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CEREAL GRAIN WITH LOW ACRYLAMIDE FORMATION POTENTIAL AS A RAW MATERIAL FOR SAFER CEREAL-BASED FOOD PRODUCTS IN SERBIA

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Abstract

The presence of acrylamide, a mutagen and a Group 2A carcinogen, in food, is a health concern that might raise cancer risk. Acrylamide is mainly formed in the Maillard reaction between free asparagine and reducing sugars, during industrial thermal food processing or home cooking, at a temperature over 120°C. The European Commission Regulation (EU) 2017/2158 established mitigation measures and benchmarks for acrylamide levels in some food categories, which were incorporated into the Serbian regulation. Research shows that cereal-based products may bring about 20-60% of acrylamide intake. In Serbia, there are no guidelines for the cultivation of cereals with a reduced potential for acrylamide formation. Knowing that the amount of free asparagine is proportional to the formation of acrylamide in the majority of food products, one of the key approaches is to select the ingredients with a lower level of asparagine. Studies indicate that applying foliar fertilizers with increased sulfur content in some cereals influences the decrease of free asparagine synthesis in grain, lowering the acrylamide potential in cereal-based foods. Furthermore, the choice of the appropriate raw material and production parameters can significantly influence the formation of acrylamide in food products. This review aims to provide insight into current strategies for the mitigation of acrylamide in cereal-based foods, as the status of acrylamide in Serbian regulations.

Key words: acrylamide, asparagine, agronomic measures, cereal-based food, food safety.

Introduction

Global trends in food safety impose that the level of specified carcinogenic and other substances harmful to human health must be kept as low as reasonably achievable (ALARA principle) in different food products (Thielecke and Nugent, 2018). In 2002, researchers from Stockholm University and the Swedish National Food Administration discovered that foods processed at temperatures higher than 120°C, such as bread, cereals, coffee, French fries, and potato chips, contained acrylamide, a highly water-soluble, flavourless, and colourless chemical (Pietropaoli et al., 2022). The European Commission Regulation (EU) 2017/2158 (European Commission (EU), 2017), which established mitigation strategies and benchmark levels for lowering the presence of acrylamide in food, was published in 2017. The databases of acrylamide were developed and updated taking into account scientific and market reports by the health institutions and food authorities, including the European Food Safety Authority (EFSA), World Health Organization (WHO), and Food and Agriculture Organization of the United Nations (FAO). In addition to these databases, toolboxes were created to show how to

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lower the acrylamide level in each food category by adjusting variables like a choice of raw material, recipe development, or process design (Aktağ et al., 2022). According to a study conducted in 2015 by the European Food Safety Authority (EFSA), typical dietary acrylamide exposures have been between 0.4 and 1.9 g kg^{-1} body weight/day, with the margins of exposure raising concerns about potential neoplastic effects (Mesias et al., 2020). The quantities of precursors (free asparagine, glucose, fructose, and maltose), the food matrix, and the type and intensity of the thermal process used all affect the amount of acrylamide present in cereal-based foods. Asparagine serves as the primary precursor for the synthesis of acrylamide in the Maillard reaction, which occurs between amino acids and reducing sugars during the thermal processing of some foods. Typically, breakfast cereals and tortilla chips made from corn (maize) are among the most popular foods consumed worldwide. The market for tortilla chips in the United States ranked first in terms of sales volume among sweet and savoury snacks. Therefore, the widespread consumption of cereal-based products exposes people to acrylamide, raising health concerns and the need for acrylamide reduction in these products (Žilić et al., 2022). Several approaches to reduce the level of acrylamide in processed foods have been discovered over the past twenty years. A graphical presentation of an example of the field-to-fork strategy to mitigate the acrylamide occurrence in cereal-based foods is shown in Figure 1.

Studies have shown that the application of certain fertilizers, can lead to a reduced content of free asparagine, and as a consequence thus contributing to a lower acrylamide content in cereal-based foods, as well as to improve the general health status of the nation, primarily the younger population,

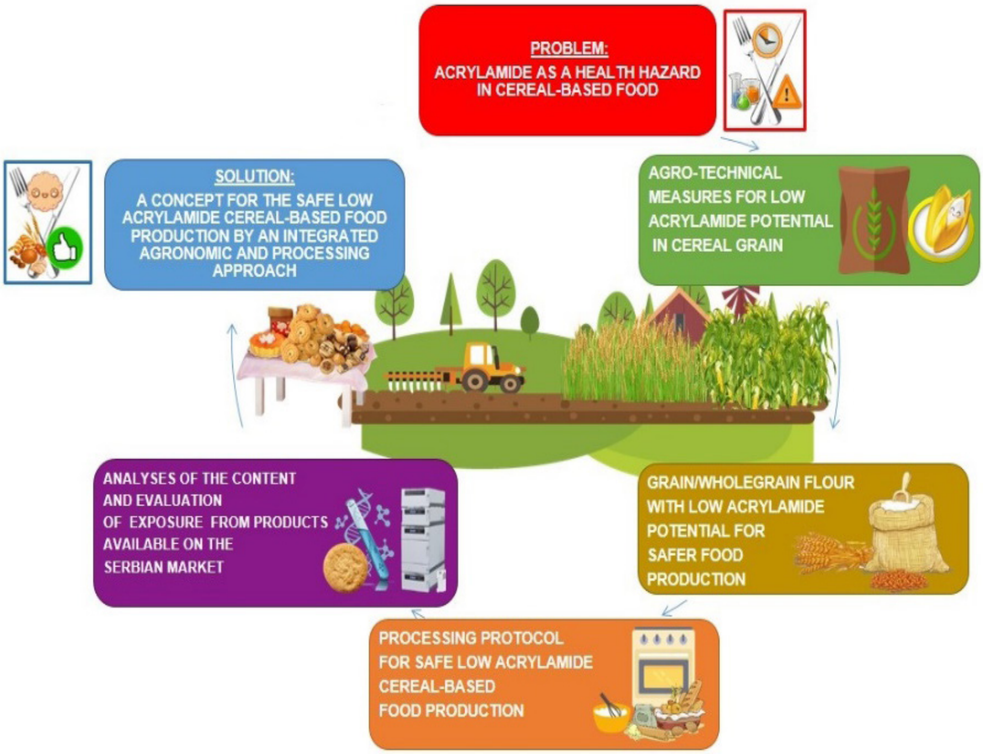

Figure 1. The mitigation measures for safer low acrylamide cereal-based food production
Grafikon 1. Preventivne mere za bezbedniju proizvodnju hrane na bazi žitarica sa niskim sadržajem akrilamida

which is predominantly a consumer of cereal-based snack products (Raffan and Halford, 2019).

One of the important issues that need to be taken into consideration is the lack of agronomic recommendations for the cultivation and the use of cereal genotypes with the reduced content of basic acrylamide precursors - free asparagine and reducing sugars, as the raw materials for the safe cereal-based food production. Delivering these recommendations is among the priorities of the research projects regarding acrylamide mitigation being conducted in Serbia at the moment.

Asparagine in cereal grains

Amino acid asparagine plays a crucial role in the delivery and storage of nitrogen in many plant species, as well as in the synthesis of proteins. Together with glutamine and glutamate, it provides up to 70% of the free amino acid content in wheat grain. In response to several biotic and abiotic stressors that act during normal physiological processes such as seed germination and nitrogen transport, high concentrations are achieved (Lea et al., 2007).

Interest in the synthesis, accumulation and degradation of asparagine in agricultural crops has been stimulated by the recent discovery that free asparagine is the primary precursor for the formation of acrylamide rather than reducing sugars (Food Drink Europe, 2019). When grains, tubers, storage roots, beans, and other agricultural products are heated at high temperatures during frying, baking, roasting, toasting, or other processing, acrylamide is formed (Raffan and Halford, 2019). Asparagine, unlike reducing sugars, regulates the formation of acrylamide in grain products.

Depending on the cereal variety, agronomic practices, and agroecological factors, the amount of asparagine in cereal grain varies greatly (Curtis et al., 2018). Žilić et al. (2020) reported that asparagine level in wheat ranged from 61.6 to 480.6 mg kg^{-1}which was similar to maize (189.7-470.5 mg kg^{-1}) and barley (297.0 mg kg^{-1}), whereas it was higher in the rye (603.2 mg kg^{-1}) and oat (859.8 mg kg^{-1}). Rapp et al. (2018) evaluated wheat varieties from central Europe and discovered a rather narrow range of free asparagine concentrations in 149 wheat varieties grown at three different locations, ranging from 1.09 mmol$kg^{-1}$to 2.98 mmol kg^{-1}. In 150 wheat genotypes produced for the HEALTH GRAIN diversification programme, a broader range, from 2.42 to 11.82 mmol kg^{-1}, was measured (Corol et al., 2016). While asparagine concentrations were evidently highly influenced by environmental conditions across the sites and years in that study, which also examined 26 genotypes cultivated over three years at four sites in Europe, it was noted that some genotypes were more consistent than others.

Different approaches for acrylamide mitigation in food products

The limited outcomes of the research and technological development initiatives conducted thus far demand more action. The use of raw materials such as grain with a low asparagine level is considered a vital strategy for addressing the issue. To establish the best cropping and production practices, identify gaps in the system, and set the future directions, it is necessary to take an integrated, fully supplied chain approach. Currently, there are three main strategies used for acrylamide mitigation in food products: agronomic, genetic and processing (Maan et al., 2022).

Agronomic approach

The main method of preventing acrylamide development in cereal is to lower the asparagine level, although this method is not always practical because of varying in grain varieties, harvesting practices, or climatic factors (Mesias et al., 2020). Cereal genotypes with little asparagine should be chosen as long as the final product's identity is preserved. The agronomic approach for reducing the potential of cereals for acrylamide formation involves agro-technical procedures, i.e. application of fertilizers and herbicides that can cause changes in the amino acids and sugars metabolism (Halford et al., 2007). Research has shown that the content of an amino acid asparagine in the grain plays the key role in the acrylamide synthesis during the Maillard reaction, while the effect of dicarbonyl compounds, predominantly reducing sugars, is important, although, not crucial. Previous studies suggested that excess sulfur negatively affects asparagine synthesis in some cereals while promoting nitrogen synthesis (Soofizada et al., 2022; Wilson et al., 2020). Studies have shown that in wheat grain, high content of free asparagine accumulates as a result of sulphur deficiency, poor disease control, and nitrogen fertilization (Curtis et al., 2009; Curtis et al., 2018). Several factors that influence asparagine metabolism in a synergetic way have been recognized such as genes, enzymes, metabolites, and environmental parameters (Curtis et al., 2018a). However, to date, glutamine-dependent asparagine synthetase, the enzyme that catalyzes the transfer of an amino group from glutamine to aspartate to form asparagine and glutamate, has received major attention (Gaufichon et al., 2010; Xu et al., 2018).

Apart from inducing changes in plants that benefit the reduction of acrylamide formation potential, agro-technical measures can have a positive or a negative impact on the grain yield and quality, as well as nutritional, functional, textural, or sensory properties of the end-food product (Raffan and Halford, 2019). Previous studies have demonstrated that it is possible to breed cereal genotypes with low potential for acrylamide formation, stabile throughout a wide variety of conditions, and with good agronomic performance, as well as to use them for cereal-based food products with low acrylamide risk (Žilić et al., 2017). According to prior research based on interrelationship analysis, bread wheat, durum wheat, and hull-less barley can be simultaneously bred for low quantities of mono-reducing sugars and free asparagine. However, there is a positive correlation between the amount of total proteins and free asparagine, i.e. the lower content of free asparagine the higher total protein content decrease (Žilić et al., 2020; Oddy et al., 2022). Accordingly, even though cereals are not the major source of protein, cereal-based food producers must alter their recipes in order to use the genotypes with lower free asparagine content (Žilić et al. 2022).

Genetic approach

A significant part of amino acid and nitrogen metabolism in plants involves the synthesis and catabolism of asparagine. In order to develop new cereal genotypes with decreased acrylamide-forming capacity, more research and comprehension of the genetic regulation of asparagine metabolism is necessary. Asparagine synthetase, asparaginase, glutamine synthetase, glutamate dehydrogenase, ferredoxin-dependent glutamate synthase, NADH-dependent glutamate synthase, aspartate aminotransferase, glutamate decarboxylase, and aspartate kinase are the main enzymes involved in asparagine metabolism. Genetic methods could target particular genes that are responsible for the activity of these enyzmes, however, asparagine synthetase has received the major attention to date (Curtis et al., 2018). To what extent crop breeding could lower the potential for acrylamide formation by decreasing the content of free asparagine in cereal grain is a crucial topic for breeders. Because of how free asparagine content responds to environmental conditions and crop management strategies, breeders may have been dissuaded from using this trait as a breeding target. As the demand from the food industry for reduced asparagine cultivars has grown, that attitude has shifted. Breeders will need genetic resources in the form of quantitative trait loci and genetic markers if they are to advance (Raffan and Halford, 2019).

Processing approach

The processing approach involves utilization of the raw materials with a lower potential for acrylamide formation, as well as the employment of optimized technological procedures that decrease the possibility of acrylamide occurrence in the end products. By utilizing flour obtained from cereal grain with lower free asparagine content as a raw material for cereal-based foods production, and by implementing optimal processing parameters (ingredients, temperature, time of baking, etc.), an integrated agronomic-processing concept can be developed for the safe cereal-based food production. The amounts of acrylamide in the final product change significantly as a result of modifications to recipes or processing conditions (Žilić et al., 2021). Moreover, the final acrylamide concentration in the end product is influenced by the relative concentration of these molecules, as well as by parameters such as temperature, heat intensity, and water activity in the recipe (Sarion et al., 2021). Baking time-temperature conditions are a crucial element in the production of acrylamide. There is no specific recommendation on the processing temperature for cereal-based foods due to the complexity of these foods, but it is suggested that the heat input, or the time and temperature combination, should be the most efficient in reducing acrylamide formation while achieving the desired product characteristics (European Commission (EU), 2017). Due to the distribution of the thermal load and the preservation of the moisture, the majority of the acrylamide in bread is located in the outer crust layer, while the crumb retains only trace amounts (Mesias et al., 2019). Ventilated baking encourages increased acrylamide production compared to static one because the heat is dissipated more slowly in vented mode. Modern innovations like vacuum baking or the application of steam-combination radiofrequency are efficient in reducing acrylamide formation (Kocadağlı et al., 2012). Temperature and thermal treatment time have been proven to be the most significant parameters influencing the production of acrylamide. To achieve the proper quality criteria for fried food, it is advised that the frying temperature should be below 175 °C and the frying duration should be no longer than necessary. Reduced frying temperature calls for longer frying times, which may have an impact on the acrylamide level (Kita et al., 2004).

Making changes to standard recipes, which usually include sugar, salt, and shortening agents in addition to flour and water, can lead to a decrease in acrylamide content in cereal-based bakery products. Sucrose can be used instead of inverted sugar syrup to minimize acrylamide in most cases (Aarabi and SeyedainArdebili, 2020), however severe thermal treatments can cause sucrose to hydrolyze (Mesias et al., 2020). Adding 1-2% NaCl and/or unsaturated vegetable oils with a high phenolic content is known to reduce the development of acrylamide in some cereal-based products, however, they may also cause downsides such as high sodium levels, unpleasant sensory effects and reduced shelf life. Yeasts that metabolize asparagine, like *Saccharomyces cerevisiae*, were shown to lower acrylamide in the final product. Some organic acids, such as tartaric, citric, or ascorbic acids, can also reduce the synthesis of acrylamide or speed up its decomposition, although particular attention should be given to the sensory qualities that arise (Sarion et al., 2021; Levine and Smith, 2005; Komprda et al., 2017).

Asparaginase, which hydrolyzes asparagine into aspartic acid and has minimal effects on the final organoleptic qualities at low dosages, is currently the most efficient mitigation approach in cereal-based foods made from doughs. Its activity is influenced by water activity, the ratio of asparagine to the enzyme, pH, time, and incubation temperature (Jia et al., 2021). For efficient limitation of acrylamide development, legume flours such as soybean, chickpea, pea, or bean can bee added to the recipe. Consumers may not necessarily appreciate the sensory qualities that follow, and an increase in lysine levels promotes the growth of additional undesirable processing pollutants (Galani et al., 2017).

Serbian regulation and research regarding acrylamide mitigation in food products

In Serbia, regulations and recommendations concerning the content of acrylamide in food came into force in January 2020, with the Regulation on allowed maximum concentrations of certain contaminants in food, and were updated last time in December 2021 (Official Gazette RS No 81/2019, 126/2020, 90/2021, and 118/2021). The set of benchmarks and guidelines are only applied to a small subset of food items. Given that Serbia is preparing to join the European Union, regulations regarding food safety, among others, should be timely harmonized.

This regulation offers recommendations for the mitigation measures for the reduction of acrylamide in food. Entities in the food business that produce and place the food with the risk of high acrylamide content on the market must apply the measures to mitigate the effects to achieve the lowest reasonably achievable levels of acrylamide below the level of reference values set by the Regulation. During the official control, these food producers and sellers are obliged to display the evidence that appropriate measures for mitigation of acrylamide's presence in food (HACCP programme) have been applied.

The foods that pose the greatest risk for the formation of acrylamide are bread, breakfast cereals, French fries, potato chips, snacks, crackers, fine bakery goods like cookies, biscuits, rusks, cereal bars, scones, etc. The following food items that fall under this category are coffee if it is roast coffee, instant coffee, or coffee substitutes; baby food if it is processed cereal-based food intended for infants and young children as defined by Regulation (EU) 2017/2158 (European Commission (EU), 2017).

A study conducted on a large subset of different food types, including cereal-based bakery and confectionary goods, as well as coffee products collected from the Serbian market, reported that acrylamide was present in 83.74% of the evaluated food items (Koricanac et al., 2021). Overall, these findings support the assertion that most of the food products that are susceptible to the development of acrylamide due to their chemical makeup and manufacturing processes are, regrettably, unavoidable. To integrate these data and estimate the health risk of acrylamide intake through food, the ongoing food safety regulations, additional research that takes into account the acrylamide levels in various food groups, and data on the average daily intake of these foods by demographic groups, are all required (Koricanac et al., 2021).

The ambition of the studies currently underway at the Maize Research Institute Zemun Polje is to provide new data regarding the future introduction of benchmarks in regulations for new groups of cereal-based products. Additionally, the new findings will provide a foundation for future research on other thermally generated food contaminants that are just as harmful as acrylamide but have not been included in the regulations or the research programmes in Serbian scientific institutions yet.

Conclusion

The results presented in this paper highlight the importance of safer cereal-based food production with low acrylamide content in Serbia. Extensive, in-depth research that includes the study of the influence of agro-ecological factors and cropping practices on the asparagine metabolism, assessment of technological properties, chemical composition, and biochemical profile of the cereal flour, as well as the improved technological process for safe cereal-based products with low acrylamide content from the most favourable cereal genotypes, has not been conducted in this part of Europe yet and it is necessary to enable the improvement within the chain of the safer cereal-based food production.

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ZRNO ŽITARICA SA NISKIM POTENCIJALOM ZA FORMIRANJE AKRILAMIDA KAO SIROVINA ZA BEZBEDNIJE PREHRAMBENE PROIZVODE NA BAZI ŽITARICA U SRBIJI

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Sažetak

Prisustvo akrilamida, mutagena i kancerogena grupe 2A, u hrani, predstavlja zdravstveni problem koji može povećati rizik od raka. Akrilamid se uglavnom formira u Majarovoj reakciji između slobodnog asparagina i redukujućih šećera, tokom industrijske termičke obrade hrane ili kuvanja kod kuće, na temperaturi iznad 120°C. Uredbom Evropske komisije (EU) 2017/2158 su utvrđene mere ublažavanja i granične vrednosti za sadržaj akrilamida u nekim kategorijama hrane, koje su uvršćene u srpsku regulativu. Istraživanja pokazuju da proizvodi na bazi žitarica mogu dovesti do 20-60% unosa akrilamida. U Srbiji ne postoje smernice za gajenje žitarica sa smanjenim potencijalom za stvaranje akrilamida. S' obzirom na to da je količina slobodnog asparagina proporcionalna formiranju akrilamida u većini prehrambenih proizvoda, jedan od ključnih pristupa predstavlja odabir sastojaka nižeg sadržaja asparagina. Istraživanja pokazuju da kod nekih žitarica, primena folijarnog đubriva sa povećanim sadržajem sumpora utiče na smanjenje sinteze slobodnog asparagina u zrnu, smanjujući potencijal akrilamida u namirnicama na bazi žitarica. Takođe, izbor odgovarajuće sirovine i proizvodnih parametara može značajno uticati na formiranje akrilamida u prehrambenim proizvodima.Ovaj pregledni rad ima za cilj da pruži uvid u aktuelne strategije za snižavanje akrilamida u namirnicama na bazi žitarica i status akrilamida u srpskim propisima.

Ključne reči: akrilamid, asparagin, agronomske mere, bezbednost hrane, hrana na bazi žitarica

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