Deliverable 3.1 – Overview of research activities in Europe to reduce acrylamide in cereal-based foods for the field of food chemistry including sensory aspects, processing, and test development

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Executive Summary

This document provides an overview of the last 5 years' research activities in Europe to reduce acrylamide in cereal-based foods for the field of food chemistry including sensory aspects, processing, and test development. Progress in agronomy, particularly the recent development of low asparagine wheat, holds promise but is likely to face supply chain challenges over the coming years. Therefore, there is still a need for better methods of acrylamide control during recipe and process development. Although current regulations provide benchmark levels of acrylamide for a range of products that require the industry to maintain levels of acrylamide as low as reasonably achievable (ALARA), a proposed shift from benchmarks to maximum limits is under discussion which could have serious consequences for the industry.

Since 2002, substantial progress has been made in reducing acrylamide in food. Approaches like enzyme usage and reformulation have shown promise, though they often impact sensory qualities and their practical application in the food industry faces challenges related to cost, regulations, and manufacturing practices. While different baking technologies provided solutions in the early days, there has been limited progress in this area. The most promising strategy for acrylamide reduction while maintaining food quality seems to involve a combination of enzyme treatments, reformulation, combined with alternative baking technologies.

The trend towards greater use of plant proteins and high protein foods requires an increase in awareness of acrylamide as many products developed as healthy and more sustainable alternatives may inadvertently result in higher levels of acrylamide.

Rapid methods, including biosensors, are being developed for acrylamide analysis with good accuracy and sensitivity. The future lies in combining technological advancements with standardization, emphasizing accuracy, portability, and consumer convenience. The development of rapid techniques for analysing asparagine and 3-aminopropionamide is still in the early stages and subject to future research.

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Introduction

The main aim and objective of the COST Action is to understand the potential for mitigating acrylamide formation in foods produced from grains by establishing a multi-disciplinary network bringing together plant breeders, the agricultural grain farming community, global grain traders, European food processors, toxicologists, academic researchers from relevant disciplines, public regulators, and relevant consumer and health interest groups and to coordinate research:

1) for a better understanding of different relationships between the profile of the raw materials and the final product quality (which includes acrylamide, sensory aspects, aroma and taste (flavour), texture, colour, etc.),

2) for novel processing technologies to reduce acrylamide formation in cereal-based foods, and

3) to define and agree upon rapid testing methods for free asparagine levels in cereals and acrylamide levels in cereal-based products.

Objectives of the document

The objective of this document is to fulfil objective 3.1: to make a detailed overview of the relevant research in food chemistry, processing, and asparagine test development which has been allocated to WG3.

Associated WG3 tasks:

Townhall meeting:

A series of townhall meetings with breakout sessions to discuss the WG3 objectives for GP1. (16th Jan, 15th Feb, 14th Mar, 2nd May 2023).

Workshop:

In person workshop 12th May 2023, Budapest, (HU) to generate working groups dedicated to researching and writing deliverable 3.1.

Webinars:

Webinar 1 "Reformulation" 6th June 2023 chaired by Christina Nowakowski (USA)

Speaker 1: Veronika Vavrová, MSc student, National Agricultural and Food Centre, Food Research Institute – Slovakia (SK)
"Valorisation of legumes by fermentation - impact on the potential of acrylamide formation"

Speaker 2: Dr Cláudia Passos, Assistant Researcher, University of Aveiro - Portugal (PT)
"Pectic polysaccharides as an acrylamide mitigation strategy – Competition between reducing sugars and sugar acids"

Webinar 2 "Industry and Acrylamide" 27th September 2023 chaired by Christine Nowakowski (USA)

Speaker 1: Jörg Cselovszky, Regulatory Affairs Manager, of Cereal Partners Worldwide, CPW

Speaker 2: Colm Campion, Senior Development Scientist, Research & Development, of CPW-UK

Writing process

In early 2023, all members of WG3 were invited to participate in the review and indicated their choice of topic. All those who accepted were asked to review the literature over the last 5 years, identify and summarise recent review articles on the topic. They were then asked to identify the most innovative research articles over the last 5 years and summarise the findings, along with their thoughts on future research in this area. JKP and CN collated and summarised the information, produced and reviewed the report.

1. Background

1.1 The acrylamide toolbox

Acrylamide, a known potential health hazard, is associated with an elevated risk of cancer and is produced during both industrial food processing and domestic culinary practices. Delatour and Stadler [1] 2022 have recently conducted an extensive 20-year review of research pertaining to dietary acrylamide, outlining three approaches to acrylamide mitigation 1) agronomy, 2) recipe reformulation and 3) advances in process technology. (Agronomy is the focus of WG2 and is not considered here.) For over 20 years, the cereals processing industry has been engaged in reducing acrylamide formation through process optimisations using guidelines established in the *Acrylamide Toolbox* [2]. This valuable resource, initially published in 2005 and now in its 15th edition, constitutes a dynamic repository of knowledge concerning acrylamide formation and its mitigation across a diverse range of food products. The toolbox serves as a source of guidance and recommendations for both the industrial sector and domestic food preparation. A previous iteration of the toolbox underwent expert review in 2016 [3], and the conclusion was that “all strategies selected in the Toolbox turned out to be useful, however, not at the same level”. Additionally, and very importantly, many of the effective solutions to reduce the levels of acrylamide in food had a negative impact on the sensory properties of the products with consequences for consumer acceptability, repeat purchase rates and viability of the product. Furthermore, we possess limited insight into which methods can be practically and cost-effectively adopted by the food industry. Part of the scope of this document is to review advances in mitigation measures developed over the last 5 years.

1.2 Advances in agronomy

Acrylamide is formed from the amino acid asparagine which is naturally present in cereals, grains and pulses, and varies significantly between cultivars, and with different agronomic practices such as climate, soil conditions and fertiliser use. With the recent development of low asparagine wheat [4-5] using CRISPR (a gene editing tool) there is scope to greatly reduce dietary acrylamide. The study by Raffan et al, 2021, [5] reports wheat varieties with up to 95% reduction of asparagine. So, is there still a need for further reformulation and development of process technologies to decrease dietary acrylamide? Economic models of low asparagine wheat are not yet available, but it is anticipated that it may take some years to develop the stable, reliable and cost effective supply chain which is required by the industry. This is likely to be a barrier for early adoption of the low asparagine wheat. One of the objectives of the AcryRed COST Action is to stimulate research to identify supply chain management models to promote low free asparagine cereals wheat and low acrylamide cereal products. This involves coordination between a network of stakeholders across the supply chain, including seed suppliers, agronomists, fertiliser industry, elevators, distributors, millers, analytical experts, and food chemists and is being addressed by WG4. Furthermore, wheat is not the only grain to contain asparagine, and low asparagine varieties of, for example, oats and rye are still to be developed.

1.3 Regulations

The current EC Regulations on acrylamide were established in 2017 [6] and are based on published benchmarks of acrylamide levels in a range of foods. The food industry is obliged to follow the ALARA principle by which they should manufacture products with acrylamide levels which are as low as reasonably achievable. In general, this was welcomed by the industry as a pragmatic approach.

However, a move from benchmark levels to maximum limits is being discussed and this is likely to have significant implications for the industry, raising concerns about the potential impact on cereal brands and their continued availability.

1.4 Rapid analytical methods

Compliance with the regulations (either benchmark or maximum levels) relies on rapid and accurate analytical methods being available. Rapid methods for acrylamide analysis (in finished product) have emerged in the last 5 years and these are reviewed in section 11, but what the industry really needs is a rapid tool for analysing asparagine levels in the raw grain. Such a tool would facilitate the screening of raw grains at reception or at the farmgate, whereby only those within a certain specification would be processed and alternative uses would be found for those which did not meet the requirements. This in itself has implications for primary producers. These techniques are in their infancy and are reviewed in section 11. Furthermore, in cereals, a key intermediate and indicator of acrylamide potential is 3-aminopropionamide and currently there are no rapid analytical techniques available.

2. Use of enzymes to mitigate acrylamide

2.1 Overview

Overall, the majority of publications focus on asparaginase, with particular emphasis on expanding thermal stability by either sourcing from thermoduric microbes or structural stabilization on chitosan, plastic scaffolding or nickel alloy nanoparticles. Study of other enzymes is emerging, namely, glucose oxidases and acrylamidases, both of which show promise in reducing acrylamide formation under specified conditions. At present, there is limited and mixed reports of successful combination of enzymes. The advantages of using an enzyme approach to reducing acrylamide are that this is typically the least invasive approach industrially, eliminating the need for changing processing conditions or ingredient formulations, thus preserving the organoleptic characteristics of a food product. Limitations for use of enzymes are cost in use, the general lack of reusability, limited means of addition to a processing system due to high heat or non-neutral pH. Not generally addressed in the literature, is the potential concerns of handling proteins in either liquid or powder form as these have potential to be contact allergens.

2.2 Recent examples

Abedi, et al (2023) [7] summarized the effect of enzymatic reaction induced by asparaginase, glucose oxidase, acrylamidase, phytase, amylase, and protease to possibly inhibit acrylamide formation or progressively hydrolyse formed acrylamide. For reduction of acrylamide, enzymatic application is identified as a healthier and safer method compared with chemical agents. Asparaginase and glucose oxidase lead to decreased acrylamide owing to depletion of acrylamide precursors. Amylase and protease provide reducing sugar and amino acid which in turn augments the acrylamide content, respectively. High-level recombinant expression of the extracellular enzymes allows for production of sufficient amounts of enzyme to enable broad industrial implementation. It can be concluded that through enzyme modification, food characteristics can be favourably altered in heated products. Moreover, it should be noted that asparaginase, glucose oxidase, and acrylamidase have advantages over other mitigation methods because the performance efficiency of acrylamide-reducing enzymes or its precursors in food has been frequently reported without compromising with the taste, texture, and flavour.

In dough-based products, key parameters influencing enzyme effectiveness investigated were enzyme dose, dough resting time, and water content. In the treatment of potato-based products and coffee, key parameters were the enzyme dose and the method of application because appropriate pretreatment, for example blanching, was indicated to be essential for optimal performance.

Ozdemir, et al (2023) [8] described a purified and immobilized asparaginase derived from thermophilic *Geobacillus kaustophilus*. However, activity and stability of this enzyme did not significantly differ from current commercially available enzymes. However, a similar approach by Patial, et al (2022) [9] sourced from a pseudomonas species, showed wider activity across pH 5-11 and temperatures of 10 – 80 °C. However, both approaches are limited by the fact that they were expressed into E. coli, which although expedient for characterization, not readily usable for the food industry.

Abedi, et al (2022) [10] described the synergic effect of phytase, amylase, galactosidase, and asparaginase activity on the mitigation of acrylamide and 5-hydroxymethylfurfural (HMF) in roll bread by co-culture fermentation of lactic acid bacteria. Specifically, they support the conclusion that phytase causes high activity in amylase and asparaginase, resulting in reduced acrylamide.

Abedi, et al (2023) [7] emphasizes the combined impact of enzymes like asparaginase, glucose oxidase, acrylamidase, phytase, amylase, and protease on acrylamide formation. Notably, asparaginase and glucose oxidase lead to decreased acrylamide levels by depleting acrylamide precursors, while amylase and protease increase acrylamide content by providing reducing sugars and amino acids. Phytase, on the other hand, catalyses the hydrolysis of phytic acid, which can contribute to acrylamide formation but also acts as an inhibitor of amylase and protease enzymes, reducing precursor formation. Glutaminase-asparaginases, which preferentially target glutamine, are highlighted as effective in mitigating acrylamide. Acrylamidase, though less widely used, is found to be suitable for coffee beverages where its effect manifests after acrylamide formation.

Kathuria, et al. (2023) [11] describes different enzyme strategies that interrupt the Maillard reaction. Enzymes like fructosamine kinase, carbohydrate oxidase, and fructosamine oxidase halt the progression of the Maillard reaction by reacting with initial substrates or intermediate products. Fructosamine oxidase, specifically, catalyses the oxidative deglycation of Amadori products, contributing to reduced glycation. Carbohydrate oxidases alter substrate specificity, impacting Maillard-derived browning in food products.

Use of lactofermentation was reviewed by Sarion 2021 [12]. The reduction in acrylamide due to lactic acid bacteria (LAB) is attributed more to the resulting decrease in pH rather than the consumption of precursor nutrients. When LAB are used in the fermentation phase of bread making, acrylamide reduction can reach up to 75%, with Lactobacillus and Pediococcus being common LAB genera for this purpose. Some Lactobacillus species possess asparaginase genes. Fermentation by LAB is safe for consumers and can be used for bread bio-preservation. LAB naturally dominate in sourdough and produce metabolites that inhibit fungal growth and acrylamide content The use of specific LAB strains in sourdough bread recipes can reduce acrylamide formation by up to 84.7% and improve bread texture and flavour. Combining *Aspergillus niger* glucoamylase and LAB strains can also reduce acrylamide formation. However, the addition of sourdough in bread recipes may weaken the dough's quality and affect bakery product quality.

2.3 Summary

While L-asparaginase remains a prominent enzyme for acrylamide reduction, there's a need for more research on alternative enzymes, such as glucose oxidase and acrylamidase, for comprehensive acrylamide mitigation. Additionally, efforts should focus on producing thermostable and cost-effective L-asparaginase, either through recombinant technology or immobilization techniques. Combining enzymes with other mitigation methods and optimizing application parameters (e.g., blanching, high-pressure treatment) can further enhance acrylamide reduction. This area of research is expected to advance in both enzyme innovation and application techniques, leading to more effective strategies for acrylamide mitigation in various food products.

3. Use of processing technologies to mitigate acrylamide formation

3.1 Overview

The simplest and most obvious method to reduce acrylamide is to reduce thermal load. Reduction in time and temperature of standard processing conditions was one of the first measures implemented. It is certainly effective in reducing acrylamide but usually to the detriment of aroma and colour. In recent years several emerging and novel techniques (either as a pre-treatment or alone), including microwave heating, irradiation, ultrasound, high hydrostatic pressure (HHP), air-jet impingement, pulsed electric fields (PEF), supercritical fluids technology (SFT), radio-frequency (RF), infrared radiation (IR), steaming, heating under-vacuum, air frying and fermentation have been used in acrylamide reduction in different food products and most of them negatively affected food quality and sensory aspects. In general, these technologies to reduce acrylamide formation behave differently depending on the type of food so each product needs to be considered on a case-by-case basis and extrapolation to different recipes and processing conditions is often not possible. It should be emphasised that most of the reduction strategies reviewed were only tested on a laboratory scale or in simple model systems, and not at an industrial level. Their feasibility in commercial food production may encounter limits which include cost, practicability and compatibility with the long established industrial procedures and recipes. Unacceptable change in the organoleptic properties (flavour and texture), nutritional profile and consumer acceptability are also a potential barrier to implementation on a commercial scale.

3.2 Recent examples

In the last few years some advances in the optimization of new techniques for reduction of acrylamide in cereal products have been reported. Extrusion cooking, vacuum baking and microwave technology and baking with modified atmosphere have been most successful in reducing acrylamide. As an example, Dong et al. (2022) [13] used microwave baking technology to make biscuits that were compared with those obtained with the traditional method. Contaminants such as acrylamide, HMF, methylglyoxal, and 3-deoxyglucosone increased. Compared with traditional baking (190 °C, 7min), microwave baking at 440W for 3min (with no significant difference in texture) successfully decreased methylglyoxal, 3-deoxyglucosone, acrylamide, and HMF content, however, the sensory liking scores of traditionally baked biscuits were slightly higher than those of microwave-baked biscuits.

The application of vacuum baking reduced acrylamide formation but at the same time affected the colour [14]. When radio frequency was applied to partially baked thin biscuits, acrylamide production was reduced [15], and the use of steam combined with radiofrequency was shown to be suitable to control acrylamide production [16].

Heat transfer mode was found to affect the acrylamide formation [17]. Comparison of static and ventilated baking modes on biscuits baked at 175 °C for moderate baking times (20–22 min) showed that the ventilated mode resulted in higher acrylamide levels.

Şimşek in 2022 [18] applied vacuum modification after partial-baking yeast-leavened wheat bread at negative pressures of 30 kPa, 50 kPa, 70 kPa and 90 kPa. After partial-baking at 220 °C, 60% less acrylamide was formed compared to conventional baking conducted in a fan oven at ambient pressure and maintained the desired texture and colour properties of bread.

Gülcan et al. (2020) [19] proposed the removal of oxygen from the baking atmosphere as a possible strategy to reduce Maillard reaction products in bread. A study on an anaerobic baking atmosphere under inert atmosphere (N_2 and CO_2) or with the use of an inhibitor (SO_2) was performed to suppress the Maillard reaction, and the formation of HMF and acrylamide in bread. Acrylamide was reduced by 50% when baking was carried out under N_2 and CO_2 and by 99% in SO_2. The highest acrylamide content was detected in the control sample as 39 µg/kg. Baking atmosphere had a significant impact on the colour values of bread with control bread showing being the darkest. Although SO_2 application almost completely prevented the formation of acrylamide, the introduction of an allergen (and the adverse sensory effects) prevents this from being a viable commercial method and an atmosphere of CO_2 is preferable.

3.3 Summary

In summary, the existing literature is limited in its coverage of emerging technologies in the context of cereal-based food products. Notably, among the available technologies, vacuum baking stands out as a promising method for mitigating acrylamide formation. However, it is crucial to acknowledge that the body of evidence demonstrating the effectiveness of these novel remains limited. Furthermore, it's important to note that all available data were obtained from laboratory experiments, necessitating further investigation into the practical applicability of these technologies in large-scale industrial production. One potential avenue for exploration involves integrating these innovative approaches with conventional methods to preserve the desired textural and sensory attributes of the products.

4. Reformulation by addition of foods as ingredients

4.1 Antioxidant-rich foods

Antioxidants may participate in several reactions during the Maillard reaction, affecting the mechanisms of acrylamide formation and elimination in multiple ways, and often behaving differently under various processing conditions whereby the same antioxidant can have a positive effect in one scenario but a negative effect in another. This is discussed in detail by Zhang and Jin [20] where it is clear that it is important to understand the mechanistic detail of the whole system when considering antioxidants for mitigating acrylamide, and more work is required in this area to elucidate the contributing mechanisms and their susceptibility to changes in pH, concentration, moisture, thermal load etc. A vast range of food ingredients with antioxidant potential have been tested with varying success such as bamboo leaves, tea polyphenols, European cranberry bush juice, olive mill wastewater, pomegranate peel, instant coffee fractions, rosemary extract, fennel seed, black cumin seed, ginger powder, olive oil mill wastewater powder, and green coffee [21].

4.2 Phenol-rich foods

One study investigated the effect of phenolic compounds in sorghum bran extract, grapeseed extract, and green tea extract on antioxidant capacity and acrylamide formation in the extract-fortified bread. The enrichment of bread with sorghum bran extract reduced the levels of acrylamide by up to 70% without compromising the end-use characteristics [22].

A reduced acrylamide donut was developed by the addition of pomegranate flower extract and vitamin B3 [23]. Pomegranate flower extract contains polyphenols (gallic and ellagic acids) and triterpenoids which are potential antioxidants. Based on the optimization process, fried donuts containing 0.07% pomegranate flower extract and 1.97% vitamin B3 had the closest attributes to the control sample and were accepted as the best treatment. In the optimised donut, acrylamide content decreased from 76 to 64 mg/kg upon incorporation.

The extracts from Tartary buckwheat seeds, Tartary buckwheat sprouts, common buckwheat seeds, and common buckwheat sprouts significantly reduced acrylamide level both in an asparagine/glucose system and in bread [24]. There were significant positive correlations between total phenolic compound content, the antioxidant activity of the extracts, and the reduction in the acrylamide level. Evaluation of the organoleptic and textural properties indicated that the addition of the extracts did not significantly affect the crust colour, aroma, taste, crumb appearance, and hardness of the bread. The study also revealed that a possible acrylamide formation inhibitory mechanism involved the Maillard reaction through the asparagine/glucose pathway and provided useful information for the further application of buckwheat in improving food safety.

4.3 Dietary Fibre

Research by Lopez-Ruiz et al [25] demonstrated that addition of 5 g/100 g of dietary fibres such as k-carrageenan, arabinogalactan and particularly pectin, provided excellent results in the reduction of acrylamide. The highest acrylamide mitigation was obtained with application of sugar-removed lyophilized apple pomace and sugar-removed lyophilized and powdered apple pomace (62% and 48% of inhibition respectively) producing cookies well within the benchmark value of 350 µg/kg.

4.4 Sprouted Grains

Yiltirak et al [26] showed that sprouting of grains initially increased the amount of reducing sugars resulting in raised HMF and acrylamide levels (even though asparagine was lower). However, acrylamide and HMF formation decreased after fermentation as sugars and asparagine were consumed by yeast. When sprouted cereal flours (bread wheat, einkorn wheat, rye, oat, barley and buckwheat) are used for producing fermented bakery products, there was no risk of higher amounts of acrylamide.

4.5 Recipe changes

Whether or not the intention is to mitigate acrylamide formation, recipe reformulation can have both negative and positive impact on acrylamide levels. The unintended consequences of switching one sugar for another, or one raising agent for another, can mean that products originally within specification, may exceed benchmark limits. The impact of recipe changes in cereal products has been well documented over the years and is summarised by Mesias et al [27]. Briefly, yeasts such as *Saccharomyces cerevisiae* play a crucial role by metabolizing asparagine, thereby reducing the acrylamide content in the end product. Ammonium bicarbonate promotes the formation of reducing sugars and dicarbonyl compounds so substituting ammonium bicarbonate with sodium hydrogen carbonate effectively diminishes acrylamide. Very early on, additional of amino acids such as glycine and sulfur amino acids was shown to reduce acrylamide generation either through competition with asparagine for reactive Maillard intermediates or reaction with acrylamide through Michael addition. Although this provides mechanistic insight, direct addition of competing free amino acids is not a solution to the problem but does indicate that careful screening of the free amino acid content of raw materials is important, not just for asparagine content. More recently Zhu et al [28] showed that glutathione had a similar effect. Organic acids such as tartaric, citric, or ascorbic acids can also mitigate acrylamide formation or enhance its degradation.

4.6 Summary

The development of effective mitigation strategies requires a detailed understanding of the constituent components of the raw material and how they may behave in the reformulated product or under different processing conditions. Many foodstuffs have been tried and tested with various results, but future research must be carried out to isolate the active ingredients, investigate the chemical mechanisms of the mitigation and further understand of how they influence the sensory properties of the product. The integration of existing knowledge into bioinformatics systems holds the potential to be a valuable resource for advancing our understanding and optimizing mitigation strategies.

5. Reformulation by addition of well-defined chemicals

5.1 Inorganic cations

The presence of monovalent and divalent cations in bakery products can significantly decrease acrylamide levels. For instance, calcium has the potential to reduce acrylamide content in bread and crackers by up to 30%, possibly due to the improved stability of asparagine/matrix interactions at high temperatures. Other plausible mechanisms involve complexation of the free amino acids with the divalent cation or diversion of glucose towards the caramelisation route (and a decrease in the formation of highly active dicarbonyl compounds) by promotion of the glucose-fructose isomerism [29-30]. Among inorganic salts, calcium chloride is one of the most effective options, although the impact on taste and appearance, as well as other processing contaminants, should be carefully evaluated. A recent paper showed significant changes in the volatile profile of baked crust when different metal cations were added [31].

Zilic [32] reports the effect of Ca and Mg in reducing acrylamide formation in tortilla chips by up to 70%. Additionally, nixtamalization (traditionally practiced) is effective in reducing acrylamide in tortilla chips and optimization is necessary but no sensory analysis was carried out.

Infant crackers and teething biscuits were found to contain some of the highest levels of acrylamide, and this raises some concerns since cereal products make up a significant proportion of a child's diet. Similar approaches to above were taken to reduce acrylamide in products for children and toddlers [33] and this has to be done on a case-by-case basis.

5.2 Polyphenols

Mildner-Szkudlarz studied addition of individual polyphenols (rather than phenol rich foods discussed in section 5.2) to bread to elucidate their impact on acrylamide generation [34]. Whilst quercetin promoted the formation of acrylamide, gallic acid, ferulic acid and catechin all reduced acrylamide formation. Caffeic acid was particularly effective but also suppressed the Maillard reaction by competing for reactive intermediates. As little as 0.1% polyphenols addition to bread resulted in a reduction of acrylamide by up to 95%. No mechanistic studies were carried out.

5.3 Polysaccharides

Reduction of pH was used to reduce acrylamide formation [35] by adding galacturonic acid or its polymeric form as pectin. Whereas the former increased acrylamide due to the presence of a reducing-sugar moiety, the polymeric form reduced the pH without providing additional precursors. Inhibition of acrylamide was also found using chitosan. Chitosan was shown to compete with asparagine for fructose in a model system [36] and in biscuits [37].

5.4 Summary

There are several examples of acrylamide mitigation with well-defined chemical compounds. Divalent cations like calcium, are effective and are believed to stabilise interactions with asparagine and promote glucose-fructose isomerism, but taste and appearance can be affected. Polyphenols can be effective, but quercetin was an exception, and a deeper understanding of the difference may lead to better selection of polyphenol rich components. Caffeic acid is effective but can suppress the Maillard reaction. Lowering pH with substances such as pectin can help reduce acrylamide and chitosan can inhibit acrylamide by competing with asparagine for fructose in a model system and biscuits.

6. Combination of reformulation and processing technologies

Nematollah [38] reviewed the use of combination of merging technologies. Effective examples in biscuits include replacement of ammonium bicarbonate with sodium bicarbonate combined with a reduction in baking temperature [39], a combination of air-frying with added chitosan [37] and a combination of steam with RF processing [15]. The effective use of sea buckthorn pomace which is rich in antioxidants relied on a combination of reformulation with enzyme treatment [40]. The sea buckthorn pomace was first treated with asparagine to reduce the high level of asparagine in the pomace achieving a 20 fold reduction. It was then added at 10% substitution into wheat, rye and triticale flours for biscuit production, producing a reduction in acrylamide compared to an untreated flours, particularly in the case of wholemeal wheat flour where a 64% reduction in acrylamide was achieved. The most promising path toward acrylamide reduction while preserving sensory attributes is likely to involve such combination of enzyme treatments, reformulation, and reduced thermal exposure.

7. Foods for a healthier more sustainable future

7.1 Overview

There has been a recent trend towards a more plant based diet, motivated by a shift to a healthier lifestyle and a lower impact on the environment. In an excellent review of two decades of research in dietary acrylamide, Delatour and Stadler [1] conclude that the data for acrylamide consumption from plant based diets are inconsistent and they recommend further work to clarify the impact of typical plant-based diets. However, evidence from a small study of 20 volunteers (10 of whom were vegan) who completed food diaries over 10 days suggested that consumers with a vegan lifestyle had a higher intake of acrylamide than the controls, (0.38 ± 0.23 µg/kg body weight/day and 0.26 ± 0.10 µg/kg body weight/day, respectively [41]. Delatour and Stadler [1] suggest that "that the current consumer trend for meat-restricted or meat-free diets will be accompanied by a marginally higher exposure to acrylamide, as legumes, seeds, cereals and nuts contain adequate precursors for Maillard type reactions under thermal conditions".

Furthermore, in search of a healthier lifestyle, products containing mixed cereals and legumes are appearing on the market, as many traditional and forgotten crops are richer in fibre, proteins and minerals than the modern triad of wheat, maize and rice. These newer substitutes can include pseudocereals and other seeds such as chia, amaranth, buckwheat, or quinoa which have been added to bakery products to improve the nutritional value or to obtain a gluten-free product. Galani et al [42] heated flour from various cereals, legumes, root and tubers, showing low levels of acrylamide in chickpeas, maize, pearl millet, sorghum and soybean acrylamide levels were similar to wheat, but caution should be exercised when heating flours alone as the presence of other ingredients and influence acrylamide levels.

7.2 Cereals and pseudograins

In recent years, new ingredients such as pseudocereals (e.g., buckwheat, amaranth, quinoa) and other seeds have been included in innovative formulations of cereal-based foods, leading to products with different sensorial characteristics, improvement of the nutritional value or to obtain a gluten-free food. Chia seeds, a pseudo-grain, are a case in point [43]: their high protein, fibre, mineral and polyphenolic content and antioxidant capacity has acquired them a "superfoods" status however the levels of process contaminants increased: acrylamide from 151 and 1188 µg/kg, and HMF, methylglyoxal and glyoxal showed similar increases.

7.3 Pulses

A growing demand for plant-based protein has seen a resurgence in the utilisation of pulses such as lentil and chickpea as a protein-rich substrate to produce food products traditionally based on animal or cereal protein. Additionally, pulses offer metabolic benefits due to the bioactivity of compounds including phenolic acids, simple and complex carbohydrates and a more complete amino-acid profile which is not found in cereal grains. This research [44] found that acrylamide increased when lentil flour was substituted into cookies but not bread. Similar results were found in fava [45]. Roasting of the wheat flour at 180 °C with incremental addition of fava flour resulted in an initial increase in acrylamide as asparagine increase, but as the proportion of wheat decreased to 25% of 0, and the reducing sugars decreased, the acrylamide was reduced to that of the original 100% wheat flour. However, when bread was prepared with 5, 15 or 25% fava incorporation, there was no significant change in the formation of acrylamide, possibly due to the enzyme activity during the fermentation.

Schouten et al 2023 [46] demonstrated this in biscuits with added chickpea or lupin where asparagine concentration was held constant. With lupin flour, acrylamide increased from 5834 up to 1443 µg/kg after 9 min of baking, while 20–40% chickpea flour reduced acrylamide to 313-354 µg/kg. The acrylamide reduction using chickpea was attributed to the lower interaction between precursors resulting from both the coarser particle size and the lower reactivity of carbohydrate in presence of chickpea proteins. Chickpea addition did not affect the colour and texture of biscuits, opening the possibility for large-scale implementation of this mitigation strategy in formulas with a similar initial asparagine content.

7.4 Insects

One increasingly popular alternative to plants proteins is the use of edible insects to enhance or replace protein. Insects or their larvae can be processed into a flour and added to pasta, snacks, biscuits and crackers. Bartkiene et al 2023 [47] found that in wholemeal bread substituted with up to 30% of cricket flour, there was an increase in acrylamide which could be reversed by fermenting of cricket flour with *Lactiplantibacillus plantarum*-No.122 which also improves the quality of the bread. Addition of fermented cricket flour had very little impact on the sensory scores. On the other hand, 20% cricket flour added to a standard biscuit resulted in only a small increase in acrylamide (111 and 122 ppb in standard and cricket biscuits, respectively) and had increased fibre and protein [48].

7.5 Fruits, nuts and spices

Ingredients such as honey, fruits, nuts are often used to "enhance" products. A review of nuts and dried fruits [49] in bakery products suggests that although they may contain acrylamide, the contribution to the overall total is generally minimal but there are exceptions where acrylamide increases. Depending on the formulation, they may provide additional precursors of acrylamide formation (free asparagine and reducing sugars).

Addition (5%) of lyophilised wild grown fruits (wild roses, elderberries, sea buckthorns, rowans, chokeberries, and hawthorns) successfully reduced acrylamide, in the case of the chokeberry by 94% [50]. The increase was attributed to the increase in antioxidant activity provided by the high levels of polyphenols in the berries. There were some changes in appearance and consistency. Most had acceptable sensory characteristics, but the taste and flavour were rated significantly worse in the biscuits containing hawthorn and elderberry.

7.6 Summary

It is important to consider new ingredients in innovative formulations of cereal-based products, whose addition should be considered not only from the nutritional point of view but also for the contribution to the formation of acrylamide and other processing contaminants.

The use of insects to enrich cereal-based products shows promise but a comprehensive review on insect enriched cereal based products is lacking. This would be a valuable contribution in this field for GP2 and should include the impact of different processing technologies and recipes on acrylamide formation, sensory evaluation, aromatic profile, nutrition value from various sources of insect material.

8. Impact of acrylamide mitigation on sensory properties

Whilst many of the above mitigation strategies have been successfully tried and tested, many do not maintain the quality of sensory properties during thermal processing leading to consumer rejection. Reduction of acrylamide during processing whilst maintaining flavour is somewhat of a challenge since both acrylamide, flavour (and colour) are generated via the same chemical pathways: much of the flavour of baked, roasted, and fried foods is developed via the Maillard reaction which involves the reaction between reducing sugars and amino acids. Acrylamide is also known to be formed in asparagine rich foods via the Maillard reaction [51-52]and also directly via interaction of asparagine and a reducing sugar [53]. The details of the mechanism vary depending on the composition of the food [54] but the obvious solution to reduce time and temperature results in an insipid pale product, as reported for example in French baguettes [55].

In some cases, different strategies have been applied where the authors report no change in sensory properties. Antioxidants such as vitamin E [56], lemon peel extract [57] and hydrocolloids such as chitosan and sodium alginate [37] have been shown to successfully reduced acrylamide in cookies with no impact on sensory scores. Whereas addition of fermented wholemeal lupin flour achieved substantial reduction in acrylamide, it also imparted an off-note to the sour dough bread [58]. There are further examples where both sensory and acrylamide have been considered in non-cereal products such as potatoes and coffee.

However, the way to solve the reduction in sensory qualities is to understand in detail the underlying mechanisms. Early papers [59] on potato cakes demonstrated how glycine could compete with asparagine for reaction dicarbonyl species thus reducing acrylamide, but also reducing pyrazine formation - pyrazines being important for the aroma of baked goods. Later work [60] showed that acrylamide increased when sulfur-deprived wheat flour was cooked, and the volatile profile changed considerably: pyrazines and other important aroma compounds such as Strecker aldehydes (malty) were higher in the sulfur-deprived flours but other important aroma compounds such as the sweet sugar degradation products were reduced.

Recent work in model biscuits [31] has shown the mitigation potential of inorganic divalent cations (e.g., Ca^{2+}) and monitored the changes in acrylamide, HMF and aroma compounds. $CaCl_2$ was particularly effective and was associated with a decrease in Strecker aldehydes (malty) and pyrazines (toasty) but an increase in sugar-derived compounds such as furaneol (sweet) 2-acetylfuran (toasty), 2,3-pentanedione (buttery) and HMF (a process contaminant).

To a certain extent, the missing flavour can be designed back in. One example is in potato snacks [61] where by first identifying very precisely the aroma-active compounds, their precursors and their formation pathways, flavour formation in low acrylamide baked potato snacks was manipulated in such a way that a sensory panel observed an increased in baked notes.

In conclusion, a holistic approach to acrylamide mitigation is required such that the sensory profile of the low acrylamide product is at least as good as the original. Equally it is important to assess the influence of mitigation measures on the nutrient and anti-nutrient profile, the formation of other process contaminants and the feasibility and cost of the new product.

9. The industry perspective

The diversity of breakfast cereal manufacturing and the diversity of the product portfolio provide a challenge for the industry, and a general approach to acrylamide reduction is difficult to define. Processing constraints, cost constraints, complexity of regulatory affairs, and the impact of dynamic manufacturing settings all have implications for the application of research efforts developed in the lab or on pilot scale. Multiple grains are often used (oat, wheat, corn, buckwheat) to which cocoa, sugars, minor ingredients like vitamins and minerals are typically added. These ingredients, typically sourced from different regions of the world, may have variable agronomic characteristics. There is also year-to-year variability of raw ingredients. Additional complexity may be due to different manufacturing locations, which, for example, may be at different elevations which, in turn, affects atmospheric cooking conditions. Different units of operation - batch (thermal cooking) vs. extrusion (mechanical cooking) can have an impact on acrylamide formation. Drying and toasting are the unit operations that primarily develop acrylamide in the finished product. Breakfast cereal production is varied, and it cannot be defined as one set processes leading to predictable acrylamide modelling for all. Production can have long or short production steps and production times, and many of those steps can have a significant impact on acrylamide generation. Large scale production lines produce vast quantities per hour. Included is the potential source of variability with smaller manufacturers, which may purchase ‘open market’ raw materials. Particularly challenging is the area of natural and organic cereal production. Oftentimes, supply chain is unreliable and raw ingredients may be naturally higher in acrylamide reaction precursors. Knowledge of both recipe and processing factors in acrylamide generation and mitigation is the key. The collaboration between functions (R&D, regulatory, quality) are critical to ensuring risk assessments are properly done for new recipes, new equipment, or new employees. Continued collaboration between academia, industry and others will lead to successfully applied novel approaches to ensure safe, nutritious, and desired products for breakfast cereal consumers.

10. Development of rapid tests for acrylamide and asparagine

10.1 Overview

There is a growing need for cost-effective, rapid, and portable detection methods for acrylamide, asparagine and 3-aminopropionic acids (an intermediate in the formation of acrylamide and a good indicator of acrylamide potential in cereal products). Traditional techniques like liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry are costly and have limitations whereas biosensors have the potential to provide simple, rapid and highly sensitive techniques which can be deployed in the field or the factory.

10.2 Biosensors for acrylamide analysis

Several reviews have been published recently on the development of biosensors for analysis of acrylamide [62-64]. Various sensing platforms with different transduction systems have emerged as efficient tools for quantifying acrylamide in food samples. An excellent review by Rayappa et al (2021) [62] explores nanomaterial-based and other bio/chemical sensor fabrication techniques, their sensing mechanisms, and the qualitative and quantitative measurement of acrylamide in various food materials. These sensors employ diverse optical, electrochemical, and piezoelectric methods. The review discusses the potential for lab-on-chip applications for acrylamide detection and quantification in food processing. With regulatory bodies emphasizing acrylamide mitigation, nanomaterial-based sensors can play a critical role in rapid, on-site acrylamide assessment in food production. A very recent review by [63] discusses applications of amperometric and potentiometric acrylamide biosensors with or without nanomaterials. The miniaturization of these bio-nanosensors can provide smart sensing devices.

The future exploration of advanced nanomaterials for acrylamide sensing is encouraged, such as carbon-based materials, metal oxide semiconductors, polymers, and metal nanoclusters. These sensors can integrate with communication channels, including the Internet of Nano Things, for efficient data transmission. While the prospects of nanomaterial sensing applications hold commercial viability, practical translation from the lab to the market requires further research and development.

The range and variety of sensors under development is vast and cannot be covered fully in this short review. Some examples are described here, but a full review of this subject is recommended for the next GP. Some of the earlier sensors, limit of detection was not low enough to be of any use, but recently a sensor based on colloidal silver nanoparticles [65] focused on the cross linking of the nanoparticles with thiourea in a colorimetric sensor. Under optimized conditions, the sensor exhibited good sensitivity to acrylamide with a low detection limit (LOD) of 2 ppb. The use of guanine and adenine as biomarkers at boron-doped diamond electrodes had Lod of a similar order of magnitude [66]. Chi et al (2022) [67] developed a Quartz Crystal Microbalance (QCM) sensor for detecting acrylamide. The sensor featured a composite modification layer with nitrogen-doped ordered mesoporous carbon modified gold nanoparticle with cross-linked 3-thiopheneacetic acid as an imprinting layer. The highly crosslinked imprinting layer improved acrylamide detection, making the sensor suitable for low-concentration food sample analysis. This sensing platform demonstrated a good linear range for acrylamide detection from 0.08 to 100 ppb, with a low LOD of 0.005 ppb and a recovery rate from 88-97%.

10.3 Smart sensing devices for acrylamide analysis

Several researchers have developed smartphone apps for measuring acrylamide. One of these was based in the reaction of acrylamide with glutathione using a fluorescence method with an incredibly low detection threshold of 0.1pM (~$8x10^{-6}$ ppb) [68]. In a very recent publication, Zhara et al [69] use a smart phone camera to readout colour changes in a micropipette with an embedded sensor based on biological recognition of acrylamide (aptamers). The synergy of nanotechnology with aptamer science could lead to automated and miniaturized aptasensors with the excellence of on-site monitoring of ultra-trace levels of acrylamide. The development of portable aptasensing arrays is substantial for on-site monitoring of targets. Smartphone sensing techniques is a promising portable device in food safety, involves in high-resolution digital camera and image analysing software which can be a professional sensing instrument in colorimetric assays. With this, the food industry's demand for rapid, sensitive, and reliable acrylamide testing could be met.

10.4 Summary of acrylamide biosensors

The absence of a universally accepted sensor-based method for rapid acrylamide analysis highlights the need for standardization to ensure consistency and reliability across different platforms and products. Miniaturizing optical sensors for high-throughput food screening faces challenges due to complex food matrices and achieving a balance between speed and accuracy. Future research will focus on polymer longevity, sensor construction, stability enhancement for electrochemical and fluorescence sensing, addressing matrix effects in food samples, streamlining sample preparation.

10.5 Biosensors for rapid asparagine analysis

Biosensors for asparagine are in development for use in agricultural practices, the food and drink industry, and even in clinical diagnosis. Knowledge of acrylamide concentrations in crops, food ingredients and intermediate products is critical for developing low acrylamide end-products. For some applications, the development of an asparagine biosensor would allow prediction of final acrylamide concentrations much earlier in the manufacturing process. This is potentially useful for cereal products where reducing sugars are abundant and asparagine is likely to be the rate limiting precursor, whereas this approach is less useful for potato products where asparagine is abundant. Biosensors for asparagine typically use a biological recognition element (e.g., enzymes, antibodies, or microorganisms) that selectively binds with asparagine. This binding event generates a signal, often in the form of electrical, optical, or chemical changes, which is then quantified to determine the concentration of asparagine in the sample. The mechanisms are reviewed by Lozano et al (2019) [70].

Some sensors are based on the enzyme asparaginase, reviewed by Nunes et al. (2021) [71]. The asparaginase catalyses the conversion of asparagine to aspartic acid with the concomitant release of ammonia, and an increase in pH which can be monitored colourimetrically. The asparaginase can be obtained from a number of different bacteria or plant species ideally immobilised on a support and there are a range of indicators available. These require further development, and the use of a more precise alternative to the colour-based assay.

Optical-based asparagine biosensors are also under development for use in clinical diagnostics where high levels of asparagine levels in blood or urine can be indicative of certain medical conditions [72].

Biosensors provide rapid analysis with high specificity and selectivity for asparagine with the potential for on-site or field-based measurements. However, there are still challenges to be overcome: sample preparation and matrix effects can impact accuracy, the stability and shelf-life of the biological components is often limited, and the sensitivity and detection limits may vary depending on the biosensor design.

11. Conclusion

Significant progress has been made in acrylamide mitigation over the past five years, with numerous reviews, including the comprehensive book "Acrylamide in Food" by Gok men and Mogol [73] providing extensive coverage of acrylamide-related aspects in food.

Enzyme usage has been well-documented, with a primary focus on asparaginase, which is effective but has limitations for commercial-scale applications. Other enzymes, like glucose oxidases and acrylamidases, are emerging as promising alternatives under specific conditions. Lactic acid bacteria have also demonstrated effectiveness in reducing acrylamide during bread baking.

Reformulation techniques, including the incorporation of antioxidant-rich foods, particularly polyphenols, divalent cations such as $CaCl_2$, and polysaccharides, have proven effective in acrylamide reduction. However, the sensory qualities of the end-product are often overlooked Given the parallels between acrylamide and Maillard-derived colour and flavour formation pathways, it is not surprising that the sensory characteristics, when measured, are often inferior. However, the success of each strategy is very dependent on the food composition, food matrix and conditions employed, so until we understand more of the underlying mechanisms, the efficacy of these approaches needs to be assessed on a case-by-case basis.

Moreover, many of these strategies have yet to undergo testing at manufacturing or even pilot scales. Their practical applicability within the industry may be constrained by processing limitations, cost considerations, regulatory complexities, and the dynamic nature of manufacturing settings. These factors all pose challenges to the translation of research findings developed in laboratory or pilot-scale settings into effective industrial practices.

Often a decrease in acrylamide is accompanied by an increase in HMF as the pathway moves away from a typical Maillard pathway towards caramelisation and breakdown of sugars. In terms of process contaminants, a risk/benefit evaluation is needed when designing novel cereal-based foods.

Different technologies for baking such as extrusion, and the use of microwave or Rf for heating were developed in the early days of acrylamide, and progress in this area alone is limited. The most promising development in acrylamide reduction while preserving sensory attributes is likely to involve a combination of enzyme treatments, reformulation, and reduced thermal exposure.

The current trend for greater use of plant proteins and high protein foods requires an increase in awareness of acrylamide as many products developed as healthy and more sustainable alternatives may inadvertently result in higher levels of acrylamide.

Development of rapid methods for analysis of acrylamide continues to expand with many prototypes of (bio)sensors reporting good accuracy and sensitivity. The future for these areas lies in the amalgamation of technological advancements, standardization, and user-friendly designs. These directions are likely to be steered by an emphasis on accuracy, portability, and consumer convenience, ensuring safety and quality assurance across the food sector. Development of rapid techniques for analysis of asparagine and 3-aminopropionamide are still in their infancy and will be reviewed in GP2.

12. Suggestions for further work

- Production of thermostable and cost-effective L-asparaginase, either through recombinant technology or immobilization techniques.
- Research on alternative enzymes, such as glucose oxidase and acrylamidase, for comprehensive acrylamide mitigation.
- Comprehensive sensory testing and sensory optimisation of all low acrylamide products. This includes appearance, aroma, taste, flavour and texture.
- Translation of laboratory-based solutions to pilot and manufacturing scale.
- Elucidation of the underlying mechanisms behind the effects of additives, especially phenolic compounds.
- Focus on wholemeal not only refined flours because of higher risk of acrylamide formation and their health benefits.
- Development of composite cereal flours for home-baking that have low acrylamide potential but are healthy, sustainable and affordable.
- Standardisation of (bio)sensors for rapid detection of acrylamide
- Development of sensors for rapid analysis of asparagine and 3-aminopropionide.
- Update of the Toolbox with most successful strategies.
- Dissemination of acrylamide reduction techniques to the hospitality industry – chefs and restaurateurs and home-cooks.
- Development of a Draft agenda for the next 5 years for food chemistry, processing, and test development research

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