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ACRYLAMIDE CONTENT IN FOODS AND FACTORS THAT INFLUENCE THE ACRYLAMIDE CONTENT IN HEATED FOODS

AKRÜÜLAMIIDI SISALDUS TOITUDES JA FAKTORID, MIS MÕJUTAVAD KUUMTÖÖDELDUD TOITUDE AKRÜÜLAMIIDI SISALDUST

Final Thesis
Curriculum in Veterinary Medicine

Supervisor: Associate Professor Terje Elias, DVM, PhD

Tartu 2023

www.emu.ee
Eesti Maaülikool
Estonian University of Life Sciences

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Abstract of Final Thesis

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Curriculum: Veterinary Medicine

Title: Acrylamide content in foods and factors that influence the acrylamide content in heated foods

Pages: 46	Figures: 2	Tables: 10	Appendixes: 1

Chair: Chair of Veterinary Biomedicine and Food Hygiene
Field of research and (CERC S) code: 3. Health, 3.2. Veterinary Medicine
B750 Veterinary medicine, surgery, physiology, pathology, clinical studies
Supervisor(s): Terje Elias
Place and year: Tartu 2023

Acrylamide (AA) is a thermal processing contaminant found in many fried and heat-treated foods. It is defined as a probable human carcinogen and neurotoxin. Most of the human population is exposed to at least some amount of AA from their diet, making it an important food contaminant to be studied. European Commission has determined benchmark values for wide product groups to control AA concentrations. Benchmark values are values used to control the effectiveness of acrylamide risk reduction measures for different product groups, and exceeding these values does not result in prohibiting the marketing of the foods. The aim of the study was to evaluate the AA concentrations in different food products and to compare these results to the benchmark values set by European Commission regulation (EU) 2017/2158. Also, to give an overview of different mitigation strategies to decrease the AA concentrations.

287 samples were analyzed in the Tartu laboratory of the Health Board with a liquid chromatographic method with tandem mass spectrometric detection. Results were compared to the benchmark values. The results give examples of AA concentrations for commonly consumed foods from Estonian retail markets. It was found that 10.3% of the tested products, which had a benchmark value set by the European Commission, exceeded the recommended AA concentration. Potato chips and French fries categories had the highest number of products exceeding the benchmark value. The results were mostly in line with findings from previous studies around Europe. From baby foods analyzed AA concentrations were found to be lower when compared to a previous study from Estonia. High variations in AA concentrations among the same product group but between individual samples were found. New studies are necessary to study the development of AA concentrations, and larger sample sizes would increase the accuracy of the results.

Keywords: Acrylamide, benchmark values, legislation, mitigation strategies

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Lõputöö lühikokkuvõte

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Õppekava: Veterinaarmeditsiin

Pealkiri: Akrüülamiidi sisaldus toitudes ja faktorid, mis mõjutavad kuumtöödeldud toitude akrüülamiidi sisaldust

Lehekülgi: 46	Jooniseid: 2	Tabeleid: 10	Lisasid: 1

Õppetool: Veterinaarse biomeditsiini ja toiduhügieeni õppetool
ETIS-e teadusvaldkond ja CERC S-i kood: 3. Terviseuuringud, 3.2 veterinaarmeditsiin
B750 Veterinaarmeditsiin, kirurgia, füsioloogia, patoloogia, kliinilised uuringud
Juhendaja(d): Terje Elias
Kaitsmiskoht ja -aasta: Tartu 2023

Akrüülamiid on toidu termilise töötlemise käigus tekkiv saasteaine, mida leidub paljudes praetud ja kuumtöödeldud toitudes. AA on IARC klassifitseerinud kui inimesele tõenäolise kantserogeeni ja neurotoksiini. Suurem osa inimestest puutub suuremal või vähemal määral toidust pärineva AA-ga kokku, seetõttu on tegemist olulise saasteainega, mille sisaldust toidus peab analüüsima. Euroopa Komisjon on kehtestanud erinevatele tootegruppidele AA sisalduse kontrollimiseks võrdlusväärtused. Võrdlusväärtused on erinevate tooterühmade AA riskivähendusmeetmete tulemuslikkuse kontrollimiseks kasutatavad väärtused, mille alusel ei keelustata võrdlusväärtusi ületanud toitude turustamist. Lõputöö käigus läbi viidud uuringu eesmärk oli hinnata AA sisaldust erinevates tooterühmades ning võrrelda tulemust Euroopa Komisjoni määrusega (EL) 2017/2158 kehtestatud võrdlusväärtustega. Samuti oli eesmärk anda ülevaade erinevatest AA kontsentratsioonide vähendamise võimalustest.

Tartu Terviseameti laboris analüüsiti 287 toiduproovi akrüülamiidi sisaldust. Analüüsid teostati kasutades Eesti Akrediteerimiskeskuse poolt akrediteeritud meetodit T73-LC/MS/MS. Lõputöös kajastati 2019. ja 2020. aastal teostatud AA analüüside andmeid ning saadud tulemused grupeeriti tootegruppidesse ning võrreldi kehtestatud võrdlusväärtustega. Selgus, et 10,7% kõigist analüüsitud toodetest, millele Euroopa Komisjoni on kehtestatud võrdlusväärtused, ületasid AA võrdlusväärtusi. Enim võrdlusväärtust ületanud tooteid leiti kartulikrõpsude ja friikartulite kategooriast. Tulemused sarnanesid varasemate Euroopas tehtud uuringute tulemustega. Analüüsitud imikutoitudest leiti, et AA kontsentratsioonid on madalamad kui varasemalt Eestis teostatud uuringus. AA sisalduse erinevused olid tootegruppide siseselt erinevad, samas leiti suuri erinevusi sama nimetusega proovide vahel. AA kontsentratsioonidest parema ülevaate saamiseks erinevates tootegruppides on vajalik teostada täiendavaid uuringuid suuremate proovihulkadega, et tagada ülevaatlikumad andmed AA sisaldusest toitudes.

Märksõnad: akrüülamiidi sisaldus, võrdlusväärtus, AA vähendamise võimalused

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LIST OF ABBREVIATIONS

AA	acrylamide
$BMDL_{10}$	lower confidence limit for a benchmark response of 10%
CONTAM	Scientific Panel on Contaminants in the Food Chain
COSMIC	Catalogue of Somatic Mutations in Cancer
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
GA	glycidamide
GC	gas chromatography
IARC	International Agency for Research on Cancer
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
LC	liquid chromatography
LOQ	limit of quantification
MOE	margin of exposure
MS	mass spectrometry
SNFA	Swedish National Food Administration
3-APA	3-aminopropionamide

INTRODUCTION

Acrylamide (AA) is a potentially carcinogenic crystalline compound formed by a heat-induced process in starchy foods. AA is mainly formed by a process called the Maillard reaction in foods when cooked at temperatures over 120 °C and in the presence of amino acids and reducing sugars (Koszucka *et al*., 2020). International Agency for Research on Cancer (IARC) has classified AA as a probable human carcinogenic agent (Rifai and Saleh, 2020). AA has been reported to cause adverse effects such as neurotoxicity, carcinogenicity, genotoxicity, teratogenicity, and reproductive toxicity (Bušová *et al*., 2020). These findings have led the EU to pass regulatory legislation considering AA concentrations in foods. This has formed a need for quick and accurate analytic methods for the detection and quantitative analyses of AA concentrations in different products (Cantrell and McDougal, 2021).

Food consumption is exposing large populations to AA, and one-third of the energy intake of humans is said to contain AA (Bušová *et al.,* 2020). European Food Safety Authority (EFSA) (2011) stated that the largest contributors to adult AA dietary intake are fried potato products, soft bread, and coffee. The aim of our study was to evaluate the AA concentrations in different food products which are commonly consumed and to compare these results to the benchmark values set by European Commission. This is to identify which product groups most often exceed the benchmark values. In the literature review, an overview is given of AA in food and possible reduction options, as well as possible health risks according to scientific literature.

Materials for the study were collected from Estonian retail markets as a part of a surveillance program of the Agriculture and Food Board and analyzed by the Tartu laboratory of the Health Board. AA concentrations were determined by a liquid chromatographic method with tandem mass spectrometric detection. Together 287 samples were analyzed.

1. LITERATURE REVIEW

1.1. Acrylamide – what is it?

AA (Figure 1) is one of the substances known as a thermal process contaminant, which means a toxic substance formed by heating (Rifai and Saleh, 2020). AA is an odorless and colorless compound having a molecular weight of 71.08 kDa. Already in 1994 industrial chemical AA was declared a probable carcinogenic agent (group 2A) and a neurotoxin by IARC (Koszucka *et al*., 2020; Cantrell and McDougal, 2021). Its presence in fried and cooked foods has become to interest the scientist and public more after 2002 when the Swedish National Food Administration (SNFA) found that lengthened heat treatment of foods created high amounts of AA. High concentrations of AA are formed in multiple different kinds of foods, especially in potato and grain products with high starch concentrations (Rifai and Saleh, 2020). Although AA is formed together with flavor and aroma-enhancing molecules in the Maillard reaction, AA itself is not a desirable compound (Cantrell and McDougal, 2021).

AA is not only a compound formed in foods during the heating process but also an industrial synthetic material. Before the SNFA reported their findings, AA was thought to be a hazard mainly from occupational exposure for the people working with it as an industrial material to produce polyacrylamide, which is used in wastewater treatment and cosmetics, as well as in other productions. AA has also been found in cigarette smoke (Koszucka *et al*., 2020).

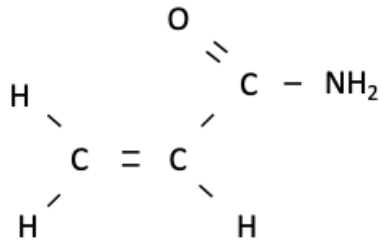

Figure 1. Chemical structure of AA (Arvanitoyannis and Dionisopoulou, 2014).

1.2. Acrylamide formation

1.2.1. Chemistry

AA is formed during the heating of foods; it is not present in raw foods readily. AA needs a temperature of 120°C or higher to form. Conditions that increase the amount of AA formed are higher temperatures and low moisture content of the food (Arvanitoyannis and Dionisopoulou, 2014; Pietropaoli *et al.*, 2022). Meat, fish, and chicken or boiled products do not seem to have AA (Arvanitoyannis and Dionisopoulou, 2014). Factors that also affect the formation of AA include the cooking method, length of heating, and composition of the food. The highest concentration of AA forms when heating raw material which has high asparagine and glucose content to 180°C (Bušová *et al*., 2020). The main pathway for AA formation is the so-called Maillard reaction, which includes a series of complex reactions taking place during heating. Maillard reaction is the one producing the brown color, crust, and known flavor for tasty baked, fried, and toasted foods (Rifai and Saleh, 2020). Two main components involved in the reaction are asparagine which is an amino acid, and the second component is reducing sugar: glucose, or fructose for example (Pietropaoli *et al.*, 2022; Rifai and Saleh, 2020). Plant-origin foods are often high on AA due to the presence of these components (Koszucka *et al*., 2019). Processing methods seem to have a significant role in determining the amount of AA formed in the final product, as it has been found that foods belonging to the same product category can have very different concentrations of AA (Arvanitoyannis and Dionisopoulou, 2014; Elias *et al*., 2017; Michalak *et al.,* 2019).

AA formation through the Maillard reaction starts by reducing sugars and amino groups interacting with each other forming Schiff bases. These bases then form Amadori or Heyns products by rearrangement. Azomethine ylide and 3-aminopropionamide (3-APA) can be further decarboxylated or hydrolyzed from Schiff bases. AA can then be formed from either of these products, from 3-APA by deamination or from azomethine ylide directly (Mollakhalili-Meybodi *et al*., 2021). Other pathways for AA formation have also been described, these include a reaction between aspartic acid and proteins, thermal degradation of amino acids and proteins, and decarboxylation and deamination of asparagine (Koszucka *et al*., 2020). Maillard reaction is mostly taking place at the surface of the product, leading to the AA to be found mainly on the bread crust and not so much in the crumb for example. This can also explain why potato chips are so high in their AA content partly due to the large surface area. The color of

the product is often also indicative of the AA content, the darker the color, the higher the concentration (Rifai and Saleh, 2020).

1.2.2. Factors affecting the formation of acrylamide

The key requirements for the AA formation in foods are that asparagine and reducing sugars are present in temperatures higher than 120°C and that moisture content is low at the surface of the food (Rifai and Saleh, 2020). Other factors that have an effect include the pH, product composition, and the type of raw material (Koszucka *et al.*, 2020). The composition of free asparagine and reducing sugars in the raw material are affected by the crop type and the cultivation and harvest seasons. Rye, einkorn, emmer, wheat, and spelt have been found to have higher asparagine content from different wheat species cultivated in the same environmental conditions. Higher content of protein and amino acids has led to a higher concentration of AA in the final product. When it comes to storage conditions, high temperature, and high humidity have been proposed to lead to increased enzymatic activity favoring AA formation, as well as increased availability of reducing sugars in damaged starch that increases the AA concentration in the final product (Mollakhalili-Meybodi *et al*., 2021).

1.3. Health risks

1.3.1. Toxicity of acrylamide

In general, toxicity is caused when the antioxidant and oxidant ratio is not balanced leading to oxidative stress in an organism. The toxic effects of AA are caused by its capability to interact with nucleophilic residues, from which cysteine is especially favored. These nucleophilic residues with cysteine include DNA and cellular proteins (Koszucka *et al*., 2020). Antioxidants can reduce both oxidative stress and breaks in DNA strands (EFSA, 2022).

1.3.2. Toxicokinetics

AA can be absorbed through the skin, respiratory tract, and digestive system. Data on toxicokinetics in humans is minor, but more data about AA distribution has been gathered from rats. In rats, when AA is absorbed through the oral route its distribution in the tissues was the following: 48% in the muscle tissue, 15% in the skin, 12% in the blood, 7% in the liver, and less than 1% in the brain and spinal cord (Matoso *et al*., 2019). Due to its small size and its

hydrophilicity AA is capable of passive diffusion in the body (Koszucka *et al*., 2020). Forms in which AA is found in the body include the original form of AA, metabolites of mercapturic acid, and DNA and hemoglobin adducts (Zhu *et al*., 2021). Most of the ingested AA is oxidized through CYP2E1 into glycidamide (GA) in the liver, which is a highly reactive epoxide (Esposito *et al.*, 2017). GA is considered the substance responsible for carcinogenicity and genotoxicity (EFSA, 2022). Neuro-, reproductive- and genotoxicity caused by GA, is caused via its capability of combining with DNA strands and causing oxidative stress (Song *et al*., 2021).

Detoxification of both AA and GA happens through conjugation to glutathione (Klaunig, 2008; Matoso *et al*., 2019), some of the GA is also detoxified via hydrolysis (Klaunig, 2008). Glutathione is resulting in mercapturic acid metabolites and is excreted through the urinary tract (Besaratinia and Pfeifer, 2007). In toxicokinetic studies of AA, mice have shown lower detoxification levels compared to rats. Detoxification was measured as AA-glutathione conjugates in excreted urine. Mice had also a 3-10 times higher formation of GA hemoglobin adducts with the same dose of administered AA when compared to rats (Besaratinia and Pfeifer, 2007).

AA is also formed endogenously in the body, and this should be considered when evaluating the effects of exogenous AA. In demonstrations, endogenous AA formation is lower than the exposure through the diet. Both should be considered for the total exposure (EFSA, 2022). Hemoglobin adducts of AA have been used as a dose marker for exposure (Besaratinia and Pfeifer, 2007; Pietropaoli *et al.*, 2022). Klaunig (2008) conclude that with experimental animals' linear dose-responsive relationship between administered AA or GA and hemoglobin adducts has been witnessed. The excretion of AA is mainly through the urinary route as glutathione conjugates or as unchanged AA (Besaratinia and Pfeifer, 2007), other routes as feces and exhaled air are also described as excretion pathways. The half-life of AA in humans is about 4-6 hours (Matoso *et al*., 2019).

1.3.3. Neurotoxicity

Worries about possible neurotoxic effects of AA have risen from the neurotoxicity seen in workers that have been predisposed to AA either through the skin or via inhalation. The same findings about cumulative toxic effects on neurological functions and peripheral neuropathy

have been witnessed in laboratory animals (Lindeman *et al*., 2021). In both humans and animals, peripheral nerve damage has been found after long-term exposure to AA (Zhu *et al.,* 2021).

1.3.4. Genotoxicity and carcinogenicity

Carcinogenesis is a multistage process, that begins from a single mutated cell. This cell is produced from damaged DNA. DNA damage can follow from spontaneous mutations or genotoxic compounds. Mutations in the DNA are irreversible processes, it is still possible that the mutated cells are removed by apoptosis before leading to cancer (Klaunig, 2008). AA-induced carcinogenicity is mainly caused by its metabolite GA. It is also suspected that AA might have carcinogenic properties through changes in hormonal balances (Pelucchi *et al.,* 2015).

Studies conducted on mice and rats have shown DNA damage from AA in multiple organs and in lymphocytes, spermatocytes, and spermatozoa. Additional information from studying the mutations was gathered from whole genome and exome sequencing. Regarding these studies, a specific AA/GA mutational signature has been found. When this signature was compared to the Catalogue of Somatic Mutations in Cancer (COSMIC) database, similarities were detected with some signatures found in the human breast, lung, ovary, and pancreas tumors (EFSA, 2022). Johnson *et al*. (1986) stated that rodents receiving high amounts of AA (2.0 mg/kg per day) had increased mortality, increase in several tumor types, and peripheral neuropathy. Statistical significance between AA intake and an increase in tumors was not witnessed in groups receiving lower doses (Johnson *et al*., 1986). Klaunig (2008) also stated that both rats and mice receiving high concentrations of AA through drinking water had an increase in tumors in multiple organ sites.

The carcinogenicity of AA in population-based studies has not yet given any clear answers. For example, many cohort studies have not been able to prove an increase in cancer risk with the higher consumption of AA (Zhu *et al*., 2021). Population-based studies through questionnaires face problems due to the uneven concentrations of AA in the same groups of food, as well as the fact that it is found in so many different food products (Besaratinia and Pfeifer, 2007). Two cohort studies in the 1980s were conducted to evaluate the cancer risk in workers that were occupationally exposed to AA. No increase in cancer risk was detected in either of the

populations (Sobel *et al*., 1986; Collins *et* al., 1989). From epidemiological studies, the relationship between cancer mortality and AA exposure is lacking evidence (Klaunig, 2008). EFSA (2015) states that the margin of exposure (MOE) causes concern about the neoplastic effects of AA, although there is no evidence from human studies to show AA as a human carcinogen. MOE is calculated by dividing the benchmark dose lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) with the daily intake of AA. $BMDL_{10}$ values are gathered from experiments in rats developing harderian gland adenomas with an AA intake of 0.17 mg/kg body weight daily (Barrios-Rodríguez *et al.,* 2021). In the case of carcinogenicity, MOE values above 10 000 are considered low risk, but in many studies, the MOE values with all studied products and population groups stay well below that value (Elias *et al.,* 2017; Esposito *et al.,* 2020; Barrios-Rodríguez *et al.,* 2021).

Pelucchi *et al.* (2015) conclude in their meta-analysis about AA dietary intake and related cancer risk that within most cancer sites, there is a lack of association. Although, in women with high AA intake that have never smoked, a potentially small increase in the risk of kidney, endometrial, and ovarian cancers was noted. Large prospective cohort studies were mostly used as sources. It is concluded that the subject needs further investigation (Pelucchi *et al.,* 2015). Breast cancer incidence and AA hemoglobin adduct levels have on the other hand shown a positive association (Klaunig, 2008).

1.3.5. Reproductive toxicity

Effects of AA on reproductive toxicity in rat includes a decrease in viability and amount of sperm cells as well as alterations in morphology (Zhu *et al*., 2021). Duarte-Salles *et al*. (2013) found a correlation between higher AA intake with decreased fetal growth in humans. Zhu *et al*. (2021) found the same correlation in rat embryo studies when the pregnant rats received AA in drinking water with doses of 5, 10, and 20 mg/kg body weight a day. Results from the high-exposure group showed a significant reduction in embryo body weight and length as well as the number of embryos (Zhu *et al*., 2021).

Studies considering AAs effects in human embryonic, fetal, and early childhood development are still missing (Lindeman *et al*., 2021). But it is known that AA can pass through the tissues to the fetus, and AA has been found in breast milk (Besaratinia and Pfeifer, 2007; Lindeman *et al*., 2021; Zhu *et al*., 2021). The dose of AA that the neonates are receiving is considered equal

to the mother when compared to the body size and relatively shorter lifespan of neonates' erythrocytes (Besaratinia and Pfeifer, 2007). Children are another group for concern as they consume more food adjusted to their body weight compared to adults and are so more likely to predispose to higher AA concentrations (Lindeman *et al*., 2021).

1.4. Legislation

In November 2017 EU Commission adopted commission regulation (EU) 2017/2158 which gives mitigation measures and benchmark levels for broad food categories to reduce the content of AA in food. Benchmark values are performance indicators and are based on experience and occurrence of AA in broad food categories. The regulation came into force in April 2018. AA is defined as a contaminant that is defined in Council Regulation (EEC) No 315/93, and in the food chain considered a chemical hazard. The Scientific Panel on Contaminants in the Food Chain (CONTAM) of EFSA gave its evaluation of AA. The main concern of AA is its possible carcinogenic effects, which have been found in animal studies. Concerns towards AA concentrations include all age groups, but children are the most affected when comparing dietary intake towards body weight. With the current dietary intake of AA, effects on the nervous system, pre-and post-natal development, and male reproduction were not considered a concern.

For controlling the AA concentrations in food products commission regulation (EU) 2017/2158 has set benchmark levels for food business operators for different food groups. Benchmark levels are for verifying the effectiveness of mitigation strategies, and these should be followed with regular sampling and analysis. Food categories to which benchmark values have been set include potato products (including French fries and chips), bread, breakfast cereals, bakery products, coffee (including instant coffee and coffee substitutes), and baby foods (including cereal-based foods that are meant for infants and young children). Benchmark levels in µg/kg for each food category are listed in Table 1. The highest benchmark value is for coffee substitutes prepared from chicory (4000 µg/kg) and the lowest value is for baby foods (40 µg/kg).

Table 1. Benchmark values of AA in different food groups (Commission regulation (EU) 2017/2158)

Food	Benchmark level (µg/kg)
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	
- Wheat based bread	50
- Other than wheat based soft bread	100
Breakfast cereals (excl. porrige)	
- Bran products, whole grain cereals, gun puffed grain	300
- Wheat and rye-based products	300
- Maize, oat, spelt and rice-based products	150
Biscuits and wafers	350
Crackers (excl. potato crackers)	400
Crispbread	350
Gingerbread	800
Products similar to the other products in this category	300
Roast coffee	400
Instant (soluble) coffee	850
Coffee substitutes	
- Exclusively from cereals	500
- Mixture from cereals and chicory	*
- Exclusively from chicory	4000
Baby foods, processed cereal based foods for infants and young children excl. biscuits and rusks	40
Biscuits and rusks for infants and young children	150

*Relative proportions of each ingredient must be considered when evaluating the benchmark value for a mixture of cereals and chicory

1.5. Acrylamide occurrence in foods

One-third of the energy intake of humans is said to contain AA, meaning that food consumption exposes large populations to AA (Bušová *et al.,* 2020). From food groups, the highest AA concentrations have been found in coffee substitutes (mean 1499 µg/kg) and vegetable chips (mean 1846 µg/kg). With coffee substitutes, chicory-based products have a lot higher mean value at 2942 µg/kg (EFSA, 2015).

AA concentrations have been studied in multiple countries and from many different food products (Hirvonen *et al.,* 2011; EFSA, 2015; Elias *et al.,* 2017; Michalak *et al.,* 2019; Bušová *et al.*, 2020; Mencin *et al.,* 2020). Some of these results are combined in Table 2. There are

large differences between different countries and the same food categories. With crisp bread, the lowest concentration is from the study made by Elias *et al.* (2017) in Estonia with a mean AA concentration of 108 µg/kg, and the highest from the study made by Hirvonen *et al.* (2011) in Finland with a mean AA concentration 674 µg/kg. Most even results are from cornflakes and baby foods, the difference between the highest and lowest mean concentration was 41 µg/kg or less. The product groups with the highest mean values were potato chips, a mean of 982 µg/kg, and biscuits and wafers, a mean of 679 µg/kg from a Czech Republic study made by Bušová *et al.* (2020). A high mean value was also found for crisp bread with 674 µg/kg from Finland in the study made by Hirvonen *et al.* (2011).

EFSA (2011) compared AA concentration trends from three years and concluded that only biscuits groups had values towards the lower mean and that crisp bread and instant coffee had increasing mean values. Many groups had no statistically significant differences and from some, the amount of data was too small (EFSA, 2011). In the study conducted by Mencin *et al.* (2020), they found that 49% of the samples had AA concentrations higher than the benchmark values for the specific group.

Table 2. Mean AA content in selected food groups from different countries

Food product	**Data origin**	**Mean AA (µg/kg)**	**Reference**
Potato chips	EFSA mean	389	EFSA, 2015
	Finland	286	Hirvonen *et al*., 2011
	Czech Republic	982	Bušová *et al*., 2020
	Estonia	529	Elias *et al*., 2017
	Poland	546	Michalak *et al*., 2019
French fries	EFSA mean	308	EFSA, 2015
	Finland	310	Hirvonen *et al*., 2011
	Estonia	299	Elias *et al*., 2017
	Poland	377	Michalak *et al*., 2019
Biscuits and wafers	EFSA mean	201	EFSA, 2015
	Finland (sweet/salted)	443/179	Hirvonen *et al*., 2011
	Czech Republic	679	Bušová *et al*., 2020

Table 2 continued

Food product	Data origin	Mean AA (µg/kg)	Reference
Biscuits and wafers	Estonia	242	Elias *et al*., 2017
	Slovenia	384.5	Mencin *et al*., 2020
	Poland	523	Michalak *et al*., 2019
Breakfast cereals	EFSA mean	161	EFSA, 2015
	Finland	100	Hirvonen *et al*., 2011
	Czech Republic	89	Bušová *et al*., 2020
	Estonia	196 *	Elias *et al*., 2017
	Slovenia	288.9	Mencin *et al*., 2020
	Poland	403	Michalak *et al*., 2019
Crisp bread	EFSA mean	171	EFSA, 2015
	Finland	674	Hirvonen *et al*., 2011
	Estonia	108	Elias *et al*., 2017
	Poland	312	Michalak *et al*., 2019
Soft bread	EFSA mean	42	EFSA, 2015
	Estonia (wheat based)	<30	Elias *et al*., 2017
	Slovenia	134.8	Mencin *et al*., 2020
	Poland	55	Michalak *et al*., 2019
Roast coffee	EFSA mean	249	EFSA, 2015
	Finland (light/dark)	15/5	Hirvonen *et al*., 2011
	Estonia	210	Elias *et al*., 2017
	Slovenia	185.5	Mencin *et al*., 2020
Baby food (excl. cereal based)	EFSA mean	24	EFSA, 2015
	Estonia (mainly vegetable/mainly fruit)	65/<30	Elias *et al*., 2017
Cereal based food for infants and young children	EFSA mean	73	EFSA, 2015
	Estonia	42	Elias *et al*., 2017

*Mean from three subgroups

1.5.1. Quantification methods

The most used methods for AA detection include gas chromatography (GC) and liquid chromatography (LC). Mass spectrometry (MS) is used together with both methods (Michalak *et al.*, 2019). Enzyme-linked immunosorbent assay (ELISA) has shown good sensitivity and high specificity for AA and is also much faster when compared to the GC and LC methods combined with MS (Arvanitoyannis and Dionisopoulou, 2014).

Michalak *et al.* (2019) concluded that AA concentration correlates with the color of potato products. This could be implemented in production lines to scan the coloring and in this way detect possible quality impairments. The color of the cereal-based products on the other hand did not correlate with the level of AA (Michalak *et al.*, 2019).

1.5.2. Dietary intake of acrylamide

EFSA (2011) concluded in their report on AA concentration monitoring that in Europe the dietary intake of AA for adults (>18 years) is between 0.31-1.1 μg/kg body weight/day, for adolescents (11-17 years) 0.43-1.4 μg/kg body weight/day, in children (3-10 years) 0.70-2.05 μg/kg body weight/day and in toddlers (1-3 years) 1.2-2.4 μg/kg body weight/day. The highest contributors to adults´ AA dietary exposure were fried potatoes, soft bread, and roasted coffee. In the case of adolescents, the highest contributors were fried potatoes, soft bread, and biscuits/potato crisps. Children and toddlers' highest exposure came from fried potatoes, soft bread, or unspecified bread and biscuits (EFSA, 2011). Similarly, JEFCA listed potato chips, French fries, coffee, bread, pastry, sweet biscuits, infant formula, and baby food as the most critical AA sources for humans (Peivasteh-Roudsari *et al*., 2022).
Timmermann *et al.* (2021) concluded that AA intake estimates are higher in Europe and the USA than in Asia. There is a high individual variation inside and between populations. Some of the factors that influence the individual dietary intake of AA include dietary practices such as recipes, consumption frequency, and portion size. Total dietary intakes have been evaluated based on dietary questionnaires and dietary records (Timmermann *et al.*, 2021). Hirvonen *et al.* (2011) concluded that the highest intake in the Finnish population is on 3-year-old children and the lowest in comparison with women aged between 65-74 years.

Young children, toddlers, and infants are a group with increased exposure and higher vulnerability toward AA. This is due to their lower body weight compared to the intake, preferences of certain food groups (for example potato products possibly high in AA), and their body's detoxification processes are not fully developed (Elias *et al.*, 2017). The main source of AA for humans is considered food, but other sources count as well. Cigarettes are also a source of AA, as mentioned earlier, in smokers this should be added to the total exposure (Esposito *et al.,* 2017).

It is suggestive that some of the dietary ingredients decrease AA toxicity in humans. Naturally occurring antioxidants such as garlic, wasabi, blueberry, blackberry, grape, rosemary, etc. have been studied to help to decrease the oxidative stress that AA is already causing in the organism. In the case of blueberry and blackberry, this is due to anthocyanin pigment that is protective against the mitochondrial dysfunction and apoptosis induced by AA. Garlic on the other hand has a radical scavenging function as well as a function to modulate an antioxidant defense system (Koszucka *et al*., 2020).

1.6. Mitigation strategies

1.6.1. Raw materials

The main strategies for AA mitigation rely on the modification of raw materials as removing precursors and pretreatment in optimized conditions (Baskar and Aiswarya, 2018). Significant for the AA formation later is the amount of reducing sugars and asparagine. In the case of potatoes, the content of reducing sugars is much higher than asparagine, decreasing reducing sugars content by selecting potato varieties with low content can help to decrease the AA concentration in the final product (Baskar and Aiswarya, 2018; Rifai and Saleh, 2020). Challenges are faced as the food must maintain its quality and desired properties (Baskar and Aiswarya, 2018).

Agronomic conditions have a high influence on the AA concentrations, from cultivation and properties of the soil to storage conditions, and climatic conditions play a role as well (Baskar and Aiswarya, 2018; Rifai and Saleh, 2020). The timing of the harvest has an impact; in the case of potatoes amount of reducing sugars is decreasing towards the end of the growing season. The optimum temperature for potato growing with the lowest sugar content has been proposed

to be 15-20°C. Temperatures above and below that tend to lead to higher reducing sugar content. Potato storage conditions for heat processing should target temperatures between 8-12°C. These temperatures have been shown to avoid the increase in reducing sugars. In temperatures below 8°C “low-temperature sweetening” takes place (Rifai and Saleh, 2020).

Managing nitrogen content during the growing period influences the asparagine content in the potato. A higher amount of nitrogen will lead to an increased amount of asparagine (Peivasteh-Roudsari *et al*., 2022). Storing products in dark, low temperatures and with low oxygen content has also been shown to decrease the final product's level of decreasing sugars. Another biological method could be breeding through genetic modification for lower concentrations of reducing sugar and free asparagine (Baskar and Aiswarya, 2018).

1.6.2. Additives

Additives or chemical methods include adding amino acids, acid solutions, asparagine enzyme solutions, salts, cations, and antioxidants aiming to decrease the AA concentration in food products (Baskar and Aiswarya, 2018). Results from using natural antioxidative extracts are unequivocal. Some additives showed a decrease in final AA concentrations, some had no effect and some even increased the AA concentration (Rifai and Saleh, 2020).

The Asparaginase enzyme is catalyzing the hydrolysis of asparagine to aspartic acid and ammonia. This enzyme has been used in fried potatoes, as the method also does not affect the final product (Rifai and Saleh, 2020). It is possible to obtain L-asparaginase from either plant, animal, or microorganism sources, with food and medical industries the microbial source is considered most appropriate (Peivasteh-Roudsari *et al*., 2022). Usage of the enzyme together with potatoes that have also been blanched beforehand (blanching at 40°C for 20 minutes), decreased the final AA concentration by 60% more compared to potatoes that were only blanched (Rifai and Saleh, 2020).

The addition of amino acids for reduction of AA concentration is based on either competition with asparagine in the Maillard reaction for carbonyl groups or reacting with already formed AA. In potato slice production lysine and glycine solutions soaking have shown an 80% reduction in AA concentration (Rifai and Saleh, 2020; Peivasteh-Roudsari *et al*., 2022). Baskar and Aiswarya (2018) claim that the AA concentration of the final product has been decreased

by 50% with the addition of non-asparagine amino acids. With potato chips, a 90% reduction has been achieved by soaking them in an acetic solution (20°C for 20 min) before frying (Baskar and Aiswarya, 2018). Adding vitamins B3 and B6 in the case of potato chips also leads to the inhibition of AA formation (Peivasteh-Roudsari *et al*., 2022). Salt solutions have also been shown to be effective in decreasing AA concentrations in the final product. Dipping potatoes in a solution of $CaCl_2$ gave a 95% reduction of AA and had no negative effect on sensory characteristics (Rifai and Saleh, 2020).

1.6.3. Processing conditions

Temperature, time, and moisture are known to affect the concentration of AA in the final product. Other factors that have an impact are pH, water activity, and fermentation. Lower pH, lower water activity, and lactic acid fermentation are less favorable to AA formation (Rifai and Saleh, 2020).

In fried potato products frying temperature and time have been found to be the most critical factors to reduce the formation of AA. An increase in either will lead to higher content of AA (Rifai and Saleh, 2020). In coffee bean roasting AA concentration was decreased with an increased degree of roasting. It seems that the AA concentration increases rapidly at the beginning of the roasting, but when continuing the concentration starts to drop. In the case of coffee, lower AA concentration follows longer roasting time and higher temperature, this on the other hand has a negative effect as off-flavor compounds are simultaneously formed (Peivasteh-Roudsari *et al*., 2022). With coffee, there are though other genotoxic compounds such as furans that increase in amount with a higher roasting degree (Hirvonen *et al.,* 2011).

Blanching of French fries before frying in specific conditions has been shown to remarkably decrease the concentration of AA (Baskar and Aiswarya, 2018). Blanching's effect is based on the reduction of reducing sugars in raw potatoes (Rifai and Saleh, 2020; Peivasteh-Roudsari *et al*., 2022). Blanching means placing the raw materials in either warm or hot water or steam for a period. Blanching potatoes before frying is often carried out to prevent enzymatic browning. Enzymes responsible for the browning reaction are inactivated during blanching (Peivasteh-Roudsari *et al*., 2022). Increasing blanching temperature and time lead to an increased reduction of AA (Rifai and Saleh, 2020; Peivasteh-Roudsari *et al*., 2022). Blanching can be used as an only method or combined with other methods for AA mitigation (Peivasteh-Roudsari *et al*.,

2022). Soaking also reduces the glucose content in the potato, which means fewer precursors of AA (Rifai and Saleh, 2020).

When it comes to fermentation, it is effective with potato and cereal products. Reducing sugars that leak from the potato will be metabolized by the yeasts resulting in fewer precursors for AA (Peivasteh-Roudsari *et al*., 2022).

1.6.4. Novel technologies

As AA mitigation in food products is an active subject in research there are many new technologies studied continuously. Some of these are air frying, genetic engineering, high-pressure processing, irradiation, pulsed electric fields, supercritical fluid extraction, ultrasound, and vacuum treatment (Peivasteh-Roudsari *et al*., 2022).

2. AIM OF THE THESIS

The aims of the study were to:

evaluate the AA content during two years in selected groups of foods and compare the results with benchmark values established in the Commission regulation 2017/2158/EU to identify product groups in which benchmark values have been exceeded;

give an overview according to the scientific literature on AA reduction options in food products.

3. MATERIALS AND METHODS

3.1. Tested foods

Samples were collected under the surveillance program of the Agriculture and Food Board in two years 2019 and 2020. Foods and products were collected from Estonian retail markets. Materials included coffee, French fries, bread, breakfast cereals, crackers, dried fruits, baby foods, olives, and peanuts. Products were categorized into the following groups: bread products, cereal products other than bread, potato products, coffee, baby foods, biscuit, wafers and crackers, dried fruits, dried berries, and nuts, and miscellaneous food products.

146 samples were collected in 2019 and 141 samples in the year 2020. A total of 287 samples were collected and analyzed for their AA concentration. Most of the products were from Estonia (170 samples), but the study also included products from Argentina, Austria, Belgium, Brazil, Chile, Finland, France, Georgia, Germany, Great Britain, Holland, Hungary, India, Italy, Latvia, Lithuania, Poland, Slovenia, Sweden, Switzerland, Turkey, United States, Ukraine, and Vietnam.

3.2. Laboratory methods

Analyses were performed in the Tartu laboratory of the Health Board. A liquid chromatographic method with tandem mass spectrometric detection was used to determine AA concentration in the samples. Samples were prepared with a QuEChERS (Quick Easy Cheap Effective Rugged Safe) method. The limit of quantification (LOQ) was 20 μg/kg.

3.3. Statistical analysis

The results of the AA concentration to a specific product were recorded in the MS Excel software for Mac version 16.71. Data management and determination of mean values were also performed in the MS Excel software. In the results, the lowest AA concentrations were reported as <20 μg/kg, these results are calculated in the middle-bound values as half of the LOQ level 10 μg/kg in the statistical analysis. Figure 2 was created in RStudio for Mac version 2023.03.0+386.

4. RESULTS

4.1. Bread products

Together 34 soft bread samples' AA concentrations were measured in the year 2019 and 29 samples in 2020. The results are presented in Table 3. The mean value with 2019 samples was 42.2 μg/kg and in the year 2020, the mean AA concentration was 69.7 μg/kg. Most of the concentrations were in the range of 21-100 μg/kg (21 samples in both years 2019 and 2020). The concentrations were compared to the Commission Regulation (EU) 2017/2158 benchmark value for group other than wheat-based soft bread. This benchmark value is 100 μg/kg. The benchmark value was exceeded by 5.9% of the samples from the year 2019 and 17.2% of the samples from the year 2020. The highest AA concentration in this product group was found in dark rye bread, produced in Estonia, with an AA concentration of 189 μg/kg. 14 of the soft bread samples had an AA concentration lower than LOQ (<20 μg/kg). Sweet pastries are presented as a separate product group in Table 3. These products include waffles, muffins, buns, cakes, donuts, and croissants. These are mostly wheat-based baked products. Among these products, there was high variation among the AA concentrations from the LOQ of <20 μg/kg to the highest concentration of 195 μg/kg found in carrot muffin (2019).

Table 3. AA concentration in soft bread

Year	Food Group	(n)[a]	Mean (μg/kg)	Samples with concentration ranges (μg/kg)			Benchmark value (μg/kg) *	Limit exceeded (%)
				<20	21-100	>100		
2019	Soft bread	34	42.2	11	21	2	100	5.9%
2020	Soft bread	29	69.7	3	21	5	100	17.2%
2019	Sweet pastries	4	94.5	1	1	2	-	-
2020	Sweet pastries	7	26.4	4	3	-	-	-

∗ Commission regulation (EU) 2017/2158
[a] n: number of samples

4.2. Cereal products other than bread

In cereal products other than bread group are collected results from AA concentration of mainly breakfast cereals. These results are shown in Table 4. Samples were further divided into two groups: bran products with whole grain cereals, and maize-based products. The benchmark value for whole grain cereal bran products is 300 µg/kg, this was not exceeded by any product in the samples from 2019. However, one of the 13 analyzed samples from 2020 exceeded this value. Together seven maize-based cereal products were analyzed, six from 2019 and one from 2020. The mean AA concentration for products from 2019 was 123.5 µg/kg, which is lower than the benchmark value of 150 µg/kg. None of the analyzed samples from maize-based cereal products exceeded the benchmark value.

In 2019 two various cereal ("kama") products were analyzed as well. These are presented at the end of Table 4. There are no benchmark values directly established for these products. The two products' AA concentrations varied a lot, the "kama" flour AA concentration was 76 µg/kg, and the "kama" balls concentration was 585 µg/kg.

Table 4. AA concentration in cereal products other than bread, and breakfast cereals

Year	Food Group	(n) [a]	Mean (µg/kg)	Samples with concentration ranges (µg/kg)			Benchmark value (µg/kg) *	Limit exceeded (%)
				<20	21-300	>300		
2019	Bran products, whole grain cereals	12	75.5	3	9	-	300	0%
2020	Bran products, whole grain cereals	13	98.1	2	10	1	300	7.7%
2019	Maize based products	6	123.5	-	6	-	150	0%

Table 4 continued

2020	Maize based products	1	64	-	1	-	150	0%
2019	”Kama”, ”kama pallid” (kama balls)	2	330.5	-	1	1	-	-

∗ Commission regulation (EU) 2017/2158
[a] n: number of samples

4.3. Potato products

Potato products were distributed to French fries and potato crisps. In Table 5 AA concentrations from French fries are presented. In both years 2019 and 2020, there were analyzed 14 samples, with mean values of 201.1 µg/kg (2019) and 324.1 µg/kg (2020). The benchmark value for French fries is set at 500 µg/kg (Commission regulation (EU) 2017/2158). The benchmark value was exceeded by 7.1% of the samples from 2019 and by 28.6% of the samples from 2020. High variation between the samples' AA concentrations can be detected as the lowest tested value was <20 µg/kg (from the year 2019) and the highest found AA concentration was 1044 µg/kg (from the year 2020). Both products were produced in Estonia. One French fries sample made from sweet potato was also analyzed with an AA concentration of 150 µg/kg.

Table 5. AA concentration in French fries

Year	**Food Group**	**(n)[a]**	**Mean (µg/kg)**	**Samples with concentration ranges (µg/kg)**			**Benchmark value (µg/kg) ***	**Limit exceeded (%)**
				<200	**201-500**	**>500**		
2019	French fries	14	201.1	8	5	1	500	7.1%
2020	French fries	14	324.1	7	3	4	500	28.6%

∗ Commission regulation (EU) 2017/2158
[a] n: number of samples

Table 6 combines the results of potato chips' AA concentrations, also vegetable chips have been added as a separate group for comparison. A total of nine potato chip samples were analyzed in both years 2019 and 2020. One sample from 2020 is a potato salt stick product categorized under the same group as other fresh potato and potato dough products in the Commission Regulation (EU) 2017/2158. The mean concentration from 2019 samples was 422.2 µg/kg and from 2020 was 629.6 µg/kg. In 2019, 11.1% of the samples exceeded the benchmark value of 750 µg/kg, while in 2020, 33.3% of the samples exceeded the benchmark value.

From vegetable chips, three samples were analyzed in the year 2019 and five samples in 2020. The mean concentration of AA in the 2019 vegetable chip samples was 281.3 µg/kg and in the 2020 samples, it was 684 µg/kg. In 2019, all samples had an AA concentration below 750 µg/kg, however in 2020, two samples had an AA concentration as high as 1257 µg/kg and 1390 µg/kg. Vegetable chips do not have a specified benchmark value.

Table 6. AA concentration in potato chips and vegetable chips

Year	Food Group	(n)[a]	Mean (µg/kg)	Samples with concentration ranges (µg/kg)			Benchmark value (µg/kg) *	Limit exceeded (%)
				<200	201-750	>750		
2019	Potato chips	9	422.2	3	5	1	750	11.1%
2020	Potato chips + potato salt sticks	9	629.6	2	4	3	750	33.3%
2019	Vegetable chips	3	281.3	1	2	-	-	-
2020	Vegetable chips	5	684	2	1	2	-	-

∗ Commission regulation (EU) 2017/2158
[a] n: number of samples

4.4. Coffee

Table 7 gathered the data from coffee and instant coffee AA concentrations from the years 2019 and 2020. In roasted coffee products in the year 2019, 16.7% of the samples exceeded the benchmark value of 400 µg/kg (Commission regulation (EU) 2017/2158) in both years 2019 and 2020. Mean values from both years were close to each other; 270.1 µg/kg (2019) and 308.2 µg/kg (2020). With instant coffee, one sample from the year 2020 exceeded the benchmark value of 850 µg/kg with an AA concentration of 963 µg/kg. Together there were four instant coffee samples from the year 2019 and six samples from the year 2020. Mean values between years varied from 450 µg/kg (instant coffee, 2019) to 702.3 µg/kg (instant coffee, 2020). In the samples from 2020, all the product concentrations were higher than 500 µg/kg, from 553 µg/kg to 963 µg/kg. One chicory coffee substitute sample was also tested, and this resulted in an AA concentration of 2113 µg/kg. This concentration was the highest measured from all the samples tested for this study. The benchmark value for coffee substitutes made of chicory is set at 4000 µg/kg.

Table 7. AA concentration in coffee

Year	Food group	(n)[a]	Mean (µg/kg)	Samples with concentration ranges (µg/kg)			Benchmark value (µg/kg) *	Limit exceeded (%)
				<200	201-500	>500		
2019	Coffee (roasted)	12	270.1	5	6	1	400	16.7%
2020	Coffee (roasted)	6	308.2	0	6	0	400	16.7%
2019	Instant coffee	4	450	1	1	2	850	0%
2020	Instant coffee	6	702.3	-	-	6	850	16.6%
2019	Coffee substitutes (chicory)	1	2113	-	-	1	4000	0%

∗ Commission regulation (EU) 2017/2158

[a] n: number of samples

4.5. Baby foods

Together 15 baby foods samples were tested for their AA concentration, seven samples from the year 2019 and eight samples from the year 2020. These results are presented in Table 8. None of the baby foods tested exceeded the benchmark values determined for them. Most of the samples are grouped into the "other baby foods" group. There are only benchmark values for cereal-based baby foods, and biscuits and rusks. Other baby foods contain fruit, vegetable, meat, and rice purees. The highest AA concentration in this group (55 µg/kg) was measured from infant rice porridge (2019).

Table 8. AA concentration in baby foods

Year	Food Group	(n)[a]	Mean (µg/kg)	Samples with concentration ranges (µg/kg)			Benchmark value (µg/kg) *	Limit exceeded (%)
				<20	21-100	>100		
2020	Cereal based foods	1	10	1	-	-	40	0%
2019	Biscuits and rusks	2	42	1	1	-	150	0%
2020	Biscuits and rusks	1	53	-	1	-	150	0%
2019	Other baby foods	5	28.4	2	3	-	-	-
2020	Other baby foods	6	17.3	4	2	-	-	-

∗ Commission regulation (EU) 2017/2158
[a] n: number of samples

4.6. Biscuits, wafers, crackers

This category combines results from biscuits, crackers, gingerbread, and crispbread, which are presented in Table 9. Altogether, 22 biscuit and wafer samples were analyzed, 10 from 2019 and 12 from 2020. Biscuits' benchmark value is 350 µg/kg (Commission regulation (EU) 2017/2158). Together five gingerbread products were analyzed, one from the year 2019 and four from year 2020. The benchmark value for gingerbreads is set at 800 µg/kg (Commission regulation (EU) 2017/2158). None of the samples analyzed did exceed the benchmark value. The maximum measured value was 354 µg/kg (2020). One cracker sample from the year 2019 was analyzed, this concentration of 82 µg/kg was well below the benchmark value set for crackers, 400 µg/kg (Commission regulation (EU) 2017/2158). Two crispbread samples were analyzed from the year 2020, these concentrations were also below the benchmark value of 350 µg/kg that is set for crispbread (Commission regulation (EU) 2017/2158). None of the samples tested in this group did exceed the benchmark values set for them.

Table 9. AA concentration in biscuits, crackers, gingerbread, and crispbread

Year	Food Group	(n)[a]	Mean (µg/kg)	Samples with concentration ranges (µg/kg)			Benchmark value (µg/kg) *	Limit exceeded (%)
				<20	21-350	>350		
2019	Biscuits	10	68.9	3	7	-	350	0%
2020	Biscuits, wafers	12	51	1	11	-	350	0%
2019	Gingerbread	1	70	-	1	-	800	0%
2020	Gingerbread	4	247.8	-	3	1	800	0%
2019	Crackers	1	82	-	1	-	400	0%
2020	Crispbread	2	123.5	-	2	-	350	0%

∗ Commission regulation (EU) 2017/2158
[a] n: number of samples

4.7. Dried fruits and berries, nuts, and peanuts

A total of 17 nut and peanut samples were analyzed, five of them from the year 2019 and 12 from the year 2020. Samples from 2019 had a mean value of 165.8 μg/kg and samples from 2020 had a mean value of 107.7 μg/kg. The highest values from each year were found from roasted cashew nuts at 525 μg/kg (2019) and medium roasted almonds, at 233 μg/kg (2020). There are no established benchmark values for nuts or peanuts in the European Commission. Six samples from dried berries and fruits were analyzed from the year 2019 and five from 2020. The mean value for the products from the year 2019 was 37.3 μg/kg and from the year 2020, it was 16.2 μg/kg. The highest values from each year were found from dried plumbs at 148 μg/kg (2019) and dried apricot at 27 μg/kg (2020). For dries fruits and berries there are no benchmark values established by the European Commission either. Nuts and peanuts, dried fruits, and dried berries AA concentrations together with standard deviation and concentration ranges are presented in Table 10.

Table 10. AA concentration in nuts and peanuts, dried fruits, and dried berries

Year	Food Group	(n)[a]	Mean (μg/kg)	Samples with concentration ranges (μg/kg)		
				<20	50-100	>100
2019	Nuts and peanuts	5	165.8	-	3	2
2020	Nuts and peanuts	12	107.7	1	7	4
2019	Dried berries and fruits	6	37.3	4	1	1
2020	Dried berries and fruits	5	16.2	3	2	-

[a] n: number of samples

4.8. Miscellaneous foods

From the total 287 analyzed samples, 15 samples from the year 2019 and 7 samples from the year 2020 did not fit any of the previously mentioned groups. These products include food

preparations, frozen vegetables, olives, porridge, and spicy nut mixes. 8 of these 22 samples were below LOQ (<20 μg/kg). The highest AA concentration from the miscellaneous group was found in whole-meal rye flour chips from Estonia with an AA concentration of 595 μg/kg, these are chips made from rye flour.

4.9. AA concentrations between different product groups

In Figure 2 AA concentrations of commonly consumed products are combined for visualization of the results. Blocks show how most of the concentrations are divided among the different product groups, visualizing the high dispersion in AA concentrations among product groups. Black dots in Figure 2 represent the mean values for the product group in the specific year. In the case of results from instant coffee from the two different years, interestingly in 2020, the measured concentrations are much higher when compared to the previous year. Mean value from 2019 450 μg/kg and from 2020 it was 702.3 μg/kg. From the same figure, it can also be noted how widely the concentrations vary inside the same product group. Very high variation is seen especially in vegetable chips from the year 2020 (lowest value analyzed was 38 μg/kg and highest 1390 μg/kg) as well as in potato chips from 2019 (lowest value in 33 μg/kg and highest 1060 μg/kg) and French fires from 2020 (lowest values in <20 μg/kg and highest in 614 μg/kg). Much less variation is seen among baby foods, soft bread, biscuits, and breakfast cereals.

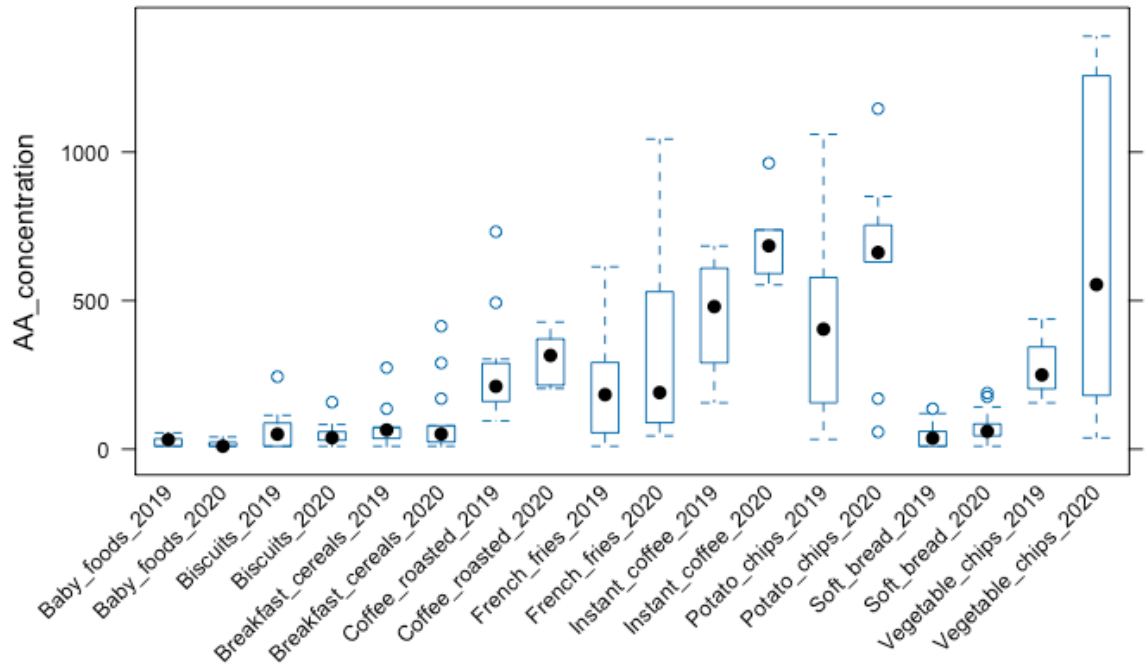

Figure 2. AA concentrations (µg/kg) in commonly consumed foods.

5. DISCUSSION

The AA concentrations of all 287 analyzed samples varied greatly. From the LOQ (<20 µg/kg) to the maximum AA concentration of the chicory coffee substitute, 2113 µg/kg. As shown in Figure 2 especially high variation among the AA concentrations was seen in vegetable chips (2020), potato chips (2019, 2020), and French fries (2020). Individual high values were found in potato chips (1060 µg/kg, 2019, and 1146 µg/kg, 2020), French fries (865 µg/kg and 1044 µg/kg, 2020), aromatic instant coffee (963 µg/kg, 2020), organic vegetable chips (1257 µg/kg, 2020), and sauerkraut chips (1390 µg/kg, 2020). From the total of 287 samples, 204 had a benchmark value established by the legislation to compare for. In total 10.3% (21 samples) of the samples exceeded the benchmark value set for them. In 2019, 106 samples had a benchmark value for comparison and six (5.7%) of the samples exceeded that. In 2020, benchmark values were available for 98 of the analyzed products, and 15 of these samples (15.3%) exceeded the established benchmark values. Interestingly in roasted coffee, instant coffee, potato chips, and vegetable chips, the mean concentrations are clearly higher from the year 2020 compared to 2019. In the current study, the sample sizes in these products have been rather small from four to 14 samples in these product groups, which most likely explains the difference.

A high mean value was found on potato chips analyzed in 2020 (629.6 µg/kg), the mean value for potato chips analyzed in the year 2019 was much lower (422.2 µg/kg). In earlier studies also high variation is seen in AA concentrations of potato chips as follows, 286 µg/kg (Finland, Hirvonen *et al*., 2011), 389 µg/kg (EFSA, 2015), 529 µg/kg (Estonia, Elias *et al*., 2017), 546 µg/kg (Poland, Michalak *et al*., 2019), and 982 µg/kg (Czech Republic, Bušová *et al*., 2020). The lowest AA concentration from potato chips analyzed in the current study was 33 µg/kg (2019) and the highest was 1146 µg/kg (2020). These results indicate that there is a high variation in AA concentrations between different products in the same food group.

In French fries, the mean AA concentrations were 201.1 µg/kg (2019) and 324.1 µg/kg (2020). There also was high dispersion among the tested products with minimum values at LOQ (<20 µg/kg) and maximum value of 1044 µg/kg (2020). These concentrations are in line with the previous findings from EFSA (2015) with 308 µg/kg, Hirvonen *et al.* (2011) from Finland with

310 µg/kg, Elias *et al.* (2017) from Estonia with 299 µg/kg, and Michalak *et al.* (2019) from Poland with 377 µg/kg.

Elias *et al.* (2017) found the highest mean AA content in coffee substitutes (721 µg/kg), in our study only one coffee substitute sample was analyzed, and it revealed the highest AA concentration from all products tested (2113 µg/kg). In the EFSA (2015) study, coffee substitutes had the highest mean concentration of AA (1490 µg/kg). The highest mean value of all the product groups was found in instant coffee products in the current study, with a mean value of 702.3 µg/kg from the year 2020. The mean value for instant coffee analyzed from the year 2019 was 450 µg/kg. Sample sizes from instant coffees were small (4 samples in 2019 and 6 samples in 2020), which most likely explains the high differences among the mean concentrations as with these few samples individual concentrations have a higher influence on the mean value. Instant coffee products are generally high in AA as found also by EFSA (2015) with a mean value of 1123 µg/kg in instant soluble coffee. With roasted coffee in the current study mean values of 270.1 µg/kg (2019) and 308.2 µg/kg (2020) were measured. This is a bit higher when compared with results from 249 µg/kg (EFSA, 2015), 210 µg/kg from Estonia (Elias *et al.,* 2017), and 185.5 µg/kg from Slovenia (Mencin *et al.,* 2020). Interestingly Hirvonen *et al.* (2011) from Finland measured mean concentrations of 15 µg/kg and 5 µg/kg of AA for light and dark roasted coffees respectively.

The potato chips category in the current study had the highest number of products exceeding the benchmark value of 750 µg/kg in the products tested in 2020 (33.3%). Of the potato chips tested in 2019, only 11.1% exceeded the benchmark value. French fries were the second group with multiple products exceeding the benchmark value of 500 µg/kg with 28.6% of the samples in 2020, and 7.1% of the samples in 2019. This is followed by coffee products with 18.2% of the samples from 2019 exceeding the benchmark value of 400 µg/kg and 16.6% of the samples taken in 2020. Also, in instant coffee samples, 16.6% of the samples from 2020 exceeded the 850 µg/kg benchmark value, but none of the samples from 2019 exceeded this value. From soft bread samples, 17.2% from 2020 exceeded the 100 µg/kg benchmark value, as well as 5.9% of the samples from 2019. The last group with exceeding values was whole grain cereal bran products with 7.7% exceeding the 300 µg/kg benchmark value from the products tested in 2020. The rest of the tested products stayed below their set benchmark values or the groups did not

have a benchmark value to compare to, such as dried fruits and berries, peanuts, and nuts. When compared to the study made by Elias *et al.* (2017) it was found that 36% of the tested whole grain breakfast cereals exceeded the indicative value for AA, as well as 23% of wheat and rye-based breakfast cereals and 14% of French fries. Whit the cereal products current results show improvement to better direction, but with French fries in the current study 28.6% of the samples from 2020 exceeded the benchmark value.

In the group of biscuits and wafers in the current study mean concentrations of 68.9 µg/kg (2019) and 51 µg/kg (2020) were measured. These are much lower when compared to older studies' results with AA mean concentrations of 201µg/kg (EFSA, 2015), 242 µg/kg (Estonia, Elias *et al.,* 2017), 384.5 µg/kg (Slovenia, Mencin *et al.,* 2020), 523 µg/kg (Poland, Michalak *et al.*, 2019) and 679 µg/kg (Czech Republic, Bušová *et al*., 2020). From Finland Hirvonen et al. (2011) found mean concentrations of 179 µg/kg for salted biscuits and wafers and 443 µg/kg for sweet ones. One reason for high differences in AA mean concentrations can be due to the very broad category of different kinds of products. But it is surprising how low mean concentration was reached in the current study.

The lowest mean values were found among the baby foods. None of the products analyzed from this category exceeded the benchmark value set for them. Most of the samples were from a group of other baby foods for which there were no benchmark values to compare. These groups' mean AA concentrations were 29.4 µg/kg (2019) and 17.3 µg/kg (2020). Benchmark values for cereal-based baby foods are set at 40 µg/kg and for biscuits and rusks for infants the benchmark values are set at 150 µg/kg. Maximum AA concentration within baby foods was found on infant rice porridge with an AA concentration of 55 µg/kg. In the study from Estonia made by Elias *et al.* (2017), it was found that 26 baby food samples from 69 samples tested had higher AA concentration than 51 µg/kg. Elias *et al*. (2017) compared the AA concentrations for indicative values set by European Commission (EU/2013/647). The indicative values have been set before the benchmark values and current benchmark values are set lower than the earlier indicative values. Elias *et al.* (2017) found that the indicative values were exceeded by more than half of vegetable-based baby foods (55%). In the current study, baby foods were well below the benchmark values. EFSA (2015) measured AA concentration of 24 µg/kg for other than cereal-based baby foods, which is like the current findings.

There are no benchmark values set by the European Commission for dried berries and fruits or nuts and peanuts. Surma *et al.* (2018) found a mean AA concentration of 21.4 µg/kg for dried fruits. This is like the results of the current study with mean AA concentrations in dried fruits and berries of 37.3 µg/kg (2019) and 16.2 µg/kg (2020). Surma *et al.* (2018) also found that raisins and dried cranberries´ AA content were below the lowest detectable limit. This same finding could be made from the current samples as well. In some of the product groups the sample sizes were small, which can affect the results quite a lot. Especially in dried berries and fruits, gingerbreads, crackers, crispbread, baby foods, instant coffee, and vegetable chips the sample sizes were between one and six. New studies with larger sample sizes would bring more accurate results.

6. CONCLUSIONS

Results of the current study show AA concentrations of commonly consumed food groups from Estonian retail markets from the years 2019 and 2020. Current findings are mostly in line with previous studies from countries close by and to the EFSA mean values. Only in the group of baby foods, it was seen that the current AA concentrations were clearly lower when compared to the earlier study from Estonia published in the year 2017. Especially in some of the groups, the sample sizes were small, which can affect the mean values a lot. This is most likely also the reason for some of the high differences in AA concentration between the two years in the same product group.

Many product groups still had products with higher concentrations than the recommended benchmark values. This was seen especially with French fries and potato chips, which also are products highly consumed by children. The fact that still, 10.3% of all tested products with comparable benchmark values were above the recommendation shows that there is still a need for control programs and the implementation of mitigation strategies. Similar studies considering current AA concentrations are also needed to ensure that the trend is toward lower concentrations. The current study showed that among multiple product groups, the AA concentrations were higher in the products analyzed in 2020 compared to 2019. If this is a real trend or caused by small sample sizes and not comparing the same producers' products, for example, should be studied more in the future. Studies with higher sample sizes would produce more reliable and accurate results.

ACKNOWLEDGMENTS

I would like to thank my supervisor Terje Elias for all the support, guidance, and comments considering the final thesis.

I would also like to use the opportunity to thank my family and friends for all the support and encouragement during the study years.

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