reported bias on the behavior of cooking such as under cooking of food items if participants are aware of the compound incriminated and its effect on the human health [14]. For staff members that agreed to participate, they were asked to fill in an Excel File in order to indicate which type of food they wanted to cook which cooking devices were available at home (fryer, oven, microwave, toaster). Then a number has been randomly attributed to all participants in order to avoid diffusion of their personal information.

Food samples management:
Once recruitment was done, food items were bought and a particular attention was paid to buy food from the same batch, when possible. Then, food items were given to all participants with an isotherm bag to avoid degradation of food and/or AA during transportation between laboratory and participant's residence location. They were asked to cook foods as they usually do, then fill a questionnaire on their cooking habits and cooking parameters such as: temperature, time, type of cooking or if they usually respect preparation indications on the labels of the product, then to store a cooked portion in their freezer. Questionnaires and filled questionnaires are all available in annex 4 & 5. After cooking, samples were returned to the laboratory and stored in a cold room at -20 °C until grinding. Pictures of the food items have been taken before grinding and encoding according to the procedure described in chapter 4.

Samples analysis

Samples were analyzed by an « in-house » developed and validated method based on Quechers extraction. This universal and simple extraction method is suitable for the wide variety of food included in this project. Extracts were then purified using a dispersive solid phase extraction (dSPE) with an adapted sorbent composition for each food type. Complex matrices such as coffee and chocolate powder have been analyzed with a more specific purification step based on mixed-mode SPE cartridges. Finally, purified extracts were filtered and then analyzed by liquid chromatography coupled with a triple quadrupole mass spectrometer (LC-MS/MS). Detailed protocols are provided in the following paragraphs.

1 MEHOD VALIDATION

The validation strategy used was to first classify food commodities into different categories according to their main constituents (protein, fat and water proportion). Then primary or full validation has been performed for one subgroup of each food category. Secondary validations have been performed for each other subgroups of this food category (see **Figure 3**). At least two operators have been involved for each validation.

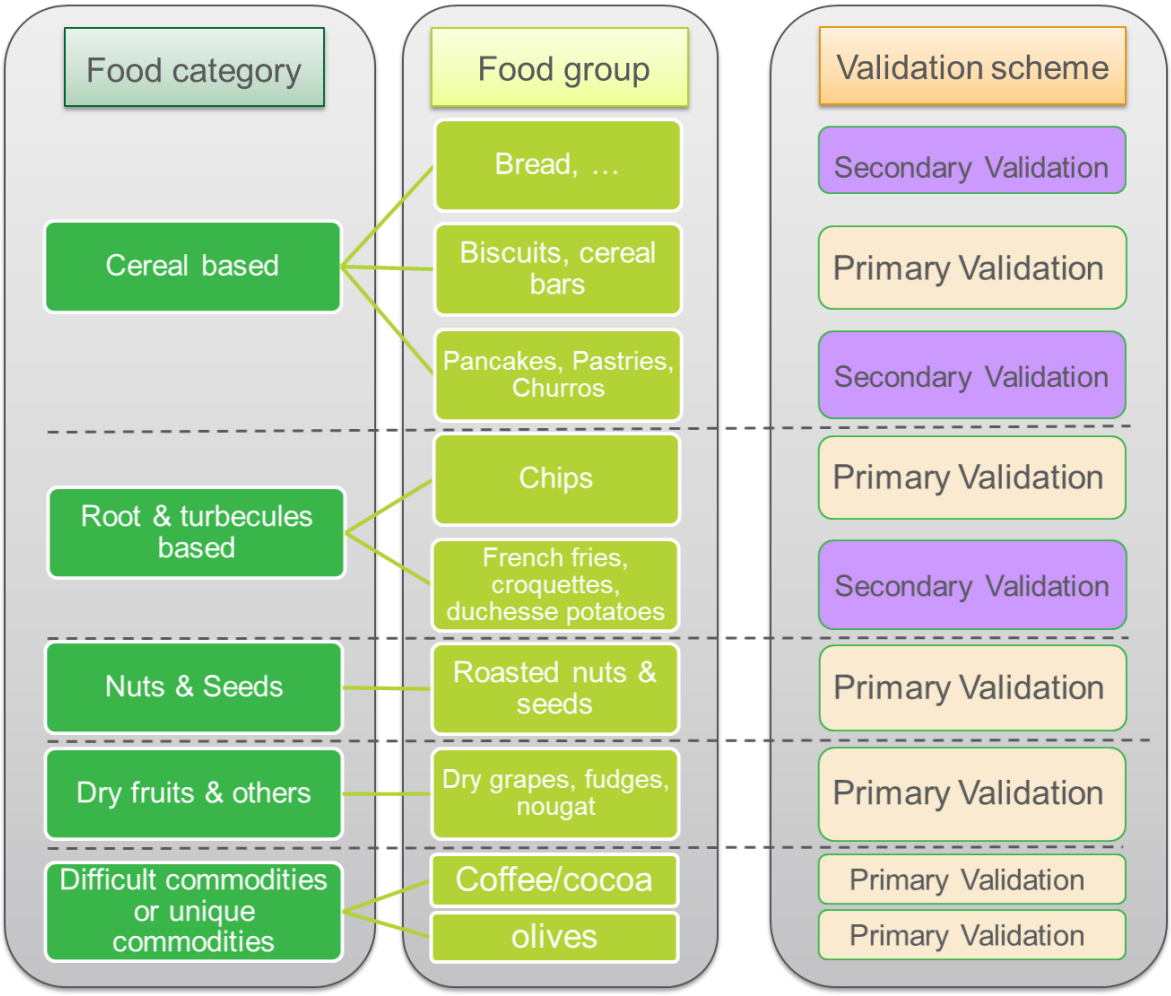

Figure 3. Food categories and associated validation scheme

1.1. Validation scheme

Validation schemes used were different for primary and secondary validations. Fortification levels have been based on the benchmark levels from the Regulation (EU) 2017/2158. If no benchmark levels were available for a food group, fortification levels have been chosen based on the typical concentration found in the concerned food matrix. As it is difficult to find food matrices without AA, the same food item without fortification of AA has been analyzed each day of validation. The value obtained was subtracted of value corresponding to the fortified sample in order to calculate the recovery.

Primary validation :

Primary validation was based on a 3 x 3 x 3 scheme: 3 concentrations, in triplicate, on 3 different days (see **Table 3**). Depending on the intra-food group variability, the same or different samples were taken. For instance, for the food category “dry fruits & others”, samples of nougat, dried grapes and fudges have been analyzed together for each validation day. This strategy was used to reflect as much as possible the variability within a food group to obtain a realistic measurement uncertainty.

Samples were fortified with an AA solution at different concentrations reflecting the range of AA that can be found in naturally contaminated samples (e.g. 50, 100 and 200 µg kg^{-1}). In the same batch, unfortified samples were also analyzed. Unfortunately, in some cases, it was impossible to find samples that were free of AA. Therefore, results were corrected for the concentrations found in the unfortified samples (pseudo-blank samples) in order to correctly calculate the AA recoveries.

Table 3. Primary validation scheme

	Without spiking	50 µg kg^{-1}	100 µg kg^{-1}	200 µg kg^{-1}
Day 1	1x	3x	3x	3x
Day 2	1x	3x	3x	3x
Day 3	1x	3x	3x	3x

Secondary validation :

Secondary validation was based on a 1 x 3 x 3 scheme: one concentration, in triplicate on 3 days Samples were fortified with an AA solution at different concentrations based on matrix type and the AA content that is usually observed. Pseudo-blank samples were also analyzed in order to subtract AA already present to the fortified value. Again, when relevant, different matrices of the same food category have been analyzed in order to take into account the variability of this group. For instance, for pastries, pastries filled witch vanilla cream, croissant and chocolate pastries have been analyzed the same day to reflect as much as possible the variability of this group.

Finally, to assess the trueness for each food category, Proficiency Tests (PTs) from European Reference laboratory for processing contaminants (EURL-PC) have been analyzed. When no PTs were available for a food category, a second line of control was analyzed: a second analyst fortified pseudo-blank samples with an AA concentration unknown by the first analyst. The match between the fortification level and the result found by the first analyst (measurement uncertainty included) demonstrates the good method trueness.

1.2. Performance criteria

The Regulation (EU) 2017/2158 established some performance criteria for analytical method dedicated to AA determination in food. When there was no criteria for a parameter to be validated, criteria from the SANTE/12682/2019 have been used. The use of this guidance document has been considered relevant, as it used in the laboratory for many years for the validation of analytical methods designed for the determination of pesticides content in food at the same range of concentration, in similar matrices and with the same kind of detector. For the determination of uncertainty, the ISO 5725-2 norm was followed. The **Table 4** shows all criteria assessed during validations and acceptance criteria.

Table 4. Parameters assessed and acceptance criteria

Parameter	Acceptance criteria
Trueness	Results +/- uncertainty measurement match spiked value
Recovery	75-110% (Regulation (EU) 2017/2158)
Repeatability	0,66 times RSDR as derived from (modified) Horwitz equation (Regulation 2017/2158)
Reproductibility	As derived from (modified) Horwitz equation (Regulation (EU) 2017/2158)
Intra-reproductibility	< from (modified) Horwitz equation (Regulation (EU) 2017/2158)
Limit of detection	< 7 µg/kg (Regulation (EU) 2017/2158)
Limit of quantification	< 20 µg/kg (Regulation (EU) 2017/58)
Linearity	Residual ≤ ± 20% (SANTE/12682/2019)
Range	25-1000 µg/kg (up to 4000 µg/kg for coffee substitute)
Selectivity	Blank matrix, S/N<3

Specificity	RRT ≤ 0.1minute and follow up at least 2 transitions. Difference between these 2 ions ratios ≤±30% between the standard and the sample (SANTE/12682/2019)
Matrix effect	Difference between the slope of the sorbent line and the slope of matrix line < |20|% (SANTE/12682/2019)
Expanded uncertainty	Assessed based on ISO 5725-2

2 SAMPLE EXTRACTION :

2.1. Samples other than cocoa and coffee substitute

1.00 g ± 0.01 g of grinded and homogenized samples was weighed in a 50 mL Falcon tube. Samples were fortified with 25 µL of a 10 µg mL^{-1} deuterated AA (AA-d3) solution. Ten mL of ultrapure water and 10 mL of acetonitrile were added, then 0,5 g of NaCl and 4 g of $MgSO_4$. Samples were shaken vigorously by hand for one minute. Finally they were centrifuged at 3900 rpm for 5 minutes.

2.2. Cocoa and coffee substitute samples

1.00 g ± 0.01 g of blended and homogenized samples was weighed in a 50 mL Falcon tube. Samples were fortified with 25 µL of a 10 µg mL^{-1} deuterated AA (AA-d3) solution. Ten mL of 60 °C water were added and samples were mixed for one minute. Then, 1 mL of Carrez I&II solutions and 5 mL dichloromethane were added and samples were vortexed for 10 minutes. Next, samples were centrifuged at 10000 rpm at 5 °C for 15 minutes. Six mL of supernatant were transferred in a new 50 mL Falcon tube and 13 mL of ethyl acetate was added. Samples were vortexed for 10 minutes and then centrifuged at 3900 rpm for 10 minutes. Supernatant was transferred in a new 50 mL falcon tube containing 1 mL of water. Ethyl acetate extraction was repeated a second time and supernatant was transferred in the Falcon tube already containing the organic phase from the first extraction. Next, the organic phase was evaporated to 1 mL under nitrogen stream at 40°C.

3 SAMPLE PURIFICATION :

3.1. Samples other than cocoa and coffee substitute

Purification based on dSPE has been used: Five ml of supernatant was transferred in a 15 mL Falcon tube and sorbents were added depending the matrix to be analyzed (see **Table 5**).

Table 5. Purification method based on food matrix

Food category	Exemple	Sorbents
Cereal based	Maize/rice crackers	300 mg PSA & 300 mg C18
Pastries	Croissant/Chocolate croissant	300 mg PSA & 300 mg C18
Pancakes		300 mg PSA & 300 mg C18
Root tuber & vegetables based	Potato crisps/vegetable crisps	150 mg PSA
Fried potato products	Potato croquette/Duchess potatoes	150 mg PSA
Black olives		150 mg PSA

Oil seeds and nuts	Sunflower seeds/sesame seeds/roasted nuts	150 mg PSA
Foods high in sugar	Dried grapes/caramel/nougat	150 mg PSA

Falcon tubes were then shaken vigorously by hand for one minute and centrifuged at 3900 rpm for 1 minute. Four mL of supernatant was transferred in a new 15 mL Falcon tube and evaporated to dryness under nitrogen stream. Next, 2 mL of ultrapure water was added and 500 µL of the solution was filtered by a 0.2 µm PVDF auto-filtration vial before LC-MS/MS analysis.

3.1. Cocoa and coffee substitute samples

Multimode SPE cartridges (strong cation exchange and strong anion exchange) were first conditioned with 3 mL of methanol, then equilibrated with 3 mL of milliQ water. Four hundred µL of sample was loaded on the cartridge in order to discard water from the equilibration step. Then, another 500 µL was added to the cartridge and the eluate was collected in a Falcon tube of 15 ml. Finally, the eluate was filtered on a 0.2 µm PVDF auto-filtrating vial prior to LC-MS/MS analysis.

4 LC-MS/MS ANALYSIS :

Analyses were performed using an Acquity Ultra Performance LC® system coupled with Xevo® TQ triple quadrupole mass spectrometry conducted under MassLynx® v4.2 software. Settings are indicated in **Table 6**, **Table 7** and **Table 8**.

Table 6. Liquid chromatography settings

Liquid chromatography settings	
Chromatographic column :	Hypercarb from Thermo (100 x 2.1 mm, 5 µm)
Mobile phase flow :	0.4 mL min^{-1}
Oven temperature :	45 °C
Elution:	Isochratic: 99.4 % water, 0.5 % methanol, 0.1 % acetic acid
Injection volume :	5 µL

Table 7. Mass spectrometry settings

Mass spectrometry settings	
Ionisation mode	Positive electrospray ionisation(ESI^+)
Electrocapillary voltage	3.5 kV
Cone voltage	20 V
Source temperature	150 °C
Desolvatation gas temperature	550 °C
Cone gas flow	80 L/h
Desolvatation gas flow	800 L/h
Acquisition mode	Multiple Reaction Monitoring (MRM)

Table 8. Acrylamide and deuterated acrylamide transitions and collision energy

Molecules	Parent ion (m/z)	Daughter ion (m/z)	Collision energy (eV)
Transition 1	72.10	55.00	10
Transition 2	72.10	72.10	2
Transition 3	72.10	44.00	10
Transition AA-d3	75.00	58.00	10

5 QUALITY CONTROL :

During sample analysis, a quality control program was established to ensure data accuracy and reliability. For this purpose, the following items were included in each batch of analysis.

- A **calibration curve** was injected at the beginning of each batch of analysis. The lowest calibration point equal to the limit of quantification (LOQ) was used to ensure the sensitivity. This point was reinjected at the end of each batch to make sure that no loss of sensitivity nor instrumental deviation occurred during the batch.
- A procedural **blank** was analyzed for each batches of analysis in order to verify that no AA contamination occurred, either at the level of the extractions or at the level of the LC-MS/MS system.
- A **control sample** was spiked at the appropriate dosage according to food matrix and was analyzed with each batch to monitor AA extraction efficiency. These results were plotted on a **control chart** to be sure that no method deviation occurred.

Exposure assessment

1 OCCURRENCE DATA

In order to perform the intake and risk assessment, the data on dietary intake and analytical concentration levels had to be coupled.

1.1. Standardization and coupling of data

The evaluation of the exposure has been realized using national representative food consumption data of the FCS2014 (for ages between 3 and 64 years). The objectives, concept and methodology of the food consumption survey have been described elsewhere [15].

Dietary assessment in adolescents and adults (> 10 years) was performed by the 24-h dietary recall method, carried out on two non-consecutive days, using GloboDiet© (former EPIC-Soft), a computerised 24-h recall program. Dietary assessment in children (3 to 9 years old) was done using two self-administered non-consecutive one-day food diaries followed by a GloboDiet completion interview with a proxy respondent. Pre-defined coded lists of foods, recipes, facets and descriptors are used in Globodiet©. Facets and descriptors describe foods and recipes in more details. Facets characterize different aspects of the dietary item such as the cooking method used, brand name and preservation method. Descriptors are pre-defined answers for the facets, e.g. grilled, fried or boiled for the facet ‘cooking method’ [16].

Food categorization in the project

Regarding the project objectives, which was evaluation of additionally defined food products to contain AA, the food groups were merged by the stakeholder and according to the Recommendation of (EU) 2019/1888 in the groups given in Table 2. List of food for monitoring of the presence of acrylamide as presented in the Recommendation (EU) 2019/1888Table 2 of this report. This type of sampling may be considered as selective due to the fact the food targets were defined prior to evaluation of the exposure.

1.2. Laboratory concentration levels

Each food product with an analytical concentration level was manually linked to a corresponding food item of FCS2014 food consumption database. For each food item analytical concentration and for the group of same products, the maximum and mean analytical concentrations are noted in annex 8.

2 INTAKE ASSESSMENT

2.1. Methodology

Data analysis and validation

The data analysis was performed following the principles of EFSA data analysis. The input data sets were cleaned, refined and validated prior to exposure assessment analyses. The left censored data were treated following the lower and upper bound approach. As recommended in international guidelines [17, 18], substitution method was used for the treatment of results below the LOD/LOQ. Under the lower bound (LB) scenario, occurrence values below the LOD/LOQ were set at 0, while under the upper bound (UB) scenario, occurrence values below the LOD/LOQ were set at the LOD/LOQ respectively.

Intake Assessment

The intake and risk assessments were performed for the Belgian population aged 3-64 years (children, adolescents and adults) using the FCS2014 food consumption database. Only respondents with two completed 24-h dietary recalls and available measured body weight were included in the exposure assessments (FCS2014: n=3096; 1529 men and 1567 women).

To assess the long-term average intake from these short-term measurements, the data required statistical modelling in order to take into account between-person and within-person variations. The daily habitual intake distributions were estimated by the Statistical Program to Assess Dietary Exposure (SPADE) [19, 20]. SPADE is freely available as an R package called SPADE.RIVM. The habitual intake distribution is modelled as a function of age. Uncertainty in the habitual intake distribution was quantified with ready for use bootstrap (n=1000) which provided confidence intervals with the required confidence level [19, 20]. The 2-part model for episodical-consumed food components was used because AA was not consumed daily by all of the subjects. To ensure representative results for the Belgian population and for the different seasons and interview days (week versus weekend days) weighting factors were used. The usual intake distribution was weighted for age, sex, province, season and day of the week.

Acute scenarios were excluded on the ground of toxicological assessment. Indeed, various studies reported Oral LD_{50} values for AA varying from 107 mg kg^{-1} bw day^{-1} for mice to >150 mg kg^{-1} bw day^{-1} for rats [1]. However, humans will never be exposed to such high concentration via food. Therefore, no ARfD or Reference point for an acute effect has been established.

Exposure scenario's

The exposure scenario describes the circumstances of the exposure. In defining the exposure possible consumption patterns regarding the food selected in the project were taken into account (high consumers and average consumer). In the scope of the project, the distinction of the source of acrylamide (baking, frying and microwaved preparation) and level of contamination were taken into

account for the food group defined in the project potato based products, in particular ready-to-eat dishes (“hachis parmentier” and gratinated potato dishes).

Scenario 1: Exposure scenario using the maximum analytical concentration (MAC)

Firstly, a maximum analytical concentration exposure assessment scenario was performed using actual food consumption data combined with the maximum actual (measured) concentration of AA. This scenario is considered to be the most conservative scenario as it assumes that the consumer will be continuously (over a lifetime) exposed to these contaminants present in foods at the maximum observed level. The individual intake of AA was estimated using the following equation:

$$Y_i = \sum_{k=1}^{n} \frac{X_{k,i} \times C_k}{bw_i}$$

where: Yi is the daily AA intake of a given individual i (ng kg^{-1} bw day^{-1}); n is the number of food items containing the AA, bw_i is the measured body weight of a given individual i (kg); $X_{k,i}$ is the amount of the food k consumed on that day (g day^{-1}); Ck is the detected amount of AA in the food item k (ng g^{-1}).

Scenario 2 : Mean exposure scenario for both LB and UB (MeAC)

Secondly, a mean analytical concentration (MeAC) exposure assessment scenario was performed using actual food consumption data combined with the mean actual (measured) concentrations for each food category. This scenario is considered to be a more realistic scenario for the chronic intake of AA. The equation as described above has been used.

Food groups contributing to the total AA exposure of the general population

The contribution of the different food categories based on the FoodEx2 hierarchy to the estimated total exposure was calculated. It has to be noted that the food groups defined in the project are not directly compatible with FoodEx2 categories. However in view of the exposure assessment, food products for which occurrence data were provided were assigned to different FoodEx2 levels and groups and contribution to exposure was calculated on the level 1. These calculations included only information from the first 24-h dietary recall. For each individual the proportion between the consumed quantity of the contaminant for a specific food group and the total consumed quantity on that day was determined. A weighted mean was calculated to estimate the mean contribution of food categories to the total exposure for the whole Belgian population.

2.2. Uncertainties

The inherent uncertainties in the risk assessment on AA have been noted and listed. Their evaluation was descriptively performed below.

Sources of uncertainty

The assessment objective was specific and was focused only on the selected food products. Some of the food products analysed in the project were less frequently consumed. These less frequently consumed products would be excluded from the analyses of chronic exposure if data are not available for both consumption days.

In the exposure scenario both upper bound and lower bound approach were considered. For the lower bound approach, the non-detected levels (“ND”) (levels below the LOD) were replaced by zero values.

Detected but not quantified levels i.e. analytical results below the LOQ were set equal to zero also. For the upper bound approach these values were set to LOD value (7 µg kg^{-1}) or to LOQ value (20 µg kg^{-1}) respectively. Missing intake and concentration values on the individual level were also replaced by zero, to avoid a missing total daily additive intake which is not allowed by SPADE. The use of maximum analytical concentration data is a certain factor of overestimation, but on the other hand the overestimation is limited as the linkage was done on the level of the food item with the necessary facets.

Type of uncertainty

Estimating the contribution of the different food categories was performed based on the FoodEx2 hierarchy to the estimated total exposure. These calculations included only information from the first 24-h dietary recall.

Several factors may influence the uncertainty of the dietary exposure assessment, such as under- or over-reporting of food consumption data, misreporting of consumed foods and wrong estimation of consumed quantities. This can contribute to an under- or overestimation of food consumption and affect the exposure assessment.

Evaluation of the exposure on the level 4 of FoodEx2 hierarchy was excluded, since this gave high overestimation of the exposure. Furthermore the classification was evaluated on the food class defined in FCS2014.

Risk assessment

AA and its metabolite Glycidamide are positive in a variety of genotoxicity tests. This indicates that AA is of concern with respect to genotoxicity. Therefore, the EFSA CONTAM Panel considered it inappropriate to establish a tolerable daily intake (TDI) [1].

The risk characterization was performed using the Margin of Exposure approach (MOE) [1]. The CONTAM Panel considered the data from studies on experimental animals to establish the reference points. The CONTAM Panel performed benchmark dose (BMD) analyses on data for neurotoxicity and on the tumour incidences induced by AA in experimental animals. They selected the value of 0.43 mg kg^{-1} bw day^{-1} as the reference point (RP) for non-neoplastic effects. This RP was derived as the lowest $BMDL_{10}$ from the data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to AA in drinking water for two years. For neoplastic effects, they selected as a reference point the value of 0.17 mg kg^{-1} bw day^{-1}. This RP was derived as the lowest BMDL10 from data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F1 mice exposed to AA for two years. Next, the MOE can be calculated using the following formula.

$$MOE = BMDL/Estimated\ Exposure\ Dose$$

According to the EFSA Scientific Committee, for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view [21]. A MOE of 100 is usually considered sufficient for non-genotoxic compounds to conclude that there is no health concern, unless there are major gaps in the toxicological database. For the risk assessment, both MAC and MeAC scenarios were considered.

RESULTS AND DISCUSSION

Sampling Plan

Table 9 summarizes all the food categories analysed in this project, the number of samples that were purchased and the number of analysis performed per food category.

A total of 217 samples have been purchased on which 254 analysis have been performed:

- 217 foreseen to collect data of food categories targeted by the Recommendation (EU) 2019/1888
- 13 additional analysis to evaluate the impact of the cooking mode
- 24 additional analysis for the pilot study on the Belgian cooking habits

Table 9. Food included in the sampling plan and number of samples and analysis done by food category

Product	Number of samples	Number of analysis done	Remarks
Potato products	**39**	**72**	
Rosti	5	7	- including 2 cooked by 2 different cooking mode (oven / deep fryer)
Potato cubes	4	4	
Duchess potatoes	5	10	- including 5 cooked by 2 different cooking mode (oven / deep fryer)
Croquettes	10	23	- including 1 cooked by 2 different cooking mode (oven / deep fryer) - including 1 cooked by 12 consumers at home
Potato balls	5	9	- including 4 cooked by 2 different cooking mode (oven / deep fryer)
Dishes made with potatoes (shepherd's pie, gratin dauphinois)	5	5	
Sweet potato fries	3	12	- including 1 cooked by 2 different cooking mode (oven / deep fryer) - including 1 cooked by 8 consumers at home
Cone shaped potato (sup15)	1	1	
Vegetable fries (sup12)	1	1	
Fine bakery product	**53**	**57**	
Hamburger Buns, Bagel, milk bread	5	9	- including 1 cooked by 4 consumers at home
Pita bread, tortillas (wrap)	8	8	
Pastries : croissant ,chocolate croissant, butter pastry with raisins, etc	14	14	
zakouski	3	3	

Donut, cream donut	5	5	
Special bread (pumpernickel bread , olives bread, onions bread ….)	8	8	
Pancakes	5	5	
Churros – fairs, markets, etc…	5	5	From fast food (fritkot/foodtruck/other)
Cereal products	**53**	**53**	
Rice crackers	11	11	
Maize crackers	10	10	
Cereal snacks (extruded corn and wheat products)	21	21	
Honey roasted muesli	11	11	
Others	**52**	**52**	
Vegetable crisps	8	8	
Roasted nuts	10	10	
Roasted oil seeds	6	6	
Dried fruits	6	6	
Roasted cocoa beans and cocoa products	5	5	
Black olives in jars	5	5	
Coffee substitutes other than those based on cereals and chicory	7	7	
caramel, nougat, …	5	5	
Products containing chia seeds	**20**	**20**	
Seed bread, gluten-free bread	6	6	
Crisps	1	1	
Cookies	10	10	
Rusks	2	2	
Tortilla	1	1	
TOTAL	**217**	**252**	

Validation results

Linearity:

Six different concentrations (20, 50, 100, 250, 500 and 1000 µg kg^{-1}) were used for both solvent and matrix matched calibration curves to investigate the linearity of the detector response. For matrices such as coffee substitutes, two concentrations (2000 and 4000 µg kg^{-1}) were added as the contamination in AA may be higher in these food categories. For each matrix, residuals were all below |±20 %| when using the linear mode. Hence, the linear model has been chosen for the ease of use.

Matrix effect:

Slopes of matrix-matched calibration curves were compared with a calibration curve prepared in neat solvent in order to test the matrix effect. According to the SANTE document [22], it can be concluded that there is no matrix effect if slopes differs less than |± 20%|. Calculations were performed without and with correction of AA signal using deuterated labelled internal standards (AA-d3). **Table 10** shows the difference (%) between slopes of calibration curves in neat solvent and in matrix for one representative matrix for each food group. These data show that no matrix effect occurs based on the criteria |±20%| with the use of AA-d3 correction. Hence, calibration curves in neat solvent with AA-d3 were used for analysis.

Table 10. Difference (%) between slopes of calibration curves in neat solvent and in matrix with AA-d3 correction

Food group	Difference between slope with AA-d3 (%)	Matrix effect (Yes/No)
Babyfood cereals	3.1	No
Crisps	0.1	No
Fried potato products	0	No
Black olives	16.7	No
Pancakes	-3.4	No
Pastries	-3.3	No
Oil seeds and nuts	-3.6	No
High sugar	3.3	No

Detection and quantification limits:

LOD and LOQ have been set at 7 µg kg^{-1} and 20 µg kg^{-1} respectively [10]. LOD and LOQ were determined using a sample contaminated at a level close to the expected LOQ inducing a peak with a signal-to-noise (S/N) ratio at least of 3 for LOD and 10 for LOQ.

Precision and recovery:

For primary validations, precision was evaluated with fortified samples at three concentration levels during three different days with at least two different operators. The same approach was used for secondary validation but only at one concentration level. A Cochran test has been used to identify outlier's. The relative standard deviations for the repeatability (RSD_r) and the within laboratory reproducibility (RSD_{RW}) were calculated for the 3 levels as described by ISO 5725-2 guidelines. All RSD_r and RSD_{RW} values were well below the maximum demanded by the Regulation (EU) 836/2011 [23], which are fixed at 14.5 % and 22 %. A summary of results is shown in **Table 11** for primary validations and in **Table 12** for secondary validations. All recoveries (whatever concentration levels) are close to 100 % (from 91.7 to 106 %) thanks to AA-d3 standard. RSD_r and RSD_{RW} were both lower than 15 % even in the case where 3 different matrices have been used during each day of the validation.

Table 11. Recovery, precision data for primary validations

	Concentration spiked (µg kg^{-1})	Average recovery (%)	RSD_r (%)	RSD_{rw} (%)
Cereals in babyfood	25	97.6	3.7	6.6
	50	100	7.4	7.4
	200	106	4.7	4.7
Crisps	100	102	3.3	4.3
	250	102	3.5	3.8
	750	99.0	2.1	3.6
Black olives	50	97.3	12.4	13

	100	98.4	4.9	9.8
	200	103	3.6	4.6
Oil seeds and nuts	50	97.8	6.8	10.7
	100	98.0	4	4
	200	98.8	2.6	5.3
High sugar content	50	99.7	7.7	7.7
	100	91.7	9.4	10.5
	200	103	2.7	5.1

Table 12. Recovery, precision data for secondary validations

	Concentration spiked (µg kg^{-1})	Average recovery (%)	RSDr (%)	RSDrw (%)
Pancakes	100	99	1.4	1.7
Fried potato products	250	102	2.2	2.2
Pastries	100	101	3	4.7

Measurement Uncertainty (MU)

The expended uncertainty with a coverage factor of 95 % (k=2) has been calculated using the following equation U = |bias| + 2 RSD_{RW}. Both bias and RSD_{RW} data came from the control chart generated with all validation data and control samples analyzed during routine analysis.

The calculated expanded MU for this analytical method is 11.3 % and the practical expanded measurement uncertainty is set at 12 % .

Food analyzed as ready to eat or prepared in laboratory environment

A total of 217 samples have been purchased and analyzed in laboratory. They have been regrouped in 12 food categories depending on their nature. The following section summarized main observations made after samples analysis. Exhaustive details on the composition of samples and their related AA content can be found in annex 6. All mean AA content in this section have been calculated by considering results <LOD or <LOQ as "0". The LOD and LOQ are equal to 7 µg kg^{-1} and 20 µg kg^{-1} respectively.

1 BREAD

In the category 'Bread', 30 samples were included. They can be subdivided in 7 sub-categories (i.e. buns, milk breads, pita breads, tortillas, special bread, seed breads (including bread with chia seeds) and rusks). The special breads sub-category contained pumpernickel, sugar, olives or onions. The results are summarized in **Table 13**. AA was detected in only 20 % of the samples. Minimum and maximum values were respectively <LOD and 211 µg kg^{-1}, while an overall mean concentration of <LOQ was found.

Table 13. Acrylamide average and maximum content for the bread food category

	Number of samples	Mean AA content (µg kg^{-1})	Maximum AA content (µg kg^{-1})
Hamburger buns	3	<LOQ	22.9
Milk bread	2	<LOD	<LOD
Pita bread	4	<LOD	<LOD
Tortilla (wrap)	5	<LOD	<LOD
Special bread (e.g. containing olives, onions, etc…)	8	24.3	98.0
Seed bread, bread containing chia seeds	6	<LOQ	47.2
Rusks with chia seeds	2	105	211

AA was detected in none of the samples of milk bread, pita bread and tortillas, it was not even detected in the tortilla containing chia seeds. However, AA was found in hamburger buns, special bread, rusks and bread containing seeds. This is consistent with previous studies as illustrated in **Table 14**. In 2004, Croft et al analysed different types of bread and AA was only detected in multigrain, rye bread and pita bread at concentrations varying from 25 to 50 µg kg^{-1} [24], which is consistent with the concentrations found for special breads in this study. In the scientific opinion on AA of EFSA, published in 2015, all results are comparable to the results obtained in the current study, even the higher concentration found for rusks [1].

Table 14. Acrylamide average content for some bread type as reported by EFSA (2015) and *Croft et al.* (2004)

Results (µg kg^{-1})	EFSA, 2015	Croft *et al. 2004*
Bread, multigrain	43	25-50
Bread, rye	57	25-50
Bread, white		<25
Bread, wholemeal		<25
Wheat soft bread	33-44	
Mixed wheat and rye bread	43	
Biscuit and rusks	106-115	
Pita bread		25-50

Only the results of the special breads are slightly higher compared to previous studies as concentrations of 76.2 µg kg^{-1} and 98.0 µg kg^{-1} were found respectively for rye-bread and for bread containing olives. However, this could originate from the impact of many different factors such as cereal type, heat treatment, formulation, product composition (e.g. asparaginase, baking agents, mono- and divalent cations, % NaCl, pH, water activity, fermentation, additional ingredient such as almonds, sesame,…) and oven type on the final AA concentration [25, 26]. Since Claus *et al.* already demonstrated that the addition of almonds, sesame and poppy seeds can significantly increase the AA level in the final product, this could be the explanation of the higher levels of AA in breads containing rye and olives [25]. Additionally, EFSA has already indicated that olives may contain high AA levels (up to 454 µg kg^{-1} [1].

Different measures can be taken in order to reduce the level of AA in bread and sweet bakery products. Examples are:

- Addition of divalent cations such as Ca^{2+} or Mg^{2+} prior to baking may reduce the AA level up to 30% [25],
- Replacing NH_4HCO_3 by $NaHCO_3$ [25],
- Prolongation of the fermentation time (at least an hour) [25, 26],
- Addition of 1 % of NaCl may reduce the AA level with 40 % [26],
- Addition of different acids in bakery products [26] and their utilization can be coupled with the use of $NaHCO_3$ to correct the eventual alkaline character [25].
- Baking at high relative humidity (for example by using steam as heating medium during final part of baking) since formation of AA occurs when the water activity is below 0.8 with the highest production at 0.4 [26].

Since AA is principally formed in the outer crust layer due to the lower temperature in the inner part of bakery products, this explains the higher levels of AA in rusks compared to bread [25, 26]. Finally, it should be noted that all samples were analyzed as purchased. Therefore, no toasting executed even though it has already been illustrated that toasting can lead to higher AA concentrations [25].

2 PASTRIES

In the category pastries, 26 samples have been analyzed and can be subdivided in the 4 following subcategories: viennoiseries, donuts & cream donuts, churros & doughnutballs and pancakes.

2.1. Viennoiseries

Fourteen samples of viennoiseries have been selected for analysis, including croissants, chocolate croissants, swiss pastry, pecan pastry, butter pastry with raisins and a mix of viennoiseries. Eleven samples were bought as "ready to eat" and 3 were baked according to cooking instructions mentioned on the packaging. A detailed overview of the results is given in Annex 6 and **Table 15** displays a summary. The minimum and maximum values were respectively <LOD and 32.5 µg kg^{-1}, while the overall mean concentration was <LOQ.

Table 15. Acrylamide average and maximum contents for the viennoiseries food sub-category

	Number of samples	Mean AA content (µg kg^{-1})	Maximum AA content (µg kg^{-1})
Pastries "ready to eat"	11	<LOD	22.2
Pastries "home cooked"	3	27.6	32.5

In most of the samples (i.e. 64%), no AA was found and it should be noted that when AA was detected, the concentration was often close to the LOQ of 20 µg kg^{-1}. These low concentrations can probably be explained by the dilution effect since AA is formed in the crust and the concentration refers to the complete food item.

Interestingly, the samples that were prepared in the laboratory often had a darker final color although the instructions on the label were accurately followed (see **Figure 4**). Consequently, higher concentrations of AA were found in the food items that were prepared by the laboratory. This is also clearly visible in
Table 15.

AOF-046 Pastries home cooked

AOF-044 Pastries home cooked

AOF-048 Pastries "ready to eat"

AOF-051 Pastries "ready to eat"

Figure 4. Resulting cooking coloration for some viennoiseries cooked by the laboratory (AOF-046 & 044) and sold as "ready to eat" (AOF-048 & 051)

The obtained results are similar to the results previously reported by Croft *et al*. (i.e. < 25 µg kg^{-1} for croissants), but lower compared to the data included in the EFSA opinion on AA (i.e. 61 to 71 µg kg^{-1} for cakes and pastries) [1, 24].

2.2. Donuts and cream donuts

Five samples of donuts and cream pastry have been analyzed. A detailed overview of the results can be found in Annex 6. No AA was detected in these samples, which is consistent with the results of Croft *et al* (i.e. < 25 µg $kg^{-}1$ for donuts, iced and cinnamon, < 25 µg kg^{-1} for sweet pastry with fruit filling and < 25 µg $kg^{-}1$ for sweet pastry, choux) [24].

2.3. Churros and doughnutballs

Five samples were included in this category of which 4 were purchased as "ready-to-eat" and one sample was prepared by the laboratory. A detailed overview of the results can be found in Annex 6. AA was detected in 20 % of the samples with an average concentration of <LOQ, while the minimum and maximum values were <LOD and 31.3 µg kg^{-1}, respectively. The sample with the highest concentration of AA was prepared by the laboratory and had a darker color (**Figure 5**).

AOF-080: doughnutballs cooked by the laboratory

AOF-076 doughnutballs "ready to eat"

Figure 5. Resulting cooking coloration for doughnutballs cooked by the laboratory (AOF-080) and product sold as "ready to eat" (AOF-076)

2.4. Pancakes

Five samples of pancakes have been analyzed. A detailed overview of the results can be found in Annex 6. AA was detected in 40 % of the samples with an average concentration of <LOQ, while the minimum and maximum values were < LOD and 28.2 µg kg^{-1} respectively which is consistent with the results reported in literature by Croft *et al.* (2004) (i.e. < 25 µg kg^{-1}) [24].

All pancakes contained sodium carbonate or a mix of sodium carbonate and diphosphate as baking powder which is reflected by low AA levels since one of the measures to reduce AA levels in sweet bakery products is to replace NH_4HCO_3 by $NaHCO_3$ [25].

3 ZAKOUSKI

Three zakouski samples were included in the study. A detailed overview of the results can be found in Annex 6. AA was detected in 33 % of the samples with an average concentration of < LOQ, while the minimum and maximum values were < LOD and 31.9 µg kg^{-1} respectively. All of them were prepared according to the instructions and had a golden color (see pictures in annex 2) which was expected to result in a low level AA. Moreover, all of these samples contained glucose syrup which is preferred compared to syrup based on fructose since fructose is considered to be a better precursor of AA compared to glucose [2]. Finally, all samples also contained citric acid which is also known to decrease the final AA level [27]. All these measures have prevented high AA levels in analyzed food.

4 CEREAL PRODUCTS

In the category pastries, 64 samples have been analyzed and can be subdivided in the 4 following subcategories: rice & maize waffles, cereal snacks, honey roasted muesli, cookies with chia seeds.

4.1. Rice & maize waffles

There are 21 samples in this category comprising 11 rice waffles (including one intended for children) and 10 maize waffles. A detailed overview of the results can be found in Annex 6, while a summary is given in **Table 16**. AA was detected in more than 90 % of the samples with an average concentration

of 105 µg kg^{-1}, while the minimum and maximum values were <LOD and 259 µg kg^{-1}, respectively. These results are consistent with literature since Croft *et al.* (2004) found 166 µg kg^{-1} of AA in rice waffles.

Table 16. Acrylamide average and maximum content for the rice & maize waffles food sub-category

	Number of samples	Mean AA content (µg kg^{-1})	Maximum AA content (µg kg^{-1})
Rice waffles	11	120	259
Maize waffles	10	84	169
Total	21	105	259

The results have shown a wide variation in contamination levels for both rice and maize waffles. However, no significant difference was observed based on the type of cereal (rice/maize). This is also illustrated in **Figure 6**. It should be noted that the sample containing a mix of rice and maize was not considered in the comparison nor the specific sample intended for children.

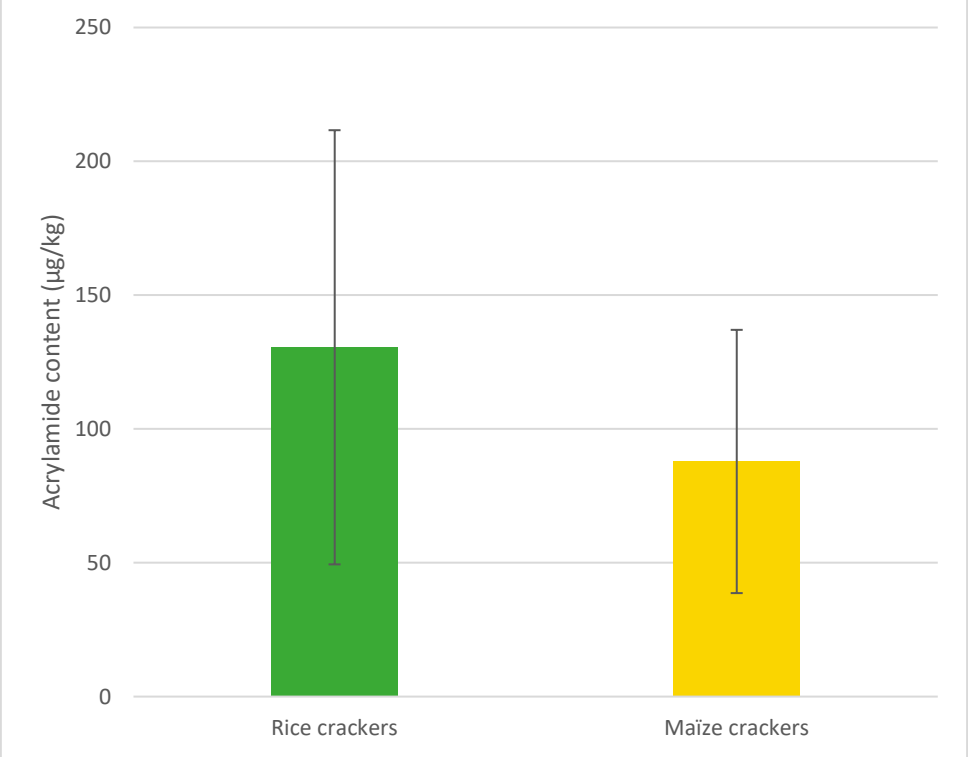

Figure 6. Difference of acrylamide content between rice crackers & maize waffles

The food category 'rice & maize crackers' is not included in Regulation (EU) 2017/2158. However, the category that is mostly related is the category of the crackers in general with a benchmark level of 400 µg kg^{-1}. Considering this benchmark level, all samples were below this value. However, a benchmark level of 150 µg kg^{-1} was fixed for biscuits and rusks for infants and young children. When comparing the results to this benchmark level, five rice crackers contained AA at higher levels. As these rice crackers might be consumed by children, an adverse health effect could not be ruled out for children, however, this should be investigated further.

4.2. Cereal snacks (extruded corn and wheat products)

Twenty two samples were analyzed including one sample intended for children. A detailed overview of the results can be found in Annex 6. AA was detected in more than 80 % of the samples with an average concentration of 115 µg kg^{-1}, while the minimum and maximum values were < LOD and 337 µg kg^{-1}, respectively. Most of these snacks were corn-based and the obtained results are consistent with literature since AA levels of 188 µg kg^{-1} croft *et al.* (2004) [24] and 100-600 µg kg^{-1} by Das and Srivastav (2012) [27] were reported for corn crisps, while levels of < 25-50 µg kg^{-1} were reported for extruded snacks [24].

When comparing the results with the benchmark level for 'Cereal crisps other than potato crisps' from Regulation (EU) 2017/2158, it can be concluded that all AA levels were below the benchmark level (400 µg kg^{-1}). If results are grouped by cereal type (maize/wheat), it can be seen that there is no significant difference between AA content depending on the type of cereal (**Figure 7**). For this comparison, one sample that was specific for children was not considered nor the samples that contained a mix of maize and wheat. In this category, also some manioc based chips were analysed and no AA was detected, resulting in the preliminary conclusion that there is a significant difference between maize/wheat samples and manioc samples.

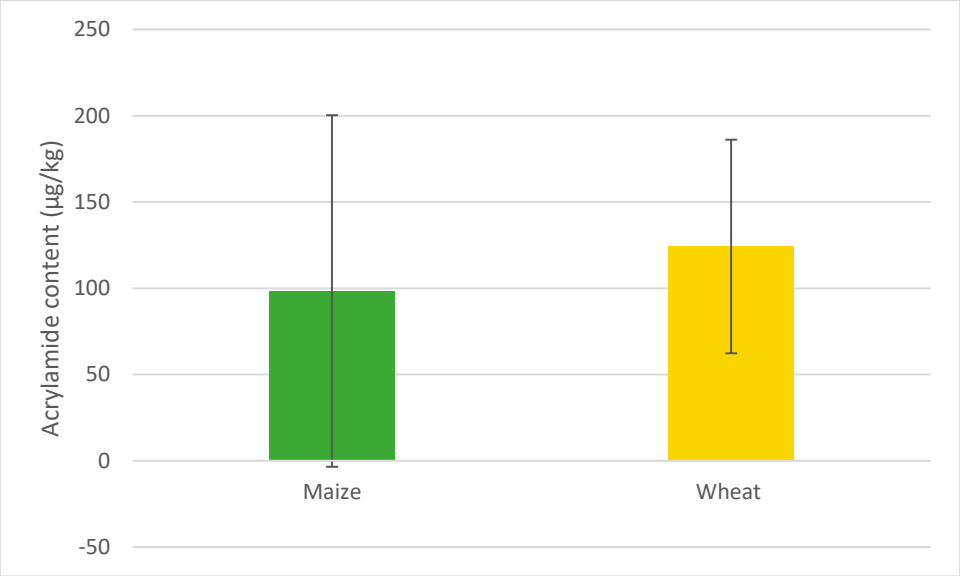

Figure 7. Difference of acrylamide content between extruded maize and extruded wheat products

4.3. Honey roasted muesli

Eleven samples of honey roasted muesli were analyzed. A detailed overview of the results can be found in Annex 6, while a summary is given in **Table 17**. AA was detected in 74 % of the samples with an average concentration of 56 µg kg^{-1}, while the minimum and maximum values were < LOD and 353 µg kg^{-1}, respectively.

Table 17. Acrylamide average and maximum content for the honey roasted muesli food category

	Number of sample	Mean AA content (µg kg-1)	Maximum AA content (µg kg-1)
Honey roasted muesli	11	56	353

These results are consistent with literature. Indeed, Croft at *al.* (2004) reported an AA level of < 25 µg kg^{-1} in untoasted breakfast cereal and muesli and a level of 25-50 µg kg^{-1} in toasted breakfast cereal, muesli and in chewy, crunchy muesli bars. In the opinion of EFSA, published in 2015, concentrations of 178 µg kg^{-1} for muesli and cereal bars were mentioned and Das and Srivastav (2012) reported a level of 50-250 µg kg^{-1} in breakfast cereals. The concentration levels of AA in breakfast cereals are influenced by the extruder technology. Direct expansion extrusion will increase the AA level compared to pellet-to-flaking extrusion [25].

In Regulation (EU) 2017/2158, benchmark levels are included for breakfast cereals are 150 or 300 µg kg^{-1} depending the cereal type considered, but muesli is not specified. When comparing the results with either benchmark level of 150 or 300 µg kg^{-1} of breakfast cereals, it can be concluded that average AA content are far below these values except for AOF-021 (353 µg kg^{-1}).

4.4. Cookies with chia seeds

Ten samples of cookies containing chia seeds in different proportion have been analysed. Chia seed is used to (partially) replace wheat flour and this could have an adverse effect on the AA content in the food [1]. A detailed overview of the results can be found in Annex 6. AA was detected in only 40 % of the samples with an average concentration of 28.6 µg kg^{-1}, while the minimum and maximum values were <LOD and 134 µg kg^{-1}, respectively.

The results obtained in this study are lower compared to previous studies since Das and Srivastav (2012) found concentrations ranging from 100 to 600 µg kg^{-1} for biscuits and crackers and Croft *et al.* (2004) reported results from 118 to 573 µg kg^{-1} in biscuits. In the EFSA opinion on AA, results ranging from 201 to 297 µg kg^{-1} are mentioned for cookies. This could originate from the mitigation measures that were implemented during the last years as a consequence of Regulation (EU) 2017/2158. In bakery products, these measures include for example the replacement of ammonium bicarbonate as a baking agent, the addition of organic acids in order to reduce the pH, addition of citric acid in its ingredients. Based on these results, it can be concluded that the presence of chia seeds has no impact on the AA level in the food.

5 VEGETABLES CHIPS

Eight samples of vegetable crisps from retail stores were analysed. A detailed overview of the results can be found in Annex 6. AA was detected in 74 % of the samples with an average concentration of 1012 µg kg^{-1}, while the minimum and maximum values were 98 and 3063 µg kg^{-1} respectively. No benchmark level exists for this food category in Regulation (EU) 2017/2158. The closest related food category is potato chips with a benchmark level set at 750 µg kg^{-1}. When comparing these results with this benchmark level, this one was exceeded in 3 samples. Therefore, these samples were notified to the Federal Agency of Safety of the Food Chain (FASFC) as they may present a risk for human health. Since these samples contained a mix of different vegetables, a new analysis was conducted with a vegetables chip sample that was sorted into the individual vegetables analysed separately. These analysis has illustrated that the high concentration was mostly caused by parsnip and beetroot (**Table 18**).

Table 18. Acrylamide content in some vegetable chips after the sample has been sorted into the different vegetables

Vegetable type	AA content (µg kg^{-1})
Sweet potato	548
Parsnip	1020
Beetroot	1117

The concentration levels of AA show a wide variation in this category. This can be due to the choice of raw materials but could also be related to the heating process that was applied. Concerning the choice of raw materials, the vegetables used in the different samples was different. Furthermore, the presence of sugar, a precursor of AA, vary according to the type of vegetable [28]. As fructose is a better precursor compared to glucose [2], higher concentration levels of AA were expected in carrot, followed by sweet potato and finally beetroot (**Table 19**).

Table 19. Amounts of glucose, fructose and sucrose (mg pe 100 g) in beetroot, sweet potato and carrot from Breitling-Utzmann and Hankele (2019)

	Beetroot R1	Beetroot R2	Sweet potato S1	Sweet potato S2	Carrot C1	Carrot C2
Glc	< 100	663	859	1010	1160	1490
Fru	< 100	615	518	740	1010	1360
Sac	9350	4330	5620	6090	3930	2200

However, the analysis of the individual vegetables did not confirm this hypothesis since the highest concentration was found in beetroot. This discordance may result from the intra-variability of precursors inside a vegetable type depending on the season, geographical area and storage conditions. Next, the heating process influences the production of AA. This was already illustrated by Breitling-Utzmann and Hankele (2019) [28] and is given in **Table 20**. Based on this table, the temperature should never exceed 130 °C. However, it should be noted that information related to the cooking process are unknown. In conclusion, this category should be investigated further as the AA content varied widely and since some samples might result in a potential health risk.

Table 20. Acrylamide amounts (µg kg^{-1}) in oven-baked vegetable crisps from Breitling-Utzmann and Hankele (2019). Acrylamide amounts exceeding the benchmark level for potato crisps (750 µg kg^{-1}) are printed in red letters

Temperature [°C]	Baking time [min]	Beetroot R1	Beetroot R2	Sweet potato S1	Sweet potato S2	Carrot C1	Carrot C2
130	40	29	435	287	97	572	455
130	50	33	539	573	181	931	887
130	60	75	1180	908	501	1360	1010
140	40	76	–[1)]	3250	–[1)]	2950	–[1)]
140	50	252	2580	4810	3550	4060	3940
140	60	605	–[1)]	4760	–[1)]	4410	–[1)]
150	40	432	2830	6590	6200	5180	7610
150	50	1060	3580	8660	7410	4930	6000
150	60	1220	3380	8290	9780	4210	5330
180	20	938	–[1)]	14200	–[1)]	11100	–[1)]

[1)] This time-temperature-combination was not used

6 BLACK OLIVES

Five samples of black olives were analyzed. Two of them were black olives in cans and 3 samples were packed in a glass jar. Although it is not starchy foods, high amount of AA may be produced during their processing [29–32]. A detailed overview of the results can be found in Annex 6. AA was detected in all of the samples with an average concentration of 239 µg kg^{-1}, while the minimum and maximum values were 31.9 and 431 µg kg^{-1}, respectively.

In the EFSA opinion on AA, the concentrations found were much higher. However, explaining this difference is nearly impossible due to the limited number of samples that have been analysed. Previously, it was already reported that AA could also partially be extracted from the brine [32]. In this study, the brine was removed prior to the analysis which could explain the lowest concentration reported in this study. Next, the different ways of processing black olives prior to being commercialized should also be taken into consideration. There are 3 main ways in which olives could processed, (i) Spanish-style, (ii) Greek-style and (iii) Californian style as illustrated in **Figure 8**. The Spanish style will not be considered as this is not applied to black olives. In the Greek style, processing consists of waiting for the green olives to ripe which turn them naturally black. Then black olives follow different steps of washing, brining and fermentation in order to get the desired organoleptic characteristics and eventually they can be pasteurized. In the Californian-style, black olives are produced from green olives that are stored with brine, followed by an alkaline treatment in order to eliminate the bitter taste from unripe olives. They are then artificially colored into black air oxidation and with E579 (i.e. ferrous gluconate). Finally, the olives are sterilized. It is interesting to note that the Greek-style & Californian-style both follow a thermal process (sterilization/pasteurization) in order to preserve the food prior to storage in glass jars or cans. Pasteurization consists of a heating process reaching in general a temperature of 90 °C. Sterilization in general uses temperature ranging from 110 °C to 121 °C up to 50 min, which is above the temperature to induce the formation of AA.

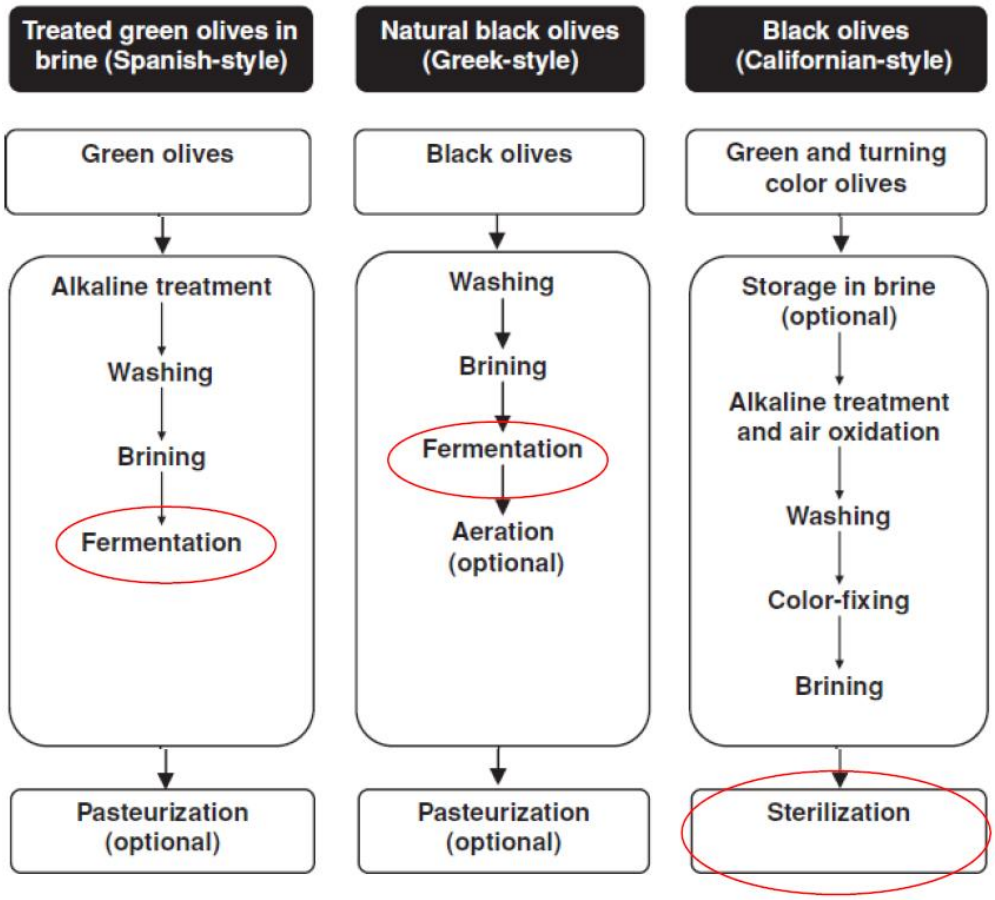

Figure 8. Main manufacturing processes for black olives (from Duedahl-Olesen (2019))

Next, different parameters that were studied in the past were investigated using the results obtained in this study. For example, Amrein *et al.* (2007) noted that olives originated from Spain tend to form more AA than others, which is confirmed by the results in this study as the highest concentration was determined in olives originating from Spain (i.e. 575 µg kg^{-1}). Francisco *et al.* (2010) also studied the influence of NaCl and acetic acid on the formation of AA. Addition of NaCl seems to reduce AA formation up to 30 %. Nevertheless, manufacturers do not have much flexibility with this parameter as salt can rapidly alter the taste of the final product. Francisco J. *et al.* (2008 and 2010) and Suthawan *et al.* (2014) investigated the impact of the addition of the coloring agent E579 on the production of AA and concluded that there was no influence [29–31]. However, this seems to be in contradiction with the results of this study as the sample containing E579 contained a very high concentration of AA (i.e. 431 µg kg^{-1} and 575 µg kg^{-1}). Furthermore, several authors reported that the sterilization process is the biggest contributor to AA formation in black olives [29, 30, 32] and that its contribution is even more important than the presence of the initial precursors (asparagine, sugar). This could also explain the higher result obtained for the sample containing E579 as they were treated following the Californian-style process in which a sterilization step is included.

Finally, it is interesting to mention a study from the European Reference Laboratory of processing contaminants (EURL-PC), investigating the effect of domestic cooking on black olives [33]. They observed an extreme AA formation (up to 5270 µg kg^{-1}). Hence it could be interesting to investigate Belgian cooking practices with this food category in the same way that it was done in this study with buns and potato-based products.

7 COCOA POWDER

Five samples with a wide range of cocoa content (i.e. 13-100 %) have been analyzed. A detailed overview of the results can be found in Annex 6. AA was detected in 80 % of the samples with an average concentration of 56.0 µg kg^{-1}, while the minimum and maximum values were < LOQ and 141 µg kg^{-1}, respectively.

No correlation could be found between the AA levels and the cocoa content as the **Figure 9** shows.

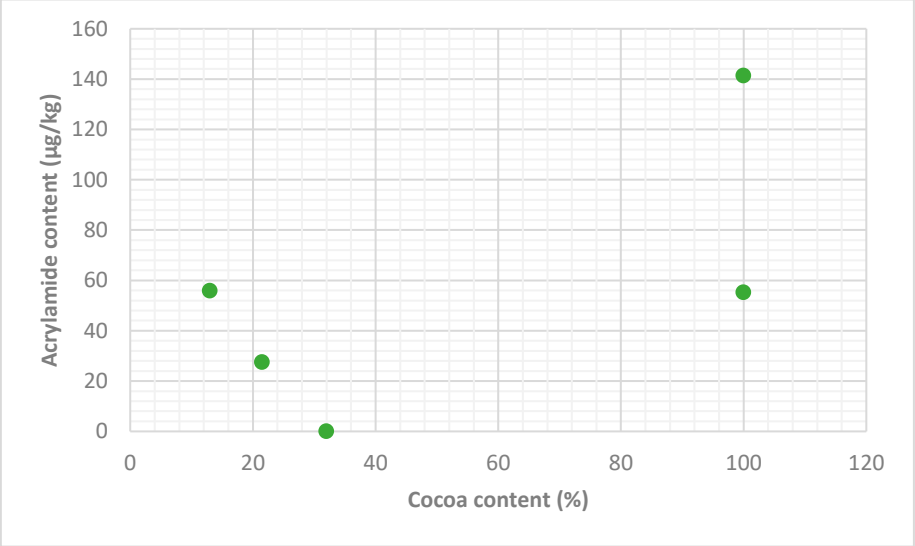

Figure 9. Acrylamide content depending cocoa content

When comparing the results with previous studies, the mean concentration in this study is approximately three times lower compared to mean concentrations included in the EFSA report (178 µg kg^{-1}) [1] and the German Study (180 µg kg^{-1}) [34]. It should be noted that the number of samples in this category is rather limited and therefore, all conclusions should be investigated further.

8 COFFEE SUBSTITUTES

Seven samples considered as coffee substitutes other than cereal and chicory-based have been analysed. A detailed overview of the results can be found in Annex 6. AA was detected in 86 % of the samples with an average concentration of 930 µg kg^{-1}, while the minimum and maximum values were < LOD and 4389 µg kg^{-1} respectively.

According to the Regulation (EU) 2017/2158, 3 different benchmark levels could be used depending on their constitution: cereal based only, chicory only, or a mix of chicory and cereal. When a mix of cereal-based and chicory-based coffee substitutes are used, the benchmark level is calculated based on the proportion of both constituents. Although the benchmark levels are not adequate for the samples selected in this study as they are based on plants, nuts and fruits, the highest concentration (4389 µg kg^{-1}) exceeds the highest benchmark level for coffee substitutes (i.e. 4000 µg kg^{-1}).

To the best of our knowledge, the only coffee substitutes that have been analyzed were always based on cereals or chicory, but never on other alternatives as included in this study. Therefore, the only comparison that can be made is using the other coffee substitutes. Claeys *et al.* (2016) reported AA levels up to 2621 µg kg^{-1} for coffee substitutes on the Belgian market between 2002 and 2013 [35],

while the concentrations reported in the EFSA opinion on AA were 510 µg kg^{-1} for malt, barley and wheat coffee, 2942 µg kg^{-1} for chicory coffee and 1499 µg kg^{-1} for other and unspecified coffee substitutes. This also shows a wide variation in the results as was also observed in this study. Different factors such as the choice of raw materials and the process could explain this variability [36]. Considering the choice of raw materials, levels of asparagine and reducing sugars have an important effect on the final AA concentration. For example, chicory roots contain twice as much fructose as glucose, leading to high AA levels in the final product [37]. Furthermore, it also seems that the part of the plant that is used influences the final AA concentration since the roots contain more sugar compared to the leaves. Concerning the process, roasting parameters such as time and temperature play a role on AA formation. Indeed, the matrix is exposed to temperatures higher than 200 °C during roasting, which can lead to AA formation [36]. However, it can be difficult to modify the roasting without changes in the organoleptic characteristics of the final product. One option proposed in literature is to roast under reduced pressure in order to obtain medium roasted coffee. In fact, it was suggested to promote AA sublimation which could lead to an elimination of AA from the matrix [36]. Storage also plays a role since a decrease up to 30 % of AA content may occur during storage [38].

AA concentrations in coffee substitutes are higher compared to natural coffee while these products are often used to replace coffee in more vulnerable population groups such as children and pregnant or breast feeding women. It is therefore vital to control the values that are established for coffee substitutes in relation to the affected groups and that targeted education is undertaken [37]. Operators have to identify sources of AA production in their process (roasting, formulation, storage) and they have to improve these steps (in particular choice of raw material and heating process). Since there is a huge difference between the different samples and it seems that difference can also appear between different batches of the same manufacturer operators also have to standardize their process [37].

9 NUTS AND OIL SEEDS

Sixteen samples in the nuts and oil seeds category have been analyzed. They have been subdivided in 2 major subcategories : (i) roasted nuts and (ii) roasted oil seeds. A detailed overview of the results can be found in Annex 6, while a summary is given in **Table 21**. AA was detected in 50 % of the samples with an average concentration of 27.0 µg kg^{-1}, while the minimum and maximum values were < LOD and 155 µg kg^{-1}, respectively.

Table 21. Acrylamide average and maximum content for the nuts and oil seeds food category

	Number of sample	Mean AA content (µg kg^{-1})	Maximum AA (µg kg^{-1})
Roasted nuts	10	< LOQ	75.6
Roasted oil seeds	6	40.8	155

AA formation in nuts and oil seeds is linked to the presence of precursors (asparagine and reducing sugar) and to roasting treatment (which is applied to enhance flavor, color and texture). There is a lot of variability in the content of precursors according to the type of nut but also according to its geographical location. Indeed, almonds contain a lot of precursors (asparagine and reducing sugar) which may lead to a high amount of AA formation, while hazelnuts contain only a low amount of free asparagine leading to lower level of AA in roasted hazelnuts. Peanuts contain asparagine precursor but low reducing sugar which also leads to lower AA production when roasted [39]. Also the geographical

location influences the AA concentration in the final product. In fact, almonds of European origin contain less free asparagine and so less AA when roasted compared to almonds from the US [38].Storage conditions also play a role since Amrein *et al.* (2005) showed that AA level in roasted almonds decrease during prolonged storage at room temperature (**Figure 10 (a)**).

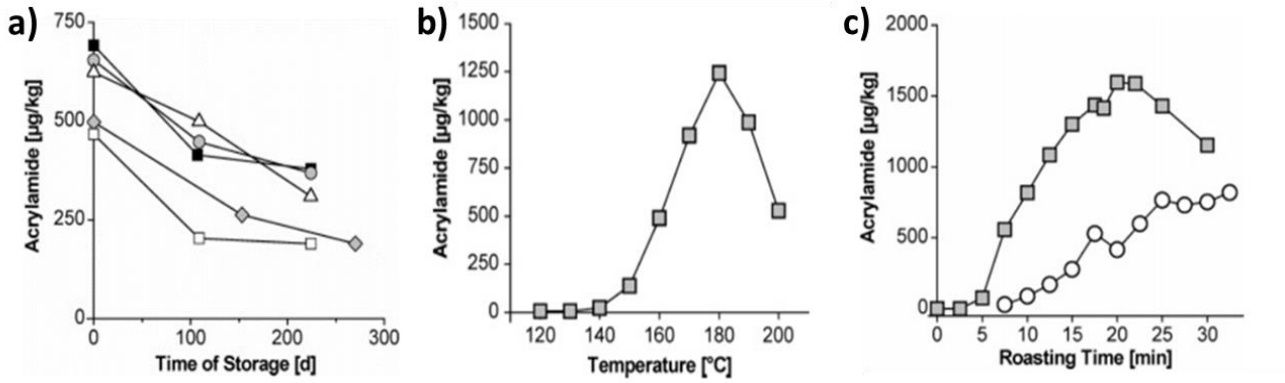

Figure 10. Evolution of AA content depending some parameters: time of storage (a), temperature for a cooking time of 10 min (b) and roasting time for a temperature of 165 °C (squares) and 145 °C (circles) (c) (from Amrein *et al.* (2005).

Finally, roasting treatment has an impact on the final AA level since AA formation increases with temperature, reaches a peak at 180°C and then decreases in almonds. However, these samples were over-roasted. Time is also important since AA level increases over time at a given temperature (**Figure 10 (b, c)**) [38, 39]. The impact of the physical form (i.e. whole, sliced and cut) and the presence of a shell on the AA levels has also been demonstrated [39, 40].

Different studies have already reported the presence of AA in nuts. However, the reported concentrations vary widely from below detection limit for almonds, hazelnuts, pine nuts and walnuts and below LOQ for pistachios up to 1000 µg kg^{-1} in roasted almonds [38, 39, 41]. Jägerstad and Skog (2005) reported the concentration of AA as 66 µg kg^{-1} in sunflower seeds [42], while a mean value of 93 µg kg^{-1} was reported in the EFSA opinion on AA for roasted nuts and seeds (EFSA, 2015). Our results seem to be in accordance with the results of De Paola *et al.* (2017) [41], since low AA levels were found for most samples. The highest results were found in sunflower seeds, although low concentrations were detected in the other sunflower seeds. This might be explained by differences in the applied roasting treatment as was already demonstrated by Süvari *et al.* (2017) [40]. However, this cannot be verified for the samples in this study as the information is not available. Although, this category is not included in Regulation (EU) 2017/2158, but it might be useful to develop guidelines in order to help the operators to standardize their processes and so to reduce variability found in this category. Eventually, this could also lead to a benchmark level.

10 DRIED FRUITS

Six dried fruit samples have been analysed. All samples have results below LOD. A detailed overview of the results can be found in Annex 6. These results are in accordance with literature since only traces of AA were found in raisins and dried figs [39, 41]. Different studies have found higher concentrations of AA in dried plums. De Paola *et al.*. reported AA levels between 14.7 µg kg^{-1} and 124.2 µg kg^{-1} and EFSA mentioned an average concentration of 89 µg kg^{-1} in the Scientific opinion on AA [1, 41]. This was not confirmed in this study, but it has to be noted that only one sample of dried plums was included.

Another interesting hypothesis is the impact of sulphur since the presence of sulphur can inhibit the enzymatic browning and retard the Maillard reaction [39]. Unfortunately, this hypothesis could not be verified in this study.

11 CARAMEL AND NOUGAT

Two nougat and three caramel samples have been analysed. A detailed overview of the results can be found in Annex 6. AA was detected in only 40 % of the samples with an average concentration of 17 µg kg^{-1}, while the minimum and maximum values were < LOD and 59.6 µg kg^{-1}, respectively. No other studies were found in literature and this category is not included in Regulation (EU) 2017/2158.

12 POTATO BASED PRODUCTS

Thirty-nine potato based products have been analyzed and can be subdivided in 7 categories (i.e. röstis, potato cubes, duchess potatoes, croquettes, potato balls, Potato-based dishes (e.g. shepherd's pie, gratin dauphinois) and sweet potatoes and vegetable fries). All these products needed to be prepared before consumption. This was performed according to the instructions of the manufacturer. Different preparation modes have been applied and a more profound discussion on the impact of deep-frying, preparation using an oven or microwave is provided in this chapter. A detailed overview of the results can be found in Annex 6, while a summary is given in **Table 22**. An average concentration of 443 µg kg^{-1} was found, while the minimum and maximum values were < LOD and 1503 µg kg^{-1} respectively.

Table 22. Acrylamide average and maximum content for some potato based products

	Number of samples	Mean AA content (µg kg^{-1})	Maximum AA content (µg kg^{-1})
Röstis	5	336	1199
Croquettes	11	272	1071
Duchess potatoes	5	322	813
Potato cubes	4	910	1503
Potato balls	5	511	1248
Potato-based dishes (e.g. Shepherds pie, gratin dauphinois, etc)	5	32.5	54.7
Sweet potatoes and vegetable fries	4	627	981

The AA levels found in this category vary widely. Different parameters can be considered to explain this variation:

(i) the presence of precursors such as asparagine and reducing sugars. Indeed, reducing sugars are the main limiting factor in potato products and their concentration varies depending on potato variety but also on climatic conditions, fertilization and storage conditions [26].
(ii) Heating treatment will also influence the final AA concentration. In this study, all food items were prepared in accordance with the instructions of the manufacturer. However, for some samples different options were given for their preparation. Both instructions were applied and more detailed discussion on the comparison of different preparation modes is provided below.
(iii) The surface-to-volume ratio also need to be considered as AA is mostly formed on the crust and is then further diluted when the complete food item is analyzed. This hypothesis was supported by the results of the potato cubes, where the highest concentration was found in the smallest potato cubes.

(iv) Dilution effect will also influence the final AA concentration. For example when a dish is prepared with only a small fractions of fried food items. This was clearly observed in the sub-category of potato-based dished and the analysis of a dish containing röstis where only 103 µg kg^{-1} of AA was found, while the average concentration for röstis was 336 µg kg^{-1}.
(v) Final color of the prepared product can be correlated to the AA level [25, 26]. An overview of the coloration of the final products is included in annex 2. In each sub-category, a clear link was observed on the AA level and the color of the final product, i.e. the highest contamination of AA was found in the final food items with the darkest color.

A specific sub-category were the 'sweet potatoes and vegetable fries'. This is a novel food category that is increasing in popularity. However, relatively high concentrations of AA were found (**Table 22**). At the moment, no benchmark levels is mentioned in Regulation (EU) 2017/2158, but the category that is most closely related is the potato fries with a benchmark level of 500 µg kg^{-1}. However, all samples included in this study for sweet potatoes and vegetable fries exceeded this level. Therefore, it is recommended to set a specific higher benchmark level for this category.

Comparison of the different cooking modes

Multiple cooking instructions were mentioned on the packaging. oTherefore, 13 samples were prepared according to the different instructions, which would allow the comparison of different cooking modes on the AA formation. An overview of the results obtained depending the cooking mode used is given in **Table 23**. **Table 24** gives information on cooking parameters (time, temperature, cooking mode), and the final coloration for some samples (other samples are available in annex 2).

Table 23. Comparison of acrylamide content between samples cooked in oven or with a Fryer

Sample number	subcategory	Oven result (µg kg^{-1})	Fryer result (µg kg^{-1})
AOF-002	Rösti	63.6	506
AOF-003	Rösti	216	1199
AOF-007	Duchess potato	<LOQ	381
AOF-008	Duchess potato	57.7	419
AOF-009	Duchess potato	27.7	755
AOF-010	Duchess potato	<LOQ	737
AOF-021	Potato balls	54.5	107
AOF-022	Potato balls	81.0	1248
AOF-024	Potato balls	<LOQ	516
AOF-025	Potato balls	<LOD	172
Add4	Duchess potato	34.7	813
Add7	Fries	46.7	981
Sup15	Cone shaped potato	199	1071

In general, it can be concluded that higher AA concentration are detected when the food is prepared using a fryer compared to oven (up to 25 times). This is also illustrated in **Figure 11**.

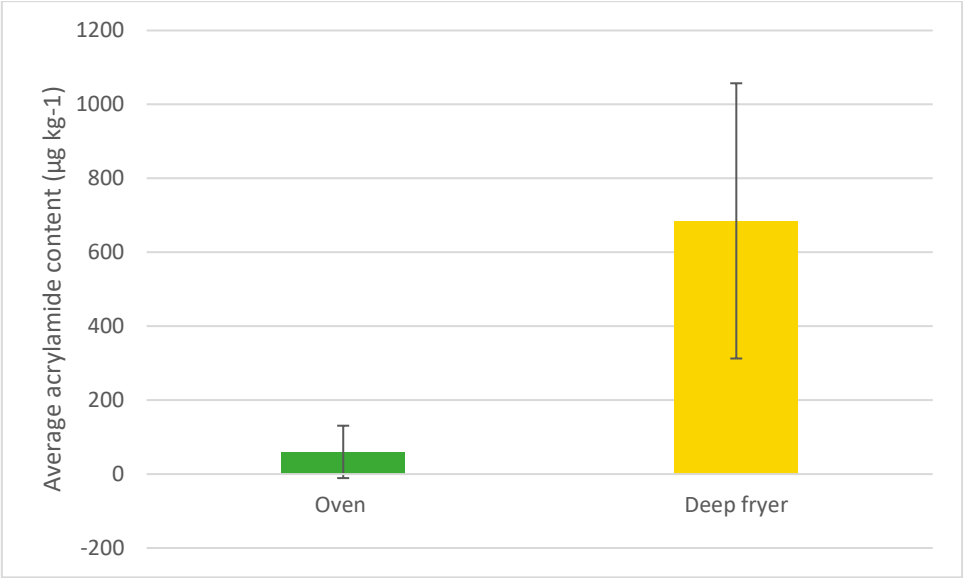

Figure 11. Comparison of average acrylamide concentration in same 13 samples depending on the cooking mode (oven/fryer)

When comparing the instructions on the label for deep-frying, the requested temperature was always 175 °C, although the required cooking time varied slightly from 3 to 5 minutes. Large variations in the AA content were observed even when very similar products were considered. However, clear link was observed between the AA level and the color of the final product. For instance, the AA level in the potato balls that were dark brown after frying, was more than 10 times higher compared to the similar potato balls that had a golden color after oven cooking. This study indicates that not only the heating process plays a role in AA formation, but that other parameters need to be considered such as the ingredients, variety of potatoes, condition of storage. However, this is information is not readily available for the consumer.

The cooking instructions for preparation in the oven show more variability in the advised temperatures (ranging from 180 °C to 220 °C) and time (ranging from 13 to 25 minutes). Nevertheless, the variation in AA levels was smaller compared to the results of frying. Moreover, all the detected levels were lower than the benchmark level (750 µg kg^{-1}) for the closest food category included in commission Regulation (EU) 2017/2158 (i.e. other potato products from potato dough). It should be noted that the preparation in the oven is less standardized compared to frying. Indeed, different cooking programs can be used (e.g. normal, rotating heat, grill), the position of the plate in the oven may vary and the food might be turned during the preparation. All these parameters may have an impact on the AA level. Same phenomenon has been observed during the study of cooking habits of the Belgian population.

Finally, it can be concluded that it is not possible to link the AA level to parameters related to the preparation of the samples. However, in the commission Regulation (EU) 2017/2158, it is stipulated that the final product should have a light golden color. Some examples are given in **Figure 12**. It can be seen that there is a link between AA content and the final color of the cooked samples. Furthermore, it can been seen that all samples with a golden color are effectively below the benchmark level, and a transition can be observed with the sample of duchess potatoes turning dark gold/orange, reaching an AA content higher than the benchmark level. This is a very interesting information as it seems that this observation is always confirmed no matter the cooking parameters that are used. Hence, information of the final color should be mentioned more often on food items packages as it seems more reliable.

After checking labels on the packaging, 50 % of the labels contained indications on the color of the food items as criteria to stop the preparation of the food.

Table 24. Final coloration for some samples depending on cooking method used (oven or fryer) and associated cooking parameters (temperature and duration)

Sample number	Oven	Fryer
AOF-021		
	200 °C | 18'00"	**175 °C | 3'00"**
AOF-022		
	180 °C | 13'00"	**175 °C | 3'00"**
AOF-024		
	200 °C | 18'00"	**175 °C 3'00"**
AOF-025		
	200 °C | 15'00"	**175 °C | 3'30"**

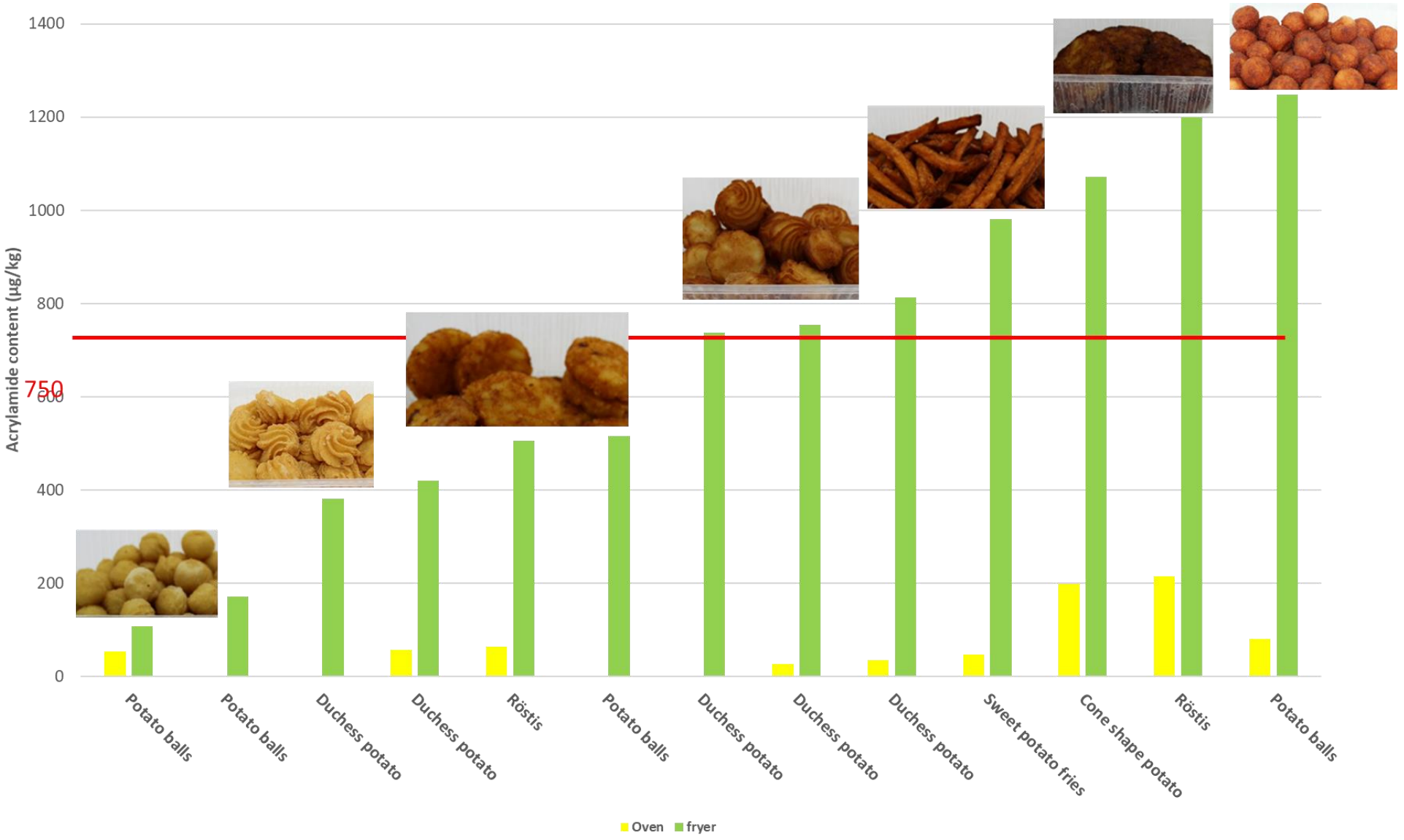

Figure 12. Acrylamide level for some food products depending the cooking mode used and resulting coloration

Study on cooking habits of the Belgians

A total of 14 participants have been recruited to study Belgian cooking practices. **Table 25** shows the number of participants, the food they chose to cook and the cooking devices they possessed. Most participants prepared the food in the oven or purchased these kind of food at a "frietkot", while only 5 participants used a fryer at home.

Table 25. Food items cooked and devices possess by participants

	Food			Cooking device		
Participant	Croquettes	Sweet potatoes fries	Buns	Fryer	Oven	Toaster
1	X		X		X	X
2	X	X	X		X	X
3		X	X		X	X
4	X			X	X	
5	X				X	
6	X		X	X		X
7	X				X	
8		X	X		X	X
9	X			X	X	X
10	X	X			X	
11	X	X	X	X	X	X
12	X	X			X	
13	X			X		
14	X				X	
Total	12	6	6	5	12	7

It should be noted that the number of participant is too small to extrapolate conclusions to the entire Belgian population but the results would allow to give a first insight in what it is actually done by the Belgian consumers. Participants were asked to complete a questionnaire (see annex 4) related to different cooking parameters and habits regarding the respect of cooking instructions mentioned on the label. The answers of all the participants are included in Annex 5. Finally, photos were taken of all the prepared dishes in order to complement the questionnaires and facilitate the interpretation of the results. All pictures of samples cooked by participants are available in annex 7. For oven cooking, participants have been asked to sample croquettes/fries from the center of the oven but also from the four outer parts in order to get a representative cooked portion since the cooking process is not homogeneous. In case of the fryer, the participants were asked to prepare the same portion as they usually do, then mixed it properly before setting aside a portion for analysis. A portion consisted of 5-6 croquettes, 10-12 sweet potato fries and one bun.

Table 26, Table 27, **Table 28** show the AA content found in each food item cooked by the participants with the associated cooking mode and cooking parameters.

1 TRENDS IN COOKING HABITS

While evaluating the answers to the questions related to the cooking habits, only the preparation of croquettes and sweet potato fries have been taken into account since no specifications are given for the preparation of buns, except that it can be done in oven or with a toaster.

Respect and considerations of cooking instructions:

Participants were asked if they usually respect the cooking instructions available on the label. **Figure 13** summarizes their answers. Five participants answered ”No” (36 %) while 64 % answered “Yes”.

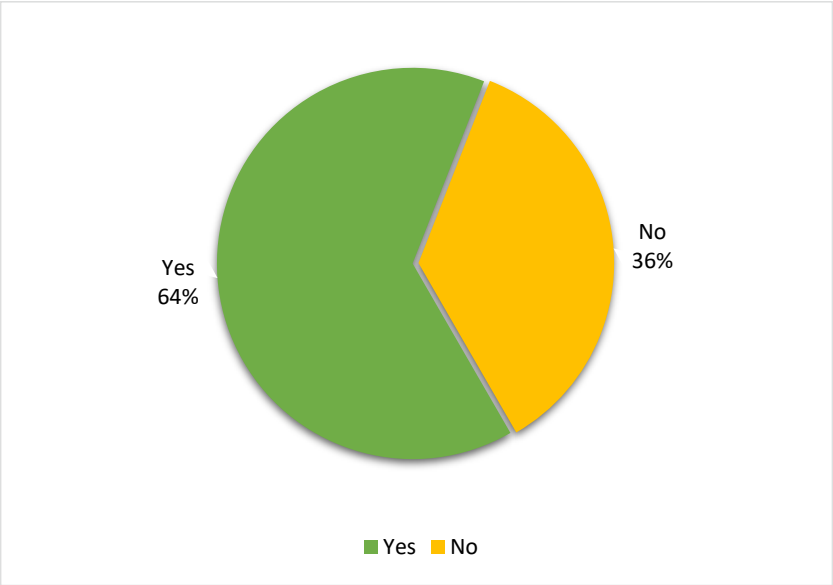

Figure 13. Answers of the participants to the questions regarding labelled cooking instructions

After questioning the respect of cooking instructions, the participants were also asked to indicate their thoughts on the labeled instructions. It has to be noted that the question was totally open in order to avoid bias. Most of the participants answered that they respect these instructions as a basis and adapted it according to their taste. Five of them clearly specified that they adapted the cooking time because the specifications on the label were insufficient to cook the food properly.

Criteria to stop the cooking:

Participants were also asked to mention what their criteria were to stop the cooking. Twelve participants out of 14 mentioned that they stopped the cooking based on the color of the food item. They used different terminology to characterize this color, but most of the time the golden color is cited which is also the preconized one in the commission Regulation (EU) 2017/2158. Four participants out of 14 mentioned also that they referred to their timer and that they respected the time mentioned on the label. Four participants referred also to non-quantitative criteria such as the crispy aspect of the food item.

2 PREPARATION OF CROQUETTES

Twelve participants prepared croquettes at home, of which 4 used a fryer and 8 used an oven.

Table 26 summarizes all cooking parameters that were used and the concentration of AA found in the prepared food. The AA content found in the food prepared by the participants showed a very wide

variation ranging from <LOD up to ~500 µg kg^{-1} although cooking parameters are, in most cases, similar. Therefore, a more thorough investigation using the pictures and questionnaires was needed.

Table 26. Cooking parameters and acrylamide content of croquettes cooked by participants

	Cooking mode	Cooking parameters		AA Content (µg kg^{-1})
Participant N°	Fryer/Oven	T (°C)	Time (min)	
1	Fryer	175	6	78.7
6	Fryer	175	4.5	127
9	Fryer	175	8	<LOD
10	Fryer	180	4	295
12	Oven*	220	16	339
13	Oven*	190	16	252
14	Oven	180	29	140
2	Oven*	220	17	500
4	Oven	220	18	173
5	Oven	220	16	288
7	Oven	220	22	52.1
11	Oven*	220	16.5	295

*Rotating heat program has been used

Croquettes – fryer cooking:

Most of the participants respected the temperature indicated on the label (175 °C). Only one participant used a higher temperature of 180 °C (corresponding to the previous temperature requirements for this kind of food) and obtained the highest AA content for fryer cooking. Concerning the cooking time, a wide range of times have been used ranging from 4 min as written on the label to 8 min (i.e. twice the preconized time). Even if most of the participants applied a cooking time that exceeded the manufacturer's instructions, AA content was in all cases much lower than the benchmark level of 750 µg kg^{-1} as mentioned in the commission Regulation (EU) 2017/2158 for potato products from potato dough category (category closest to croquette). When plotting the AA content versus the cooking time,

without taking into account the temperature (

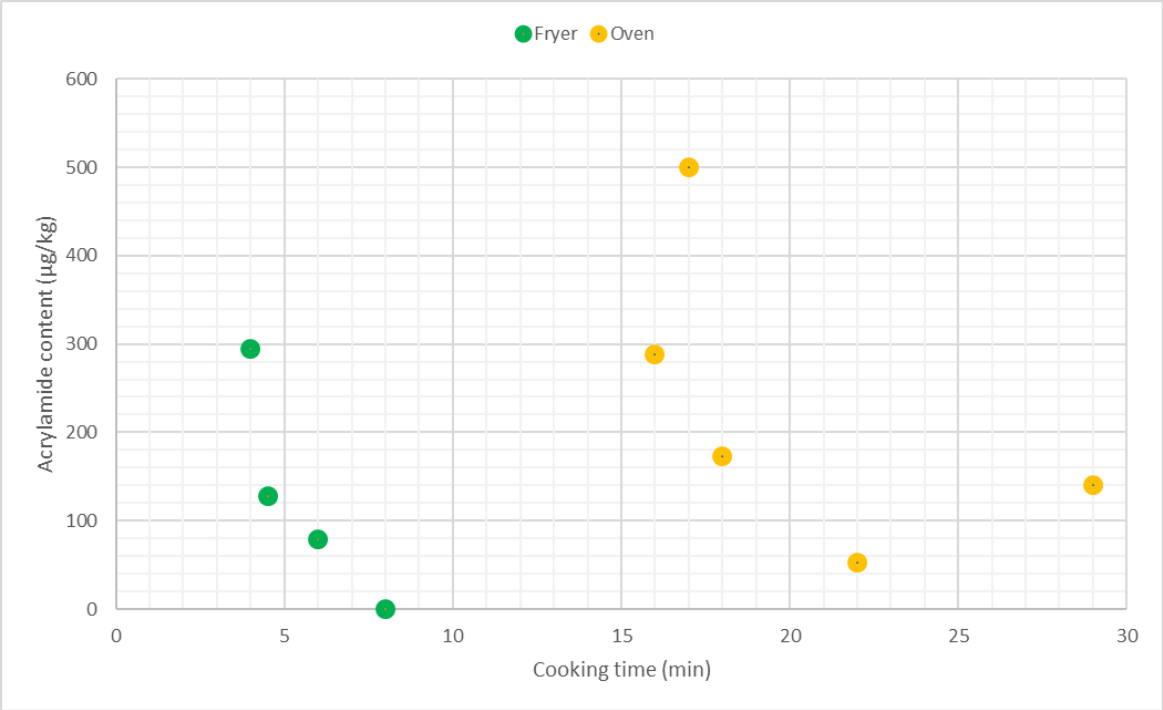

Figure 14) as they are all very similar, it could be noted that there is an inverse correlation between these two parameters. When looking to the pictures of the corresponding cooked items (annex x), colors ranging from golden to dark orange have been obtained and are positively correlated to AA concentration. For participant 9, AA content seems to be an aberrant value as the croquettes were cooked for 8 minutes and no AA was found. Nevertheless by checking the corresponding pictures, it can be seen that croquette is very pale compared to the others. This may result from a mistake in the answers or a malfunction in the temperature controller itself. This last point can be also the conclusion for other participants to a lesser extent: as it is impossible to link time cooking to AA content. The explanation may be that the accuracy of the thermostat ion domestic fryers is limited and may differ from model to model.

Croquettes – oven cooking:

The concentration of AA detected in the croquettes prepared in the oven varied widely, but was in all cases below the benchmark level of 750 µg kg^{-1}. When looking at the cooking parameters of the participants, most of them respected the temperature indicated on the label (220 °C), while two participants (i.e. participant 13 and 14), cooked the croquettes at a lower temperature, i.e. 190°C and 180°C respectively. The manufacturer Recommendation for cooking time is 16 min which has been respected by most of the participants with cooking times ranging from 16-18 min. Participants 14 and 7 exceeded the preconized time for several minutes (i.e. 29 and 22 min, respectively). Even tough, only 140 µg kg^{-1} of AA has been found in the sample prepared for 29 minutes by participant 14. This could be explained by the temperature used for the preparation that is far below the 220 °C as recommended by the manufacturer. By checking more thoroughly the questionnaire of this participant, it can been seen that the criteria to stop the cooking is listed as the gold color but, the participant also indicated that in general he finds the cooking time specified on the label are too short. Finally, candidate 7 cooked croquettes during 22 min at 220 °C and only 52.1 µg kg^{-1} of AA has been found. This cooking time is more than 5 min longer than the preconized cooking time ~16 min. When checking the questionnaire,

participants 7 also answered that it is necessary to extend the cooking time in order to improve the 'crispy' character, although the cooking process is also stopped based on the color. Both of these participants do not have the rotating heat mode. The wide range of cooking temperatures used can be explained by the oven cooking program used. Half of participant used rotating heat (participants 2, 11, 12, 13) while other participants use a normal cooking program (information available in the questionnaires in Annex 5). As the rotating heat program is very efficient, the cooking time is in general lower compared to the classical program, this point is also confirmed in the Regulation (EU) 2017/2158. It is also illustrated in **Figure 15** where it can be seen that the average level of AA is higher with rotating heat for a shorter period of time compared to the classical program. Hence it explains why there is no positive correlation between cooking time and AA content. Again, a positive correlation between the color obtained ranging from gold to dark orange and AA content (see pictures Annex 5). This confirms that the cooking parameters are more linked to the device used (quality of the model, program used) than the AA content. Some participants also report (information not in questionnaires) that the cooking time depends strongly of the oven used. These declarations support these results. Pictures of prepared samples in combination with the applied temperature can indicate the presence of AA, regardless of the cooking time.

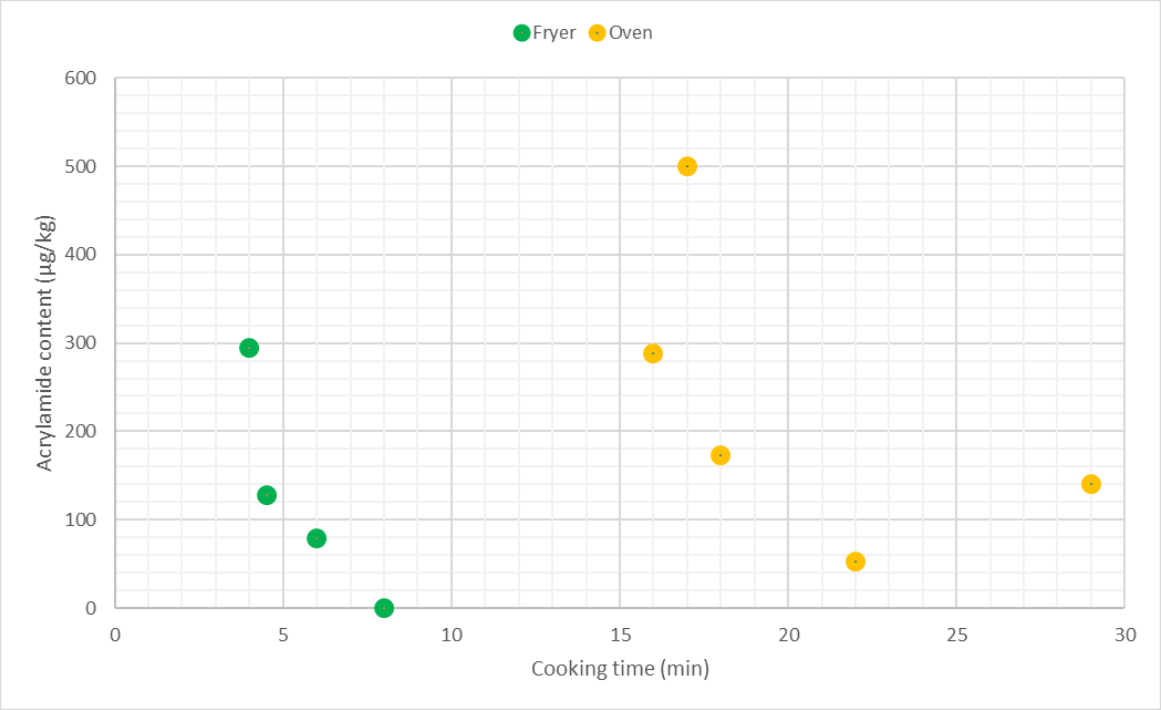

Figure 14. Acrylamide content depending in function of the cooking time and cooking mode

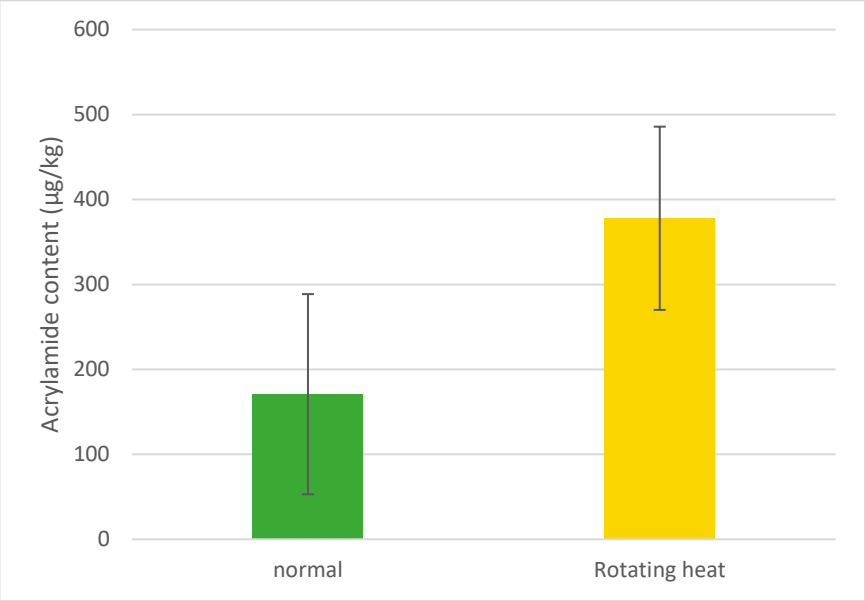

Figure 15. Acrylamide content depending the oven program used

Comparison of the cooking modes:

As the **Figure 16** below shows, the cooking mode does not seem to have a significant impact on the AA content. This may be explained by the fact that most of participants respected the preconized temperature and tend to get a golden color. This golden color coupled to a good temperature are adequate criteria to guarantee a safe AA content for the consumer, regardless of the cooking time and cooking mode.

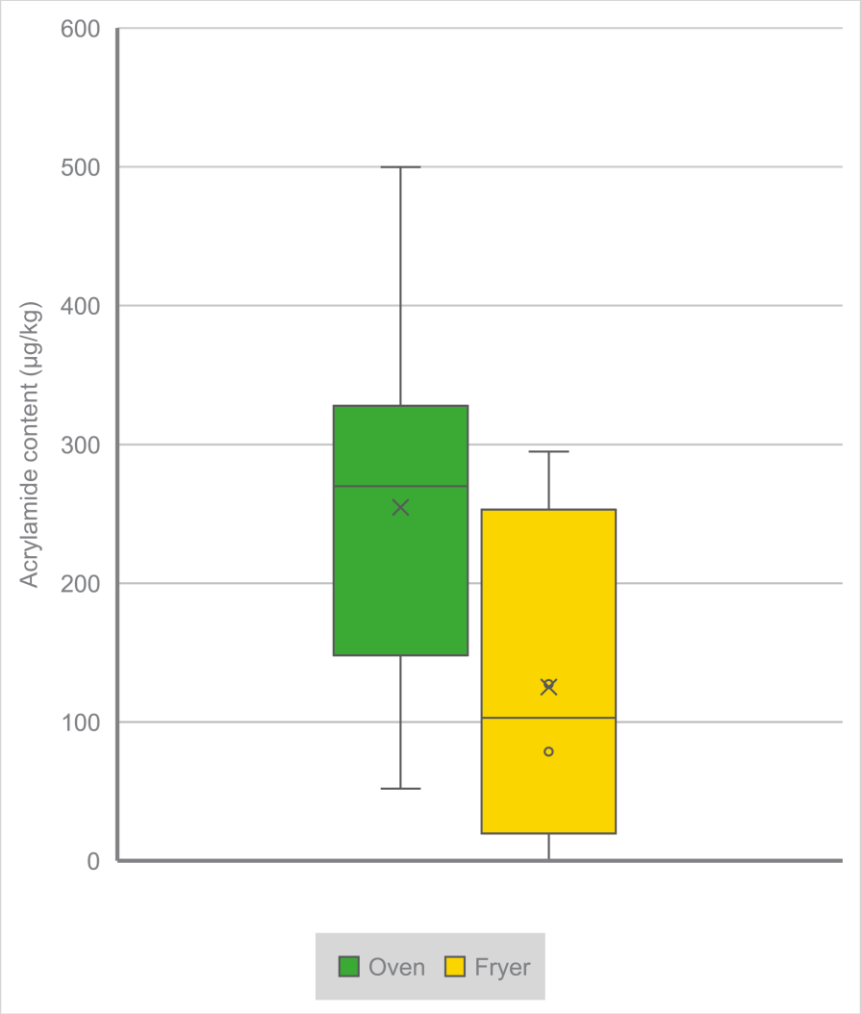

Figure 16. comparison of acrylamide content in croquettes according the mode of cooking

Conclusion:

Based on these results, it can be concluded that it is challenging to find a correlation between the cooking parameters and the AA content detected in the prepared food as all participants possessed different oven models oven and use different cooking programs such as rotating heat. Nevertheless, the most important finding is that participants respect in majority the instructions on the label, especially the temperature. Even if the cooking time is not respected, most participants stop the cooking based on the golden color of croquettes. If temperature and adequate color are respected, acceptable levels of AA are found. The non-respect of cooking time is not considered to be a problem and depends more on characteristics inherent to model of oven/fryer used.

3 PREPARATION OF SWEET POTATO FRIES COOKING

A total of 8 samples of fries have been analyzed, 4 with a fryer and 4 in oven. **Table 27** summarizes all cooking parameters and the detected AA content. Most of the participants declared that they are not familiar with sweet potato fries. Hence, it was requested to attempt several times in order to obtain the adequate color and texture (according to their own taste).

Table 27. Cooking parameters and acrylamide content of fries cooked by participants

Participant	Cooking mode	T°(°C)	Time (min)	AA Content (µg kg^{-1})
2	Fryer	170	6.5	34.5
9	Fryer	170	6.3	35.1
10	Fryer	180	2.0	134
3	Fryer (air-)	180	12.0	179
3	Oven	220	20.0	166
8	Oven	220	11.0	<LOD
12	Oven	220	17.0	76.3
11	Oven	220	16.0	59.3

Sweet potatoes fries – fryer cooking:

One candidate used an air-fryer. As this cooking mode is different from classical fryer, it is difficult to include it for comparison. Cooking instructions, available on the packaging, were a cooking temperature of 175 °C during 2.5-3 min. Fifty percent of the participants used a cooking temperature of 170 °C, the others applied 180 °C. Nobody followed the Recommendation of the cooking temperature. This could be explained by the absence of this graduation on devices. Nevertheless, only low AA concentrations were determined, even for participants that applied very long cooking times. Furthermore, none of the results exceeded the benchmark level of 500 µg kg^{-1} as stated in the Regulation (EU) 2017/2158 for French fries, which was considered to be the closest food category to sweet potato fries. Plotting the cooking time versus AA content has not been judged necessary as it will result in the same observations as discussed for the croquettes. A good correlation was found between the color of the fries and its AA content. For instance, pictures of candidate 2 are very light and the lowest AA content (34.5 µg kg^{-1}) was detected while, the fries of participants 3 were slightly burned on the extremities and they contained the highest amount of AA.

Sweet potatoes fries – oven cooking:

For the preparation of sweet potatoes in an oven, the same order of magnitude of AA content was found as for the fryer. The preconized temperature was 220 °C and the cooking time 15-20 min. All participants respected indications for temperature and time, except the candidate 8 who baked fries during only 11 min. No AA was detected in this food item. By checking the questionnaire, the mode used for the preparation was very specific, i.e. “turbo pizza” specifically designed to get food brown and crusty. This description does not seem to link to the absence of AA. By checking the picture of this cooked sample (Annex 5), it can be seen that the sample is lightly orange, pale and absence of burned parts which could indicate that no AA has been generated during the short cooking time. Furthermore by checking the questionnaire more thoroughly, the participant declared that the taste was good but that the fries were

not crusty at all, which is coherent with the absence of AA. Therefore, it can concluded that it was logical that no AA was found. When comparing the pictures it can be seen that the most colored and burned fries (candidate 3, oven and fryer) are linked to the highest AA content. This confirms that the color is a good indicator of the AA content if we consider samples cooked at the same temperature

Sweet potatoes fries – comparison of cooking mode:

Figure 17 shows that there is no significant differences between cooking in the oven and in the fryer. No link was found between the AA content and the cooking parameters as they reflected more the quality of the equipment used during the cooking. Furthermore, AA concentration was low and in the same order of magnitude, regardless of the technique used. Therefore it was not relevant to continue further the interpretation of these results.

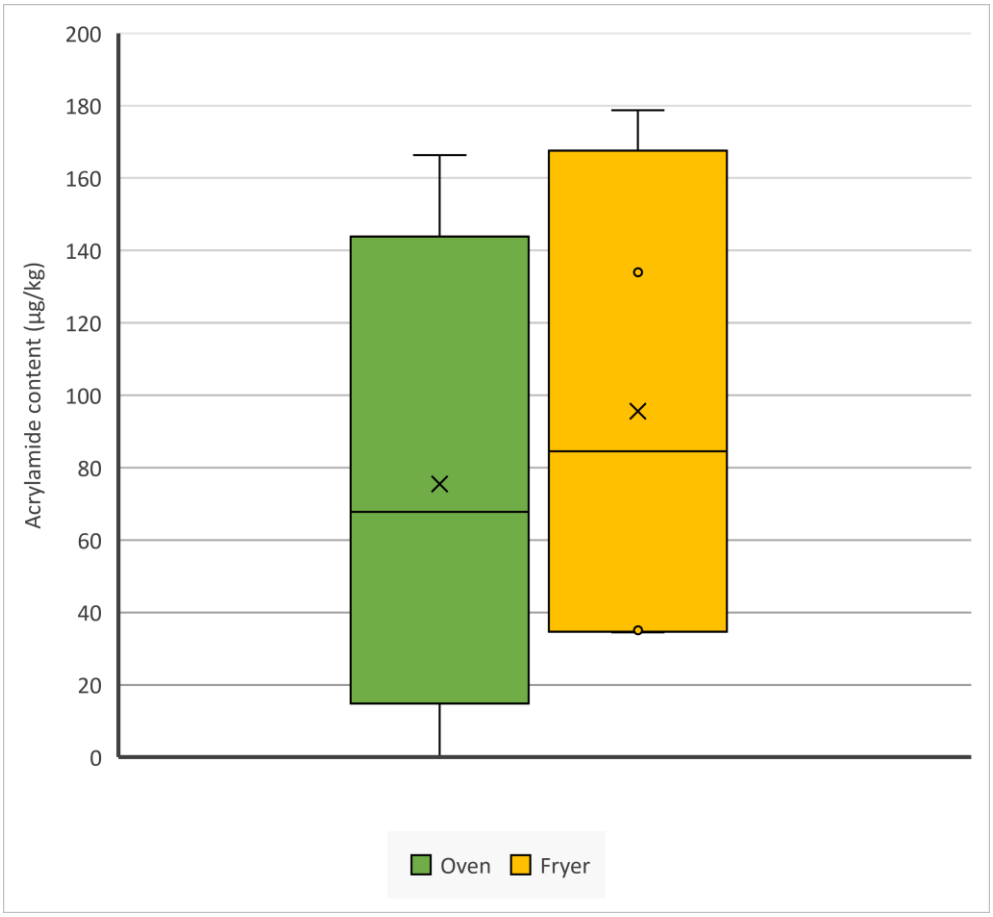

Figure 17. Comparison of acrylamide content in sweet potatoes fries according the mode of cooking

Conclusion:

The conclusion is similar to the evaluation of the croquettes. When consumers respect the golden colors and an adequate cooking temperature, safe AA content will be generated. It is not relevant to make a link between the cooking time and AA content. Cooking time is more reliant on cooking model devices, cooking programs and others non-controllable parameters.

4 PREPARATION OF BUNS

A total of 4 buns have been analyzed by participants since 2 participants were not able to cook the samples as they were too thick to enter in their toaster. Three of them have been cooked with a toaster, and 1 with oven in grill mode. No correlation can be made between cooking parameters and AA content as it depends strongly of the power of the device, the design of the device such as position and number of heating resistances. However, this food group enables to have an overview of the AA concentration that may be generated during buns cooking.

Table 28. Cooking parameters and acrylamide content of buns cooked by participants

Participants Number	Cooking mode	Cooking program	Time (min)	Conc. AA (µg kg^{-1})
7	Oven	grill	4	52.1
8	Toaster	Not mentioned	2	43.0
10	Toaster	4 on a scale between 1-8	1	< LOQ
13	toaster	4 on a scale between 1-6	-	24.9

As the **Table 28** shows, the maximal concentration found in buns was 52.1 µg kg^{-1} after 4 min of cooking in an oven with grill mode. This value is slightly higher than the benchmark level (50 µg kg^{-1}) for the closest regulated food category (wheat based bread). Nevertheless, attention must be paid before drawing conclusion as this benchmark level doesn't concern bread samples that follow a toasting process. Values obtained for this food group are very low considering the aggressive cooking mode (toaster/grill mode) as they reach a very high temperature (~300 °C) and generally burn the surface of the food item. However, it can be assumed that the burned surface is very thin in comparison with the whole volume of the bun. The AA content generated at this specific location is somewhat diluted in the total food item. Comparison between samples pictures and AA content can be made. For example, participant 7 and 8 obtained the highest AA concentration and pictures shown that these samples are clearly overcooked. Sesame seeds on the tops are roasted and the inner sides are brown. On contrary, the sample of participant 10 that has been toasted during only one minute does not show burning trace, nor brown color on its inner or outer sides, which is also reflected by its AA content (i.e. < LOQ). Sample 13 is barely above the LOQ level with 24.8 µg kg^{-1} of AA. When checking the picture, the outside surface does not seem burned, nor the sesame seeds, but the inside is clearly burned on a wide but distinct zone. Hence the toasting is not homogeneous and very local which can be explained by the design of the toaster (position of the heating resistance) and explained also why the AA content is low.

Conclusion:

Although only 4 participants participated to this small study, we can conclude that no abnormal AA content have been generated during the cooking of buns. The toasting is not always homogeneous and strongly depends of course of the design of the toaster (positions and number of heating resistance). But even If the food item is burned at some local points, it concerns a very thin surface in comparison of the entire volume of the buns in the same way that has been observed for pastries.

5 GENERAL CONCLUSION:

Although the number of subjects in the study was too low to extrapolate to the entire Belgian population, these results are still very interesting and informative.

It can be concluded that low concentrations of AA were generated and were in general far below benchmark levels for regulated food categories closest to food items studied (i.e. croquette, sweet potatoes and buns). Most participants respected the preconized temperature and generally stopped the cooking when a « golden color » is reached. They declare that this golden color is the most suitable for their taste, and luckily is also the preconized color in the Regulation (EU) 2017/2158.
This study highlights that the cooking time parameters in “domestic” conditions cannot be linked to AA content, but reflects more the quality of model cooking device, the cooking program used, the position in the oven, etc...

If temperature and color parameters are respected together by consumers, safe AA level should be reached no matter the cooking time followed and variation in other parameters. It was considered that situation is under control and not necessary to pursue this further investigation.

Exposure assessment

1 PREVIOUS INTAKE ASSESSMENTS BY EFSA

In 2015, the CONTAM Panel of EFSA performed an intake assessment for AA [1]. Occurrence data of the following categories were used for the intake assessment:

- Potato fried products (including potato crisps and snacks)
- Soft bread
- Breakfast cereals
- Biscuits, crackers, crisp bread and similar
- Coffee (dry)
- Coffee substitutes (dry)
- Baby foods, other than processed cereal based
- Processed cereal-based baby foods
- Other products based on potatoes, cereals and cocoa (i.e. porridge, cake and pastry, savoury snacks other than potato, other products based on cereals, other (non-fried) products based on potatoes, other products based on cocoa)
- Other products not based on potatoes, cereals, coffee and cocoa (i.e. roasted nuts and seeds, black olives in brine, prunes and dates, vegetable chips, paprika powder)

The results of the intake assessment is given in **Table 29**. It displays of the ranges (minimum, median and maximum) of the mean and 95th percentile exposure levels across the different surveys and age groups representing the general population.

Table 29. Exposure to AA in µk kg^{-1} bw day^{-1} (EFSA, 2015).

Age group	Mean		P95	
	Median [Minimum-Maximum]		Median [Minimum-Maximum]	
	LB	UB	LB	UB
Infants	0.8 [0.5-1.3]	1.0 [0.7–1.6]	1.8 [1.4–2.3]	2.1 [1.6–2.5]
Toddlers	1.3 [0.9-1.9]	1.4 [0.9–1.9]	2.3 [1.4–3.4]	2.4 [1.5–3.4]
Other children	1.2 [0.9-1.6]	1.2 [0.9–1.6]	2.2 [1.4–3.2]	2.3 [1.4–3.2]
Adolescents	0.7 [0.4–0.9]	0.7 [0.4–0.9]	1.4 [0.9–2.0]	1.4 [0.9–2.0]
Adults	0.5 [0.4–0.6]	0.5 [0.4–0.6]	1.0 [0.8–1.3]	1.0 [0.8–1.3]
Elderly	0.4 [0.4–0.5]	0.5 [0.4–0.5]	0.8 [0.7–1.0]	0.9 [0.7–1.0]
Very elderly	0.4 [0.4–0.5]	0.5 [0.4–0.5]	0.9 [0.6–1.0]	0.9 [0.6–1.0]

Infants, toddlers and other children were the most exposed groups. The mean exposure levels ranged from 0.5 (minimum LB) to 1.9 µg kg^{-1} bw day^{-1} (maximum UB), and the 95th percentile from 1.4 (minimum LB) to 3.4 µg kg^{-1} bw day^{-1} (maximum UB) depending on the age group. Adolescents, adults, elderly and very elderly had mean exposure estimates ranging from 0.4 (minimum LB) to 0.9 µg kg^{-1} bw day^{-1} (maximum UB), and the 95th percentile estimates from 0.6 (minimum LB) to 2.0 µg kg^{-1} bw day^{-1} (maximum UB) [1].

Other exposure assessment have also been conducted by other international bodies. The highest estimates were reported by JECFA, which concluded in 2005 that AA dietary exposure estimates were 1 µg kg^{-1} bw day^{-1} at the mean, and 4 µg kg^{-1} bw day^{-1} for a consumer at a high percentile of the distribution [43]. In 2010, these estimates were updated using data submitted to JECFA and taken from the literature. However, the JECFA further concluded that no changes had occurred in dietary exposure to AA between 2005 and 2010 [44].

In this context, the intake assessment conducted for Belgian population will be compared, as much as possible, with the results of the intake assessment by EFSA.

2 INTAKE ASSESSMENT

2.1. Maximum analytical concentrations scenario (MAC)

The results of the chronic exposure assessment to AA using the maximum analytical concentrations and the upper bound (UB) approach, are presented in **Table 30** and **Figure Figure** 18.

Table 30. Estimated exposure to AA (µg kg^{-1} bw day^{-1}) in the Belgian population using the maximum analytical concentration – UB.

Age (years)	N	Mean	P50	P95	P97.5	P99
3-9	737	0.102549	0.048949	0.368702	0.535999	0.844115
10-17	725	0.081844	0.037962	0.294502	0.427364	0.68441
18-64	922	0.049027	0.022255	0.178776	0.261278	0.409787

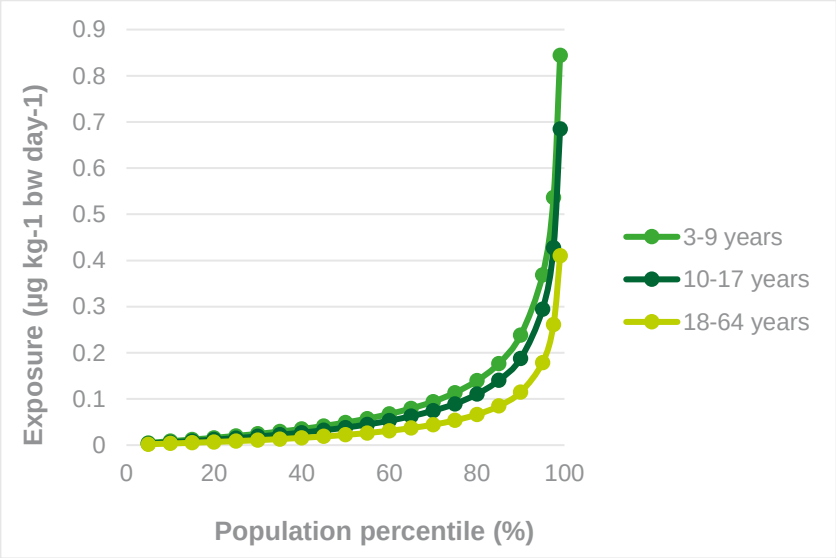

Figure 18. Estimated exposure to AA in the Belgian population (3-64 years) using the MAC-UB.

Children are the most exposed groups, followed by the adolescents and the adults are the least exposed. The mean exposure levels ranged from 0.05 (Adults) to 0.1 µg kg^{-1} bw day^{-1} (Children), while the P95 varies from 0.2 to 0.4 µg kg^{-1} bw day^{-1}. This is lower compared to the values reported by EFSA. This can be explained by the fact that in the Belgian intake assessment, very specific food categories were included. Indeed, the specific food items have been selected according to Recommendation (EU) 2019/1888 concerning the monitoring of the presence of AA in certain foodstuffs [6]. This Recommendation is complementary to Regulation (EU) 2017/2158 that includes Benchmark levels of the most important contributors to AA exposure. Therefore, the contribution to the exposure of the food items analysed in the framework of this project is expected to be lower. This is confirmed in the results, i.e. the exposure values reported in **Table 30** are lower compared to the values reported by EFSA (**Table 29**).

2.2. Mean analytical concentrations scenario (MeAC)

The chronic intake assessment was not only calculated using the maximum analytical concentration, but also with the mean analytical concentrations and the UB approach, are presented in **Table 31** and **Figure 19**.

Table 31. Estimated exposure to AA (µg kg^{-1} bw day^{-1}) in the Belgian population using the mean analytical concentration – UB

Age (years)	N	Mean	P50	P95	P97.5	P99
3-9	977	0.074691	0.040091	0.257338	0.356127	0.528146
10-17	918	0.061452	0.031795	0.21172	0.299192	0.448622
18-64	1201	0.036494	0.018391	0.129141	0.184227	0.271968

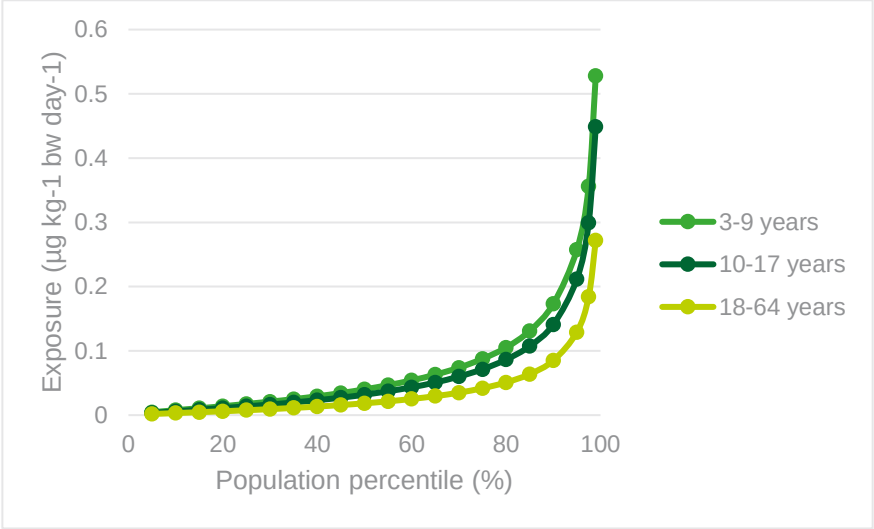

Figure 19. Estimated exposure to AA in the Belgian population (3-64 years) using the MeAC – UB.

The results of the chronic exposure assessment to AA using the mean analytical concentrations and lower bound are presented in **Table 32** and **Figure 20**.

Table 32. Estimated exposure to AA (µg kg^{-1} bw day^{-1}) in the Belgian population using the mean analytical concentration - LB.

Age (years)	N	Mean	P50	P95	P97.5	P99
3-9	977	0.064476	0.033559	0.21774	0.314809	0.46913
10-17	918	0.054103	0.027729	0.190381	0.271557	0.391734
18-64	1201	0.034512	0.016109	0.126278	0.180908	0.275419

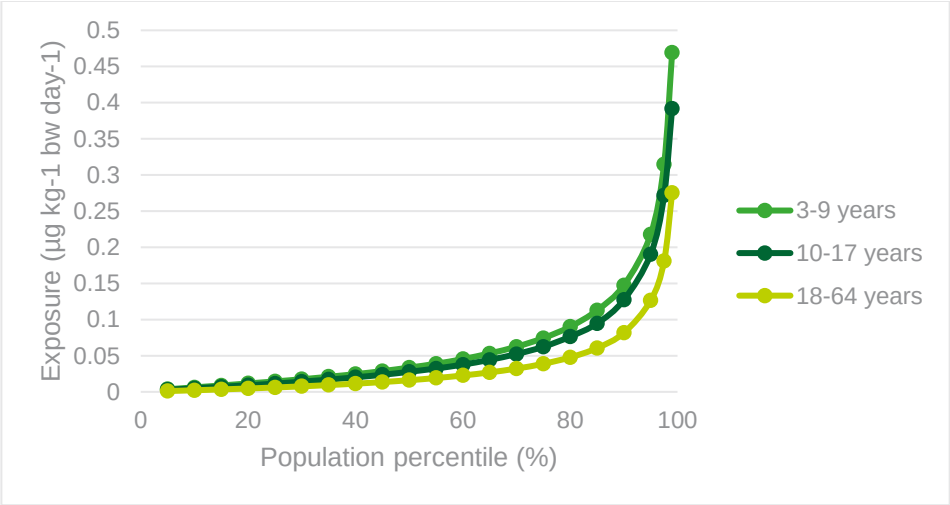

Figure 20. Estimated exposure to AA in the Belgian population (3-64 years) using the MeAC – LB.

Similarly to the intake assessment using the MAC, the children are also the most exposed group when the MeAC is used. The mean exposure levels ranged from 0.06 (LB) to 0.07 µg kg^{-1} bw day^{-1} (UB), and the 95th percentile exposure level from 0.1 (LB) to 0.3 µg kg^{-1} bw day^{-1} (UB). The exposure of the adolescents follows closely the exposure level of the children, while the adults are the least exposed group with a mean exposure level ranging from 0.03 (LB) to 0.04 µg kg^{-1} bw day^{-1} (UB) and the 95^{th} percentile exposure level of 0.2 µg kg^{-1} bw day^{-1} (LB & UB). It has to be noted that only very small differences are observed between the LB and UB. This can be explained by the fact that AA was detected in most food categories

that were included in this study and significant levels (>>> LOQ) were quantified. Furthermore, the food categories for which no AA was detected represent only a small fraction of the food consumption pattern of the Belgian population. As a result, the further interpretation of the results will be based on the UB scenario.

When comparing the results of the intake assessment to the results reported by EFSA, the same conclusion as for the intake assessment with the MAC can be drawn, i.e. the intake assessment is lower compared to EFSA, but the most important contributors for AA exposure were not included in this study (e.g. French fries).

3 IDENTIFICATION OF THE MAIN CONTRIBUTORS

The percentage contribution of the different food categories to the exposure were calculated for each age class based on the FoodEx2 hierarchy (level 1) for the intake assessment with the mean or maximum concentration levels. The results are illustrated in **Figure 18** and **Figure 21**.

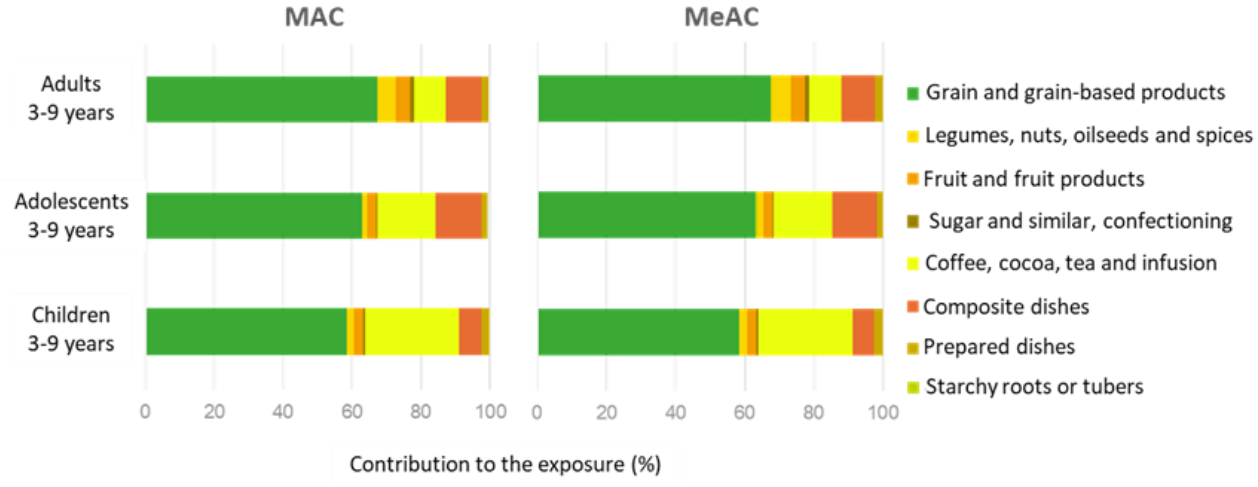

Figure 21. Relative contribution of the different food groups to the daily intake of AA.

The main contributors for the AA exposure are the grain and grain-based products. The relative contribution of this category is very similar for the three populations. However, for the second most important contributor is 'coffee, cocoa, tea and infusion' for the children and adolescents, while the composite dishes are most important for the adults, closely followed by coffee, cocoa, tea and infusion. In the intake assessment carried out by EFSA, the potato fried products were the main contributor, but it has to be noted, that the most important contributor in this category, i.e. French fries, were not included in this study. Another important contributor according to EFSA were the grain- and grain-based products which is confirmed by the results in **Figure 21**.

Risk assessment

1 ESTABLISHMENT OF REFERENCE POINTS FOR RISK ASSESSMENT.

The EFSA CONTAM Panel performed a risk characterization of AA. The data from human studies were not considered adequate for dose-response assessment, therefore, the CONTAM Panel considered the data from studies on experimental animals to establish reference points. Based on all the available data,

four possible critical endpoints (i.e. neurotoxicity, effects on male reproduction, developmental toxicity and carcinogenicity) for AA toxicity were further investigated and resulted in the establishment of two Reference points:

- Non-neoplastic effects for which a $BMDL_{10}$ value of 0.43 mg kg^{-1} bw day^{-1} was calculated for the most relevant and sensitive endpoint for neurotoxicity, i.e. the incidence of peripheral nerve (sciatic) axonal degeneration observed in F344 rats exposed to AA in drinking water for two years in the NTP study [45].
- Neoplastic effects for which a $BMDL_{10}$ of 0.17 mg kg^{-1} bw day^{-1},was calculated i.e. the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F1 mice exposed to AA for two years. Furthermore, the CONTAM Panel noted that the Harderian gland is an organ absent in humans, but that in rodents this organ is a sensitive target tissue to detect compounds that are both genotoxic and carcinogenic. Taking into account that target tissues for tumour formation by a given genotoxic carcinogen may differ between rodents and humans, the CONTAM Panel considered the most sensitive target tissue in rodent bioassays, the Harderian gland, a conservative endpoint for assessment of the risk for neoplastic effects of AA in humans.

The CONTAM Panel concluded that using the $BMDL_{10}$ for neurotoxicity as the reference point is conservative when considering possible non-neoplastic effects (effects on male reproduction, developmental toxicity).

The fact that AA and its metabolite GA are positive in a variety of genotoxicity tests indicates that AA is of concern with respect to genotoxicity. Therefore, the CONTAM Panel considered it inappropriate to establish a tolerable daily intake (TDI). The MOE approach was applied. According to the EFSA Scientific Committee, for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view [21]. A MOE of 100 is usually considered sufficient for non-genotoxic compounds to conclude that there is no health concern, unless there are major gaps in the toxicological database.

According to the EFSA Scientific Committee, a MOE of 100 is usually considered sufficient for non-genotoxic compounds to conclude that there is no health concern, unless there are major gaps in the toxicological database. However, for substances that are both genotoxic and carcinogenic, a MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view [21]. As a result, a MOE of 100 was considered for the evaluation of non-neoplastic effects, while a MOE of 10 000 was considered.

2 RISK CHARACTERIZATION

Risk characterization of AA was performed by comparing the exposure values for the three different age groups to the $BMDL_{10}$ for neurotoxic and neoplastic effects, i.e. 0.43 mg kg^{-1} bw day-1 and 0.17 mg kg^{-1} bw day^{-1}, respectively. MOE will be evaluated across age groups and using the maximum and mean analytical levels.

2.1. Neurotoxic effects

In order to calculate the MOE for neurotoxic effects, the $BMDL_{10}$ value of 0.43 mg kg^{-1} bw day^{-1} was used in combination with the data of the intake assessment included in **Table 30** and **Table 32**. It has to be noted that only the UB scenario was considered since the differences between LB and UB were considered negligible. The results are given in **Table 33** and **Figure 22**.

Table 33. MOE values for neurotoxic effects of AA, estimated for the Belgian population.

Age group	MeAC		MAC	
	Mean exposure	P95 exposure	Mean exposure	P95 exposure
3-9 years	5757	1671	6669	917
10-17 years	6997	2031	7948	1098
18-64 years	11783	3340	12456	1561

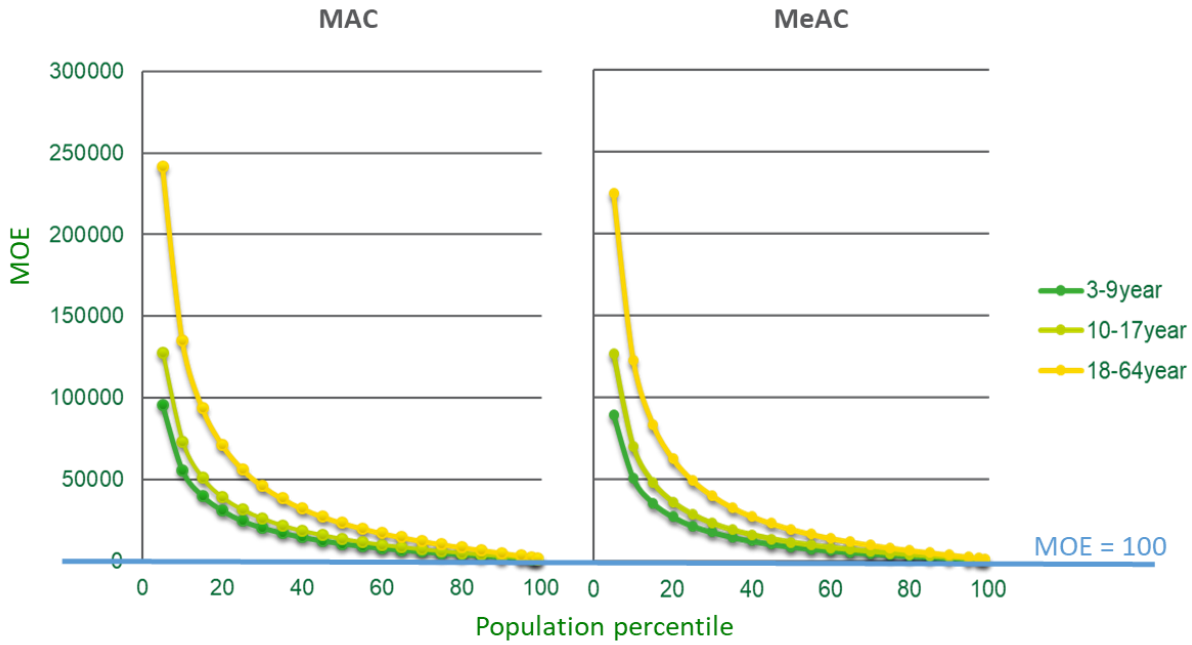

Figure 22. MOE for neurotoxic effects of AA using the exposure values based on MAC and MeAC

For neurotoxic effects, a MOE of 100 is considered to be sufficient to conclude that there is no health concern. Since all MOE values are well above 100, it can be considered that exposure to AA is not of concern regarding neurotoxic effects. This is in accordance with the conclusion of EFSA [21]. However, it must be noted that EFSA also mentioned that the MOEs for the 95th percentile for children are close to the value that might be of concern. This is also the consequence of the selection of the food matrices that was in accordance with Recommendation (EU) 2019/1888 and does not consider the highest contributors to AA exposure.

2.2. Neoplastic effects

In order to calculate the MOE for neoplastic, the $BMDL_{10}$ value of 0.17 mg kg^{-1} bw day^{-1} was used in combination with the data of the intake assessment included in **Table 30** and **Table 32**. It has to be noted that only the UB scenario was considered since the difference between LB and UB were considered negligible. The results are given in **Table 34** and **Figure 23**.

Table 34. MOE values for neoplastic effects of AA, estimated for the Belgian population based on MeAc exposure scenario.

Age group	Mean		Mac	
	Mean exposure	P95 exposure	Mean exposure	P95 exposure
3-9 years	2276	661	1658	461
10-17 years	2766	803	2077	577
18-64 years	4658	1316	3468	951

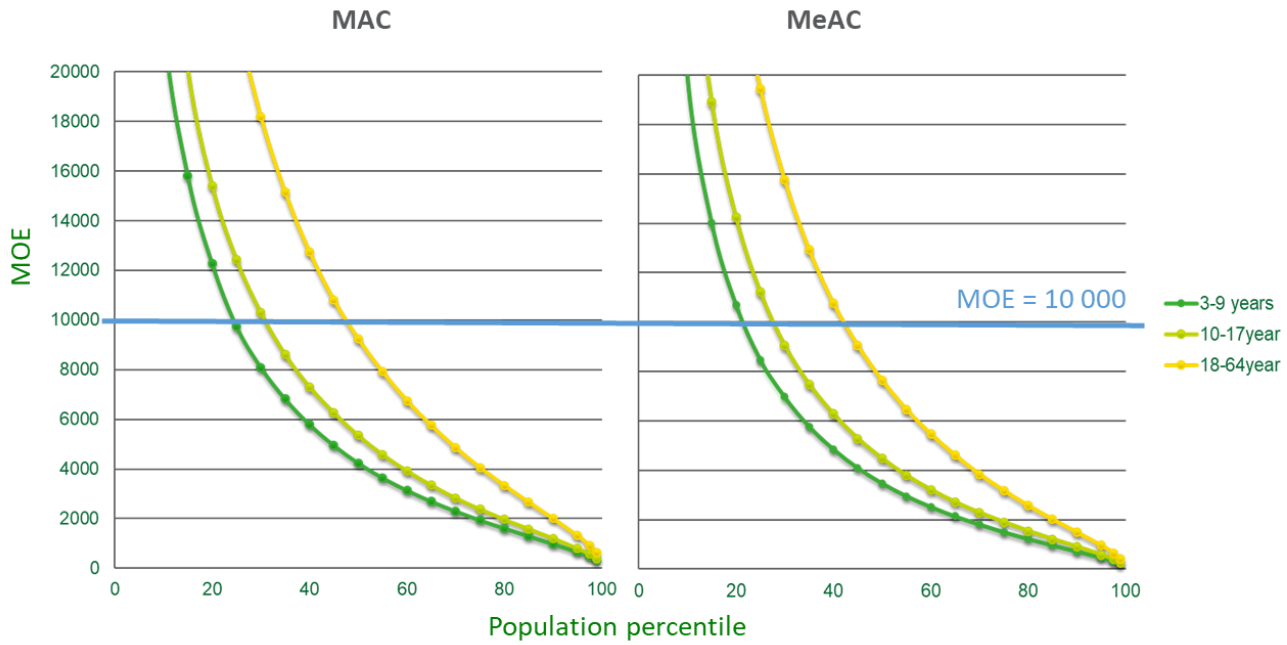

Figure 23. MOE for neoplastic effects of AA using the exposure values based on MAC and MeAC.

For the neoplastic effects of AA a MOE of 10 000 or higher would be of low concern from a public health perspective [1]. Since the MOEs are all substantially lower than 10 000, it should be concluded that although the available human studies have not yet demonstrated AA to be a human carcinogen, the MOEs based on the current levels of dietary exposure to AA indicate a concern with respect to neoplastic effects. This is completely in accordance with the conclusion of EFSA.

CONCLUSION AND RECOMMENDATIONS

In the project 'acrylamide in other foods', monitoring of acrylamide has been performed as requested by Recommendation (EU) 2019/1888 [6] and the EFSA call of 2019 [8]. In order to supplement the risk assessment on dietary exposure to acrylamide, occurrence data for acrylamide in less studied food groups were investigated, followed by an intake assessment and subsequent risk assessment.

In total, 217 food items were purchased on the Belgian market and analyzed using sensitive, validated analytical methods. Overall, the determined concentration were in accordance with the EFSA opinion of 2015 [1] and other scientific studies. However, some food groups need particular attention or need to be investigated further and they are advised to be included in the European Regulation. For example, very high acrylamide levels were detected in vegetable chips. Moreover, 3 out of 8 samples have been notified to the FASFC since their concentration was above 1500 µg kg^{-1}. Also, black olives prepared according to "Californian style" process should be considered for further investigation.

The samples selected in this project have allowed the comparison of different preparation modes on acrylamide formation. When the potato based products were prepared by frying, the acrylamide concentration were up to 25 times higher compared to the preparation in the oven. Based on this study, it was demonstrated that the preparation should be stopped when a 'golden color' is obtained. This is also recommended in Regulation (EU) 2017/2158 [10].

Afterwards, the analytical results (i.e. mean and highest concentration in every food category) were used for a dietary intake assessment of the Belgian population for AA by combining them with the results of the latest Belgian Food Consumption Survey in a lower and upper bound scenario. Overall, it can be concluded that the exposure to AA is lower compared to the intake assessment reported by EFSA [1]. This originates from the food items analysed in the framework of this study since the major contributors included in Commission Regulation (EU) 2017/2158 [10] and these foods were not analysed. Next, the major contributors among the analysed food products were determined. For all age groups, the intake assessment originates mostly from grain- and grain-based products.

Finally, the risk associated with dietary exposure to AA was evaluated for the Belgian population. As AA is potentially genotoxic, no Tolerably Daily Intake (TDI) could have been established. Therefore, the risk characterization was performed using the Margin of Exposure approach (MOE). Two reference points were considered as critical. (i) Non-neoplastic with a $BMDL_{10}$ value of 0.43 mg kg^{-1} bw day^{-1} and (ii) Neoplastic effects with a $BMDL_{10}$ of 0.17 mg kg^{-1} bw day^{-1}. For both reference values, the MOE was calculated using the exposure data of the mean and maximum occurrence levels. For neurotoxic effects, all MOEs were well above 100, so it can be concluded that exposure to AA is not of concern regarding neurotoxic effects. However, for the neoplastic effects, almost all MOEs were almost all substantially lower than 10 000, it should be concluded that although the available human studies have not yet demonstrated AA to be a human carcinogen, the MOEs based on the current levels of dietary exposure to AA indicate a concern with respect to neoplastic effects.

The results obtained in this study are completely in accordance with the conclusions of EFSA.

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