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Abstract

Acrylamide is a possible carcinogen to humans, formed upon heat-treatment of carbohydrate-rich foodstuffs via the Maillard reaction. Reducing sugar and the amino acid asparagine are the primary precursors for acrylamide formation, of which free asparagine is the limiting factor. Among the foods being subjected to acrylamide formation, breakfast cereal is one. Substitution of sucrose with alternative sweeteners such as fruit juice concentrate in extruded breakfast cereals has been linked to increased content of acrylamide. Therefore, this literature study aimed to review how the usage of alternative sweeteners can affect acrylamide formation in the manufacture of extruded breakfast cereals. Furthermore, a fruit juice analysis was done in order to determine whether concentrated fruit juice may contribute to the formation of acrylamide.

Free asparagine was the major amino acid determined in both of the concentrated apple juice samples. The content of free asparagine was considerably lower than the content of reducing sugars, confirming that free asparagine is the limiting factor in the formation of acrylamide. Acrylamide was formed in concentrated fruit juice upon analysis, indicating that there is a possibility for developing acrylamide under right conditions in the final product containing concentrated fruit juice. Thus, to reduce the amount of acrylamide being formed in extruded breakfast cereals, the sources of free asparagine should be minimised. As the content of free asparagine varies between different fruits, a concentrated fruit juice with lower free asparagine content should be favoured in the manufacture. Also, a sweetener absent in free asparagine could be chosen in front of concentrated fruit juice. Treating the concentrated fruit juice is another possibility to reduce the content of free asparagine. This study confirms that the concentrated fruit juice may influence the total amount of acrylamide being formed in extruded breakfast cereals. More research is warranted to examine the correlation between alternative sweeteners and acrylamide formation in breakfast cereals. Research on the physiological and metabolic function of free asparagine in plants is also needed.

Keywords: acrylamide, breakfast cereals, free asparagine, fruit juice concentrate, sweetener

Sammanfattning

Akrylamid är ett potentiellt karcinogent ämne för människor som bildas genom upphettning av kolhydratrika livsmedel genom Maillardreaktionen. För att akrylamid ska bildas krävs aminosyran asparagin i fri form samt ett reducerande socker, varav asparagin utgör den begränsande faktorn. Akrylamid kan bildas i flertalet livsmedel, varav frukostcerealier är en produktkategori. Användning av alternativa sötningsmedel såsom fruktjuicekoncentrat har kopplats till en ökad halt av akrylamid i extruderade frukostcerealier. Syftet med den här litteraturstudien var därför att undersöka vilken inverkan alternativa sötningsmedel har på bildandet av akrylamid i extruderade frukostcerealier. En analys av koncentrerad fruktjuice gjordes för att fastställa huruvida denna kan bidra till bildandet av akrylamid i slutprodukten.

I de två analyserade fruktjuicekoncentraten var fritt asparagin den dominerande aminosyran. Halten av fritt asparagin var betydligt lägre än halten av reducerande socker, vilket bekräftar att fritt asparagin är den begränsande faktorn för bildandet av akrylamid. Akrylamid bildades i de analyserade fruktjuicekoncentraten, vilket visar på att akrylamid potentiellt kan bildas även i extruderade frukostcerealier som innehåller koncentrerad fruktjuice. För att minska halten akrylamid som bildas i extruderade frukostcerealier bör källorna till fritt asparagin minimeras. Antingen kan fruktjuicekoncentratet bytas ut mot ett annat fruktjuicekoncentrat som innehåller lägre halt fritt asparagin, detta eftersom asparaginhalten varierar mellan olika frukter. Alternativt bör fruktjuicekoncentratet substitueras med ett annat sötningsmedel som inte innehåller fritt asparagin. En annan möjlighet vore att behandla fruktjuicen på ett sådant sätt att halten fritt asparagin minskar. Den här studien bekräftar att koncentrerad fruktjuice kan påverka den totala mängden akrylamid som bildas i extruderade frukostcerealier. Mer forskning behövs för att undersöka sambandet mellan alternativa sötningsmedel och bildandet av akrylamid i frukostcerealier. Därutöver behövs forskning kring asparaginets fysiologiska och metaboliska effekt i växter.

Nyckelord: akrylamid, frukostcerealier, fritt asparagin, fruktjuicekoncentrat, sötningsmedel

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Abbreviations

AQC	6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
ADAM	1-amino-adamantane hydrochloride
ALARA	As low as reasonably achievable
Asn	Asparagine
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FBO	Food business operator
FMOC-Cl	9-fluorenylmethol-chloroformate
HPLC	High-performance liquid chromatography
IARC	International Agency for Research on Cancer
LC-MS/MS	Liquid chromatography tandem mass spectrometry
RTE	Ready-to-eat
SC-FOS	Short chain fructo-oligosaccharides
WHO	World Health Organization

1. Introduction

Health awareness is subjected to great attention. Health-claims, studies and authority-stated information guides people to make well-grounded choices in order to improve well-being and thus increase longevity without diseases. Alongside, the prevalence of cancer is steadily increasing. Scientists are eager to find and determine its underlaying causes. However, a lot of factors seems to contribute in the development of cancer (WHO 2021). Hence, more research is prerequisite in order to impede the progression and reduce the number of future cancer cases.

Food labelling commonly states 'no added sugar' on food product packages nowadays. The development of several health disorders, primarily the metabolic syndrome, is associated with added sugar (Stanhope 2016). As sugar is considered to be noxious, it is often substituted with alternative sweeteners. Both natural and added sweeteners such as fruit juice concentrate, honey and oligofructose, like inulin, are used as replacements in order to emphasise healthier alternatives (Fidler Mis et al. 2017).

The discovery of acrylamide deprived food was made by Swedish scientists in year 2002. Since then, work has been continuously ongoing in order to try mapping the contributing factors of acrylamide formation in heated foodstuffs (Hellenäs et al. 2013). This low molecular weight organic substance does not exist naturally in food but is rather formed through a reaction between free asparagine and reducing sugars at high temperature. These precursors naturally occur in the raw material, such as cereals (ibid), but is also suspected to be present in alternative sweeteners.

In year 1994, International Agency for Research on Cancer (IARC) designated acrylamide as a Group 2A carcinogen (IARC 2021). Since then, the concern regarding the potential risk of acrylamide intake from cereals and human cancer development has increased (Williams 2014). The classification stresses that the correlation might be factual and thus troublesome. Consumption of food, in which acrylamide has been formed, may thereby contribute to the overall health status in a negative sense (FAO & WHO 2011). Consequently, it is of great importance to reduce the intake of acrylamide. Minimising the contribution in food by hindering formation ability will reduce its concentration. As stated by the EU Commission, food industry should reduce the acrylamide content in processed food (Commission Regulation (EU) 2017/2158). Also, the consumers should be informed and updated with new findings in order to be able to make well-established choices and reduce their acrylamide intake.

1.1. Problem Approach

The Swedish agricultural cooperative Lantmännen has been dealing with increased levels of acrylamide in their manufacture of cereal based products in which alternative sweeteners are being used instead of traditional sucrose. Sweetening agents contain reducing sugar but is suspected to also contain free asparagine, two substantial precursors for developing acrylamide. The experienced concern together with the ascending pressure from EU regulations to lower the concentration of acrylamide in food, made Lantmännen wonder how their cereal based products influence the overall concern. Despite this, they also wondered how the acrylamide concentration can be reduced.

1.2. Aim

Based on the problem approach, the aim of this study was to review whether the concern experienced by Lantmännen is studied in the scientific literature. Moreover, this study aimed to compile the available findings and determine how the usage of alternative sweeteners can affect acrylamide formation in the manufacture of extruded breakfast cereals.

The following questions were intended to be answered in this literature study:

- *Which alternative sweeteners are commonly used in the extruded breakfast cereal production and does its usage influence the formation of acrylamide?*
- *Which of the sweeteners contributes least to the formation of acrylamide and are the safest to use from this perspective?* and
- *Does apple juice concentration affect the level of formed acrylamide*?

1.3. Delimitations

This study will only scope the acrylamide concern in extruded breakfast cereals. Acrylamide is also formed in several other starch-rich foodstuffs such as potato products, coffee, fine bakery products, bread and baby jar food. However, this will not be considered in this literature study. With regard to this, the result cannot with certainty be applicable to these other foodstuffs.

2. Background

In year 2002, Swedish researchers discovered substantial amounts of acrylamide in carbohydrate-rich foods that were heat-treated (Tareke et al. 2002). Previously, acrylamide had numerous usage applications, including purifying water as well as within the industry of papermaking, textiles and petroleum (Pacetti et al. 2015). Furthermore, it was used as sealing in tunnel constructions (Mousavi Khaneghah et al. 2020). During the 1990s, the Hallandsås railway tunnel was built in southwestern Sweden. In the middle of the construction process, a water leak was discovered (Eriksson 2005). Hence a sealant was needed. The product Rhoca-Gil, which contained acrylamide, was used to seal the leakage. However, acrylamide was subsequently leaking out into the environment causing contamination of the water in a creek. In 1997, dead fish and cattle being affected with severe neurological symptoms were observed, which could be traced back to the polluted water (Hagmar et al. 2001). Nevertheless, tunnel workers also showed nerve symptoms similar to those caused by exposure of acrylamide, leading to intoxication (ibid.). Once acrylamide is absorbed into the body, either through the skin, gastrointestinal tract or through inhaled air, it spreads fast (Raffan & Halford 2019). Further on, it can be metabolised into glycidamide, another toxic compound that may influence the carcinogenic effect of acrylamide (Svensson et al. 2003).

Acrylamide is classified as a processing contaminant (Curtis et al. 2014). According to the authors, a processing contaminant is formed in food upon cooking or processing. Alongside, it is not naturally present in the raw material, which is substantiated in another study made by Tareke et al. (2002). Furthermore, the processing contaminant is undesirable (Curtis et al. 2014). Either it is possibly noxious, or it has disadvantageous product quality effects. In the study conducted by Tareke et al. (2002), acrylamide was not detected in boiled food. This is also confirmed in another study (Svensson et al. 2003). Thus, the formation of acrylamide is mainly associated with foodstuff that are either roasted, baked, fried or toasted.

2.1. EU regulation

In 2017, the European Commission published a regulation concerning the establishment of benchmark levels and mitigation actions in order to lower the concentration of acrylamide in food (Commission Regulation (EU) 2017/2158). Food business operators (FBOs) that are producing and putting breakfast cereals (porridge excluded) on the market ought to comply with this regulation. The applied mitigation actions stated in this regulation should lead to acrylamide concentration below the benchmark levels.

2.1.1. Benchmark levels

The benchmark levels in breakfast cereals (porridge excluded) differ between different categories. The content of acrylamide should be lower than 300 µg/kg in whole grain cereals, bran products and gun puffed grains, as well as products based on wheat and rye (Commission Regulation (EU) 2017/2158). Products based on maize, oats, spelt, barley and rice have an acrylamide benchmark level of 150 µg/kg. These benchmark levels are rather guidelines than maximum limits. The reason for having different benchmark levels in breakfast cereals could not be found.

2.1.2. Mitigation actions

For breakfast cereals, mitigation actions can be subdivided into several areas, such as agronomy, recipe and processing (Commission Regulation (EU) 2017/2158). Sometimes, cereals are received from the agricultural producer without any intermediaries. FBOs should then ensure that the provided cereals fulfil the requirements to avert increased levels of asparagine. The recipe can be modified in order to reduce the risk of acrylamide formation. Maize and rice-based products normally have lower acrylamide levels than products made from oat, wheat, rye and barley (ibid.). Reducing sugars and ingredients, for example honey, in which reducing sugars are included, have an impact on the formation of acrylamide when added to the cereal mixture before heat treatment. Likewise, other ingredients may contribute to acrylamide formation if being heat-treated prior to addition. Lastly, all critical steps in the manufacture that may imply formation of acrylamide have to be identified. Acrylamide concentration increases with temperature and heating time. Therefore, an efficient combination of these two parameters is required. Keeping in mind, neither the product's taste nor the "texture, colour, shelf-life and safety" should be compromised (ibid.). Despite this, moisture content has to be controlled and burnt products should be avoided.

2.2. The FoodDrinkEurope acrylamide toolbox

FoodDrinkEurope is an organisation representing the food and drink industry in Europe (FoodDrinkEurope 2019). Together with national authorities within European Union (EU), they have composed a toolbox. The toolbox is based upon the Commission Regulation (EU) 2017/2158, enabling implementation of the legislations. It is used as a guideline for manufacturers to monitor in which production step and in which product they might be able to reduce the acrylamide formation. The toolbox includes methods for FBOs to implement in the production in order to lower the levels of acrylamide to as low as reasonably achievable (ALARA) in the food products (Hellenäs et al. 2013). By adapting mitigation actions from the toolbox, FBOs ought to take into consideration factors that might affect the production feasibility, such as product quality, organoleptic properties and the risk of contribution from other contaminants (FoodDrinkEurope 2019). The tools are neither applicable to all production methods nor for all product categories. Hence, it is not meant to be restrictive in any sense. Instead, it is intended to assess each manufacturer, encouraging the identification of the most appropriate method for their own manufacturing.

2.2.1. Acrylamide in breakfast cereals

In breakfast cereals, the composition of reducing sugars in the raw material, such as cereal grains, is not the most definitive critical component for the acrylamide formation (FoodDrinkEurope 2019). On the contrary, the presence of free asparagine has more impact on the acrylamide levels in food products. Hence, free asparagine is of more relevance regarding the desired reduction. Based upon research, the fact that free asparagine is a key determinant for the formation of acrylamide in cereal products is confirmed (Taeymans et al. 2005).

2.2.2. Methods for acrylamide reduction in breakfast cereals

According to the FoodDrinkEurope (2019) acrylamide toolbox, there are several mitigation actions to reduce the acrylamide content in breakfast cereals. As for the Commission Regulation (EU) 2017/2158, these methods can be divided into agronomy, recipe and process design. In cereals, acrylamide formation can be hindered by avoiding elevated levels of free asparagine through cultivation. Also, the choice of cereal being used in the production of breakfast cereals may influence the amount of produced acrylamide. As previously mentioned, some cereals tend to yield more acrylamide compared to others. Moreover, the asparagine concentration is higher in the bran, meaning that reducing the content of bran would be favourable in regard to the formation of acrylamide. However, disadvantages may occur thereof and should thus be considered. Further on, usage of reducing sugars prior to heating may be reduced as a mitigation strategy. This is because reducing sugars are able to act as precursors in the formation of acrylamide (FoodDrinkEurope 2019). Also, other ingredients such as nuts and dried fruits may contribute to the final acrylamide concentration in the food product. When producing the breakfast cereal, this has to be considered. During processing, mitigation actions can be taken as well by optimizing the temperature and heating time.

3. Material and Method

This literature study is mainly based upon information gathered and compiled from scientific articles, books and reports. The literature was found by searching in several databases, primarily Web of Science, Google Scholar, Scopus, PubMed and ResearchGate. In addition, reports and other information from authorities such as Swedish Food Agency and FoodDrinkEurope among others have been used. Furthermore, EU regulations were also used.

The following searching words were used separately or in different combinations in order to find appropriate information in the literature: *acrylamide, asparagine, "breakfast cereal*", extrud*, extrusion, sugar** and *sweet**.

Alongside, a fruit juice analysis was performed. The procedure is described below.

3.1. Fruit juice analysis

Two concentrated apple juice samples received from Lantmännen were sent to Curtis Analytics Ltd. for analysis. Free sugars were extracted from the sample and analysed using ion chromatography. A Phenomenex® Amino acid Kit was used to analyse the content of free amino acids, including asparagine, according to Curtis et al. (2016). Both methods were done in triplicates.

To analyse the acrylamide content, a model system was set up. In each of the two analyses, 30 ml of concentrated apple juice was placed into a glass vial and baked at 165 °C for 20 minutes. Afterwards, the samples were re-adjusted back to 30 ml as around 10 ml of juice was evaporated respectively during the baking. This procedure was done in order to imitate the reality. Thus, the acrylamide results represent the amount of acrylamide formed per ml of unbaked apple juice. For the extraction, a 1,2,3-13C-acrylamide was used as an internal standard. The volume was then adjusted with deionized water and the samples were agitated for 20 minutes, followed by centrifugation for another 20 minutes. A 2 ml sample was then taken from each of the concentrated apple juice samples for filtration and analysis by LC-MS/MS (liquid chromatography tandem mass spectrometry) according to Elmore et al. (2015).

4. The Concern of Acrylamide

Since the discovery in 2002, acrylamide has been flourished in media in parallel to substantial findings regarding its formation in food when being heated. Although the knowledge about this organic substance is greater nowadays, it has probably been present in food ever since heating developed as a useful cooking technique (FoodDrinkEurope 2019).

4.1. Acrylamide formation

According to literature, the formation of acrylamide is the result of several chemical reactions whilst the reaction between the free amino acid asparagine and a reducing sugar (for example glucose and fructose) in the Maillard cascade is the most predominant one (FoodDrinkEurope 2019). In addition, carbonyl compounds may contribute to the acrylamide formation as it is involved in the asparagine decarboxylation, a crucial step in the development of acrylamide. Decarboxylation and deamination may lead to the formation of a Schiff base, which in the Maillard cascade consequently can result in acrylamide formation (Mousavi Khaneghah et al. 2020).

Maillard is a complex series of reactions which is not well-established yet (Curtis et al. 2014). It is however important in the development of pleasant attributes in heated food, such as colour, flavour, aroma and texture. These characteristics are desirable and thus, nutrients such as reducing sugar and amino acids present in the food are necessary for the reaction to occur (Stadler et al. 2002). The resulting compounds affects the heated food and is dependent on process conditions such as temperature and time at which the food is exposed to heat treatment, as well as the composition of reducing sugar and free asparagine in the raw material (Curtis et al. 2014). In particular free asparagine is essential in the participation of acrylamide formation as it is the limiting factor (Mottram et al. 2002).

Acrylamide is formed when food rich in carbohydrates are processed upon heat treatment exceeding 120 °C (Özer et al. 2012). In one study, "rye-based flat bread doughs, flat bread and bread" as well as dry wheat starch were baked at different settings of time and temperature (Bråthen & Knutsen 2005). The aim with this study was to investigate the correlation between acrylamide formation, maximum temperature and time in a dry model system and in dry food products based on cereals. Moreover, the aim was to determine whether the temperature-time dependence was influenced by different amounts of glucose and asparagine. The acrylamide content increased with elevated levels of asparagine for the model system with dry wheat starch. Such an increase was not notable for glucose, indicating that asparagine is the limiting factor over reducing sugar. For both flat bread, flat bread dough, normal bread and dry starch model system, an increase in acrylamide was observed up to approximately 190 to 210 °C, where a maximum limit was reached. However, the acrylamide content continued to increase with increasing baking time in the bread crust. On the contrary, the acrylamide formation decreased for the dry starch model system and flat bread with increasing baking time.

The type of raw material being used in the food manufacturing, together with quantities and quality influence the amount of acrylamide being formed (Taeymans et al. 2005). Other factors affecting the acrylamide concentration are time and temperature during heat treatment, as well as moisture content and pH (Kristersson et al. 2017; Mousavi Khaneghah et al. 2020). Furthermore, the content of precursors such as asparagine and reducing sugars affect the formation of acrylamide.

5. Breakfast Cereals

Breakfast cereals are grains that are being processed to a low moisture content and intended to be eaten by humans (Caldwell et al. 2016). Alongside hot cereals, that are requiring some sort of cooking prior to ingestion, breakfast cereals are predominantly ready-to-eat (RTE) which does not require any further cooking. The RTE breakfast cereals normally compose different grains and may be flavoured, sweetened and “fortified with vitamins and minerals”, either prior to processing or subsequently after (ibid.). The recipe may alter depending on which product type being produced, thus preservatives, nuts and dried fruits may also be added. In an ordinary Swedish diet, breakfast cereals are a common feature. Cereal grain-based products are primarily included in the category of breakfast cereals (FoodDrinkEurope 2019). Generally, these are consumed together with milk or other dairy products, for instance sour milk or yoghurt. The cereal grains used for the manufacture of breakfast cereals are particularly barley, maize, oat, rice and wheat (Caldwell et al. 2016). Rye is also being used (FoodDrinkEurope 2019).

Among different process techniques, breakfast cereals are commonly extruded. Extrusion cooking is a method in which the cereal material is being pre-conditioned and mixed, followed by cooking and cooling through a screw barrel and finally it emerges through dies where the product is cut and cooled down (Ruiz-Gutiérrez & Quintero-Ramos 2017). The starting material, having a moisture content of 25 to 30 percent is subjected to temperatures above 100 °C in the extruder. Breakfast cereals are commonly extruded at 140 to 180 °C (Fellows 2017). When emerging the dies, the ambient surrounding having different conditions compared to the extruder, causes the final product to expand (Ruiz-Gutiérrez & Quintero-Ramos 2017). Finally, it is oven-dried to lower the moisture content to a desired value. Alongside the dies, the product is influenced by the temperature, pressure and mechanical shear (Smith 1976). Dwell time is another factor affecting the final product (Ruiz-Gutiérrez & Quintero-Ramos 2017).

5.1. Contribution to acrylamide intake

According to a meta-analysis, breakfast cereals contributed to the highest concentration of acrylamide after potato-based foods and fried foods (Mousavi

Khaneghah et al. 2020). In contrast, breakfast cereals contributes to a lesser extent to the acrylamide intake in Sweden (Hellenäs et al. 2013). Only seven percent of the Swedes intake of acrylamide was originated from breakfast cereals (ibid.).

In a Swedish study, 23 different samples of breakfast cereals were analysed (Abramsson Zetterberg 2017). The result showed that the mean concentration of acrylamide was 140 µg/kg, ranging from 23 to 313 µg/kg. The observed differences in acrylamide concentration within the food group was according to Abramsson Zetterberg (2017) explained by differences in time and temperature settings as well as cereal type being used. In another study, Swedish scientists investigated the amount of acrylamide found in different food groups (Svensson et al. 2003). In 14 different samples of breakfast cereals, the analysed mean content of acrylamide was 220 µg/kg, ranging from <30 to 1400 µg/kg. Observed in some samples from both of the Swedish studies, the concentration of acrylamide transcends the EU benchmark levels of 150 µg/kg and 300 µg/kg, depending on product type.

The European Food Safety Authority (EFSA) has compiled information based on monitoring made by European member states on current acrylamide concentrations in food (Hellenäs et al. 2013). The results indicate that no significant acrylamide reduction was seen during 2007 to 2010 in Sweden. Likewise, no reduction was seen between 2005 and 2012 in Sweden (Kristersson et al. 2017).

5.2. Alternative sweeteners

In order to write 'no added sugar' on the package food labels, FBOs substitutes the sugar (i.e. sucrose) in favour of alternative sweeteners to sweeten the products. Different sweeteners are being used in different products, of which concentrated fruit juice, inulin, chicory root, malt extract and honey are the commonest in Sweden (author's observation). When honey is being used, the label states either 'no added sugar', 'sweetened with honey' or 'only natural ingredients'. According to legal act, the nutrition claim 'no added sugar' is only valid for products absent in "added mono- or disaccharides or any other food used for its sweetening properties" (Regulation (EC) No 1924/2006).

5.2.1. Fruit juice analysis

From the analysis of the amino acid composition of the two samples of apple juice concentrates (A and B), a mean value was taken for each of the triplicate respectively. In Figure 1, the mean values are provided. In both of the samples, asparagine clearly dominates. This result is consistent with the distribution of amino acids found in apple juice (Ma et al. 2018). In this study, 20 different amino acids were analysed and quantified, out of which free asparagine was the dominating amino acid, present in abundance. Regarding amino acid content, apple juice concentrate is a rich source of free asparagine.

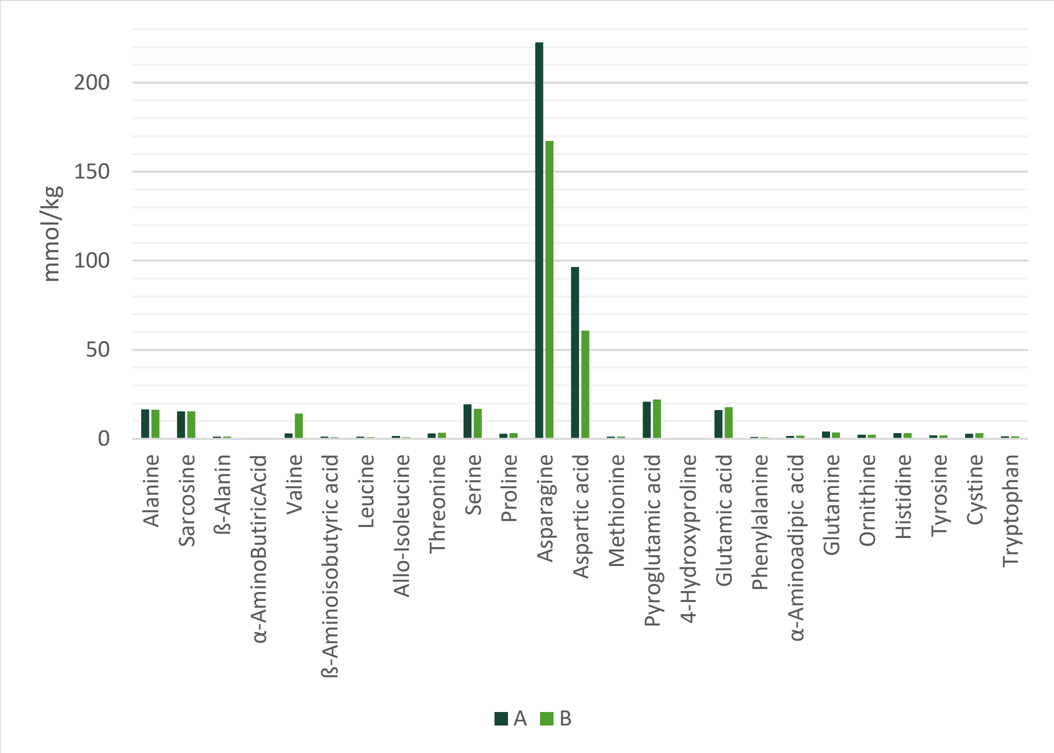

Figure 1. Concentration (mmol/kg) of individual amino acids in two samples of apple juice concentrate (A and B). A mean value was taken for each triplicate.

Withal, it is notable that both samples primarily contain free asparagine and aspartic acid (Figure 1). Moreover, sample A consists of more free asparagine than sample B. Additionally, acrylamide was formed in both samples upon heating (Figure 2). Sample A developed more acrylamide compared to sample B. Hence, a correlation between free asparagine content and acrylamide concentration seems apparent.

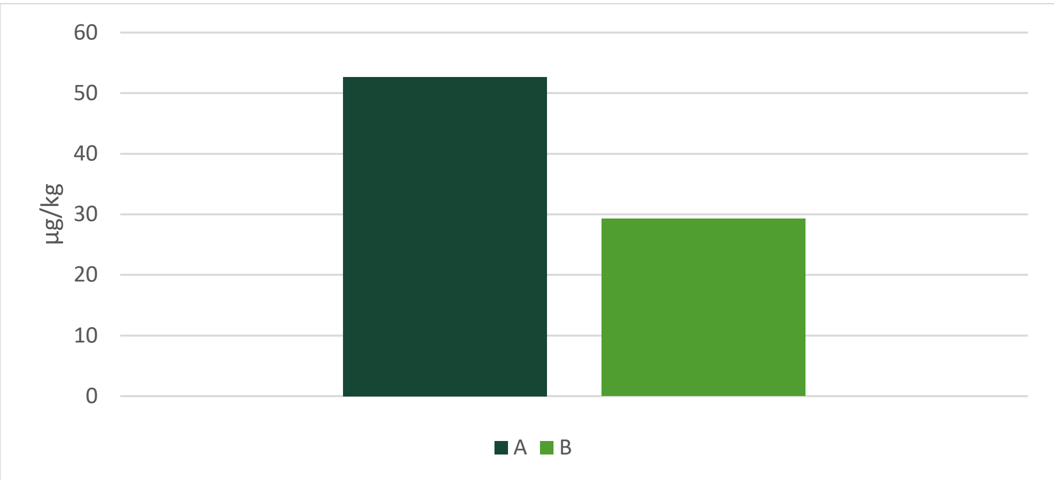

Figure 2. Acrylamide concentration (µg/kg) in two samples (A and B) of apple juice concentrate.

In Figure 3, differences between the distribution of reducing sugar are illustrated for the two concentrated apple juice samples. Sample B has marginally greater content of both glucose as well as fructose and sucrose. Maltose was not detectable in either sample. Even though sample A has less reducing sugar, it accounts for more acrylamide formation. Despite this, the sugar content is substantially higher than the concentration of free asparagine. Altogether, this confirms the fact that asparagine is the limiting factor and not reducing sugar. Thus, asparagine determines how much acrylamide that is formed.

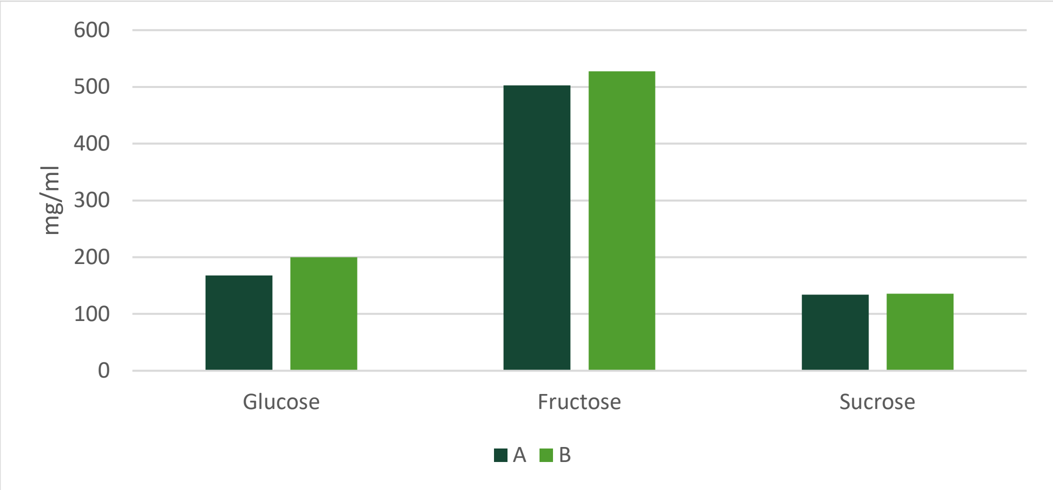

Figure 3. Content (mg/ml) of glucose, fructose and sucrose detected in two samples of concentrated apple juice.

However, the amount of acrylamide being formed in the concentrated apple juice samples is extremely small. Whether the acrylamide content formed from the apple juice concentrate is negligible or considered to be significant in the final breakfast cereal depends on the amount of free asparagine in both the fruit juice but also in the other ingredients. How much free asparagine that is enough to be considered troublesome in the food product can only be determined by further analyses.

5.2.2. Other fruit juices

In comparison with concentrated apple juice, other fruit juices may contain varying concentrations of free asparagine. This has been examined in a study conducted by Chinese researchers (Zeng et al. 2015). In their study, 21 different free amino acids originating from six samples of fruit juice were analysed simultaneously using a modified procedure including a "pre-column derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)" followed by high-performance liquid chromatography (HPLC). The performance was done in triplicates. In Table 1, the determined content of free asparagine is presented. Out of these 21 amino acids, the concentration of free asparagine was determined.

Table 1. Concentration (mg L^{-1}) of free asparagine (Asn) in six samples of fruit juice. The values are presented as mean±SD. Table content is derived from Zeng et al. (2015).

Amino acid	Apple	Litchi	Longan	Orange	Pear	Strawberries
Asn	162.9±6.4	36.4±1.5	148.1±4.4	363.5±7.9	443.1±3.5	1311.9±20.7

Based on the fruit juices being determined in the above-mentioned study, litchi juice contained the lowest amount of free asparagine followed by juice from longan, apple, orange, pear and strawberries. The result indicates that varying amount of free asparagine is present in different kinds of fruit juices.

In another study, asparagine was found to be the predominant amino acid present in apricot juice (Salehi 2020). In total, 26 samples of apricot juice were analysed for different nutritional compounds, including amino acids, using HPLC. On average, the content of asparagine was ranging between 820 to 1570 mg L^{-1} (Versari et al. 2008).

In a further study, fruit juices from apricot, grapefruit, orange, peach, pear and pineapple were being analysed simultaneously using reversed-phase HPLC after pre-column derivatization with 9-fluorenylmethol-chloroformate (FMOC-Cl) combined with 1-amino-adamantane hydrochloride (ADAM) (Fabiani et al. 2002). Each fruit juice contained varying amount of free asparagine. Foremost, asparagine was present in abundance in apricot and peach juice on the chromatograms. Despite this, pear and pineapple juice also contained high amounts. The content of asparagine in grapefruit and orange juice was on the other hand considerably lower. No numeric values were given in the paper but the authors claimed that the received result with its amino acid profile was consistent with previous findings.

In one study, apple juices used for cider making were analysed based on the content of free amino acids (Burroughs 1957). Using paper chromatography, six varieties were examined, in which asparagine accounted for the majority of amino acids in four. Two of the samples contained higher amount of free asparagine than the others. The explanation for this was their naturally higher content of soluble nitrogen. The remaining four samples had a lower amount of soluble nitrogen; hence they were lower in asparagine. Thus, a correlation between the level nitrogen and asparagine was apparent.

5.2.3. Oligosaccharides, inulin and chicory root

Short chain fructo-oligosaccharide (SC-FOS) is a potential prebiotic stimulating a healthful gut (Chikkerur et al. 2020). Moreover, it is regarded as a functional food, serving favourable attributes during food processing. SC-FOS are classified as carbohydrates consisting mainly of fructose molecules linked together in a chain by ß-1,2 bonds, with a terminal glucose unit (Bouhnik et al. 2006). Oligosaccharides are low in energy due to reduced digestibility and are therefore added in food products as a bulking agent (Bouhnik et al. 2006; Flores et al. 2016). Thus, they might be replacing sugar.

Inulin is a polysaccharide composed of the same chemical components as SC-FOS (Tiefenbacher 2017). Whereas inulin is a fructan, SC-FOS is a short-chain fructan that can be manufactured through enzymatic degradation of inulin. Inulin function as sugar substitution (Roberfroid 2000). The relative sweetness of inulin is 1 to 14 percent of the sweetness of sucrose (Tiefenbacher 2017). Besides having nutritional function in breakfast cereals, inulin also contributes to browning in the Maillard reaction. Commercially, inulin is most commonly extracted from chicory root (Chikkerur et al. 2020).

Addition of pure chicory root is sometimes included as an ingredient in breakfast cereal products that usually states ‘no added sugar’ on the package. Chicory root consists of 5 to 10 percent oligofructose and 15 to 20 percent inulin (Kumar et al. 2018). One study conducted by Polish researchers showed that elevated concentration of acrylamide was formed during roasting with increased temperature of roots from chicory (Zięć et al. 2015). This pattern was seen in the temperature range of 100 to 175 °C. Interestingly, the formation was inhibited at 210 °C. This is in agreement with the maximum temperature limit attained in the study conducted by Bråthen & Knutsen (2005). Despite this, the concentration of acrylamide also increased with increasing time of roasting (Zięć et al. 2015).

Another study has investigated the correlation between the amount of free asparagine in chicory and different factors potentially affecting the formation of acrylamide (Loaëc et al. 2014). In this study, five different cultivars of chicory were grown in different field trials and analysed accordingly. Between the varieties and growing years, there was a significantly difference in the concentration of free asparagine. An observed decrease in the range of 31 to 50 percent in free asparagine content was seen when raw chicory root was being dried prior to analysis. The concentration of free asparagine was also significantly influenced by nitrogen fertilization in all five cultivars. The chicory roots used for the trial with nitrogen fertilization were chosen to be roasted. A significant correlation was seen between the concentration of free asparagine and the formation of acrylamide. This study showed that selecting varieties with lower content of free asparagine as well as limiting the amount of nitrogen fertilization being used during cultivation are important factors for the mitigation of acrylamide in chicory root.

5.2.4. Glucose syrup

Fructose and glucose are both reducing sugars contributing to the formation of acrylamide. Fructose, in comparison with glucose, is however more effectively forming acrylamide (Amrein et al. 2003). This is in accordance with a study where the correlation between sweeteners and acrylamide formation in biscuits was examined (Mustatea et al. 2015). The biscuits that were sweetened with fructose contained higher levels of acrylamide compared with biscuits sweetened with sugar, glucose or honey, which contains the monosaccharides glucose and fructose. This explains why glucose together with non-reducing sugars such as sucrose are designated as replacement of fructose or ingredients containing fructose as a mitigation action to formation of acrylamide (Commission Regulation (EU) 2017/2158). Based on this, glucose syrup is to be used in front of ingredients rich in fructose such as honey and invert sugar syrup. Upon extrusion, syrup contributes to sweetness and viscosity by addition of moisture (Della Valle et al. 2011), making the starch gelatinize (Singha et al. 2017).

5.3. Free asparagine content in other breakfast cereal ingredients

Besides sugar or other alternative sweeteners, breakfast cereals consist of other ingredients. Depending on which product is being produced, the recipe varies. Some ingredients are added prior to heating, whereas others are added afterwards. The concentrated apple juice being analysed in the present study is mainly used to produce extruded oat puffs. Despite this alternative sweetener, other major ingredients in the recipe are oat flour and rice flour. These might as well as the concentrated apple juice impart in the acrylamide formation, depending on the presence and amount of free asparagine.

In one study, the content of free asparagine and sugar in cereal grains were being analysed (Žilić et al. 2017). This was done in order to investigate the potential formation of acrylamide. Out of eight species, hull-less oats had the second highest content of asparagine of the different cultivars being analysed, following rye. Four different varieties of hull-less oats were being analysed. The asparagine content ranged between 510.7 to 1196.6 µg/g with a mean of 893.85 µg/g. Thus, hull-less oats had the second highest acrylamide formation potential, whereas wheat had the lowest with a mean asparagine content of 426.05 µg/g. According to this study, the content of free asparagine varied within a specie but also between different species. To mitigate the formation of acrylamide, selecting varieties with low amounts of asparagine was designated as a good strategy (ibid.).

Rice has also been studied regarding acrylamide formation. Cookies made of rice flour was shown to have significantly lower levels of acrylamide in comparison with those made of wheat flour (Chen et al. 2020). White rice flour had the highest content of free asparagine compared to brown rice, whole wheat and white wheat flours. However, cookies made of white rice flour contained the lowest amount of acrylamide. During baking, the content of free asparagine decreased considerably. The authors concluded that the free asparagine was not a limiting factor for acrylamide formation in the cookies. In another study, free amino acids in eleven different rice varieties were being analysed (Liyanaarachchi et al. 2020). Among the varieties, different amount of free asparagine was detected. The mean asparagine content ranged between 4.5±0.6 to 16.6±3.2 mg/100 g on a dry matter basis. The asparagine content detected in this study was considerably lower than those detected in the cereal grains, including hull-less oats, in the study made by Žilić et al. (2017). The scientists therefore draw the conclusion that acrylamide formation upon heating is lower in rice compared to other cereals having higher content of free asparagine (Liyanaarachchi et al. 2020).

Since hull-less oat has higher acrylamide formation potential than rice, it indicates that oat flour either alone or together with rice flour influence the amount of acrylamide formed during the manufacture of extruded breakfast cereals. From an acrylamide context, rice flour is therefore a better option compared with oat flour when producing extruded breakfast cereals. However, other factors do also have to be taken into consideration during product development and manufacture, not only the potential of forming acrylamide. This might influence the overall decision on which cereals that are being included in the final product.

6. Discussion and Conclusion

Acrylamide is formed upon heat-treatment of food rich in carbohydrates, including breakfast cereals. In Sweden, seven percent of the total intake of acrylamide originates from breakfast cereals (Hellenäs et al. 2013). Notwithstanding the relatively low contribution, the acrylamide intake is troublesome. This low-molecular compound is regarded as probable carcinogenic to humans and actions must thereby be taken to reduce the intake of it. This is why European Union Commission has adopted a regulation concerning this issue, which is directly binding in all member states. This legislative act has also been interpreted by FoodDrinkEurope to address the issue and guide manufacturers in the food and drink industry to reduce their acrylamide formation upon production. Based on the assessments given by several authorities, it is of great importance to reduce the levels of acrylamide in breakfast cereals to ALARA. It is clearly denoted though that there is no unambiguous solution to this issue.

Breakfast cereals are commonly produced by extrusion cooking, a method at which the material is exposed to temperatures above 100 °C. Acrylamide is formed at temperatures exceeding 120 °C. Depending on which temperature the material is subjected to, different amount of acrylamide may be formed. The formation is also time-dependent, meaning that the material should not be exposed for too high temperatures for a certain amount of time without increasing the risk of acrylamide formation.

There are limitations with this study, concerning the analysis of acrylamide in the concentrated fruit juices. First, the amount of acrylamide formed during the set-up model system for fruit juice analysis is based on the conditions under which they ran the experiment (20 minutes at 165 °C). These conditions differ completely from the reality during the process making of extruded breakfast cereals. The time at which the batter is exposed to temperatures above 120 °C is fairly short in an extruder. Nevertheless, acrylamide is still formed. Worth mentioning is that the levels of acrylamide formed in the fruit juice samples were considerably low compared to those attained in the actual products. Moreover, only a third of the sample was evaporated and thus not concentrated enough, explaining the fact that only a small amount of acrylamide was formed in the experiment. Also, the treatment was done in an unknown temperature. If re-doing the experiment, the samples would first have been completely evaporated in an oven at lower degrees, followed by baking at the temperature and time that were used in this analysis. Nonetheless, in this experiment something was still formed only by heating the fruit juice, accounting for that there is a possibility for developing acrylamide also in the final product by using this concentrated fruit juice.

Concentrated fruit juice, including both free asparagine and reducing sugar, is regarded as a contributing factor to the formation of acrylamide in breakfast cereals. As being confirmed in the fruit juice analysis, asparagine is the limiting factor. It would be interesting to dig deeper into the question of to what extent the concentrated fruit juice contributes to the total formation of acrylamide. By limiting the concentration of free asparagine, the acrylamide content might perhaps be reduced. Usage of the analysed concentrated fruit juice was assumed to be problematic in the context of acrylamide formation. Thus, another alternative might be valid to ensure a reduction. Either, the amount of free asparagine could perhaps be reduced in the fruit juice by fermentation, which has been successively done in bread (Mustafa et al. 2009). Another suggestion is to minimise ingredients rich in free asparagine by substituting those with ingredients that contain lower amounts. As the content of free asparagine varies between different fruits, a concentrated fruit juice with lower free asparagine content should be favoured in the manufacture. Preferably, a sweetener absent in free asparagine should be chosen in front of concentrated fruit juice. Ingredients like inulin, glucose syrup or another fruit source might be useful to substitute the concentrated apple juice in order to reduce the content of acrylamide. Lastly, addition of the enzyme asparaginase could perhaps reduce the content of free asparagine in the concentrated fruit juice (Xu et al. 2016).

Moreover, other ingredients may also imply an extensively increased amount of formed acrylamide. It has been shown that products made of maize and rice contain lower amount of acrylamide compared to other cereals, indicating an advantage with respect to the need of keeping the formed amount below the benchmark levels as a mitigation action. Based on this, using maize or rice in front of oat, wheat, rye and barley seems advantageous. However, it is difficult to evaluate how these cereals might affect the manufacturing process and the organoleptic properties of the breakfast cereals. More information on the impact of cereal specie being used for the final quality of the breakfast cereals is therefore needed. Acrylamide formation might be influenced by the supplement of asparagine from other constituent ingredients as well. Based on this, it would be of significant interest to investigate the amount of asparagine in the final extruded products. This has not been done yet. It would enhance the comparability between the model system set up in the present study and the actual process conditions. Based on the received result, it is challenging to draw conclusion in terms of how equal the fruit juice analysis is to the real conditions.

Studies on the impact of concentrated fruit juice on the acrylamide formation in breakfast cereals are scarce, but more specifically non-existing. Therefore, research on this relation is warranted. However, this study indicates that the concentrated fruit juice contains free asparagine, the limiting factor for acrylamide formation. Also, this study confirms that the concentrated fruit juice may influence the total amount of acrylamide being formed in extruded breakfast cereals. In the context of acrylamide formation, free asparagine is unwanted. However, since free asparagine is the predominant amino acid in some plants, it should reasonably have a function in the plant. Thus, research on the physiological and metabolic function of free asparagine in plants is also needed.

References

Abramsson Zetterberg, L. (2017). *Akrylamid och andra värmeinducerade ämnen i livsmedel, kunskapsöversikt*. (Rapport nr 11 del 2). Uppsala: Livsmedelsverket. https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2017/kunskapsoversikt-akrylamid-och-andra-varmeinducerade-amnen-livsmedelsverkets-rapport-nr-11-2017-del-2.pdf

Amrein, T.M., Bachmann, S., Noti, A., Biedermann, M., Barbosa, M.F., Biedermann-Brem, S., Grob, K., Keiser, A., Realini, P., Escher, F. & Amadó, R. (2003). Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *Journal of Agricultural and Food Chemistry*, 51 (18), 5556–5560. https://doi.org/10.1021/jf034344v

Bouhnik, Y., Raskine, L., Simoneau, G., Paineau, D. & Bornet, F. (2006). The capacity of short-chain fructo-oligosaccharides to stimulate faecal bifidobacteria: a dose-response relationship study in healthy humans. *Nutrition Journal*, 5, 8. https://doi.org/10.1186/1475-2891-5-8

Bråthen, E. & Knutsen, S.H. (2005). Effect of temperature and time on the formation of acrylamide in starch-based and cereal model systems, flat breads and bread. *Food Chemistry*, 92 (4), 693–700. https://doi.org/10.1016/j.foodchem.2004.08.030

Burroughs, L.F. (1957). The amino-acids of apple juices and ciders. *Journal of the Science of Food and Agriculture*, 8 (3), 122–131. https://doi.org/10.1002/jsfa.2740080304

Caldwell, E.F., McKeehen, J.D. & Kadan, R.S. (2016). Breakfast cereals. *Reference Module in Food Science*. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.00143-8

Chen, Y., Wu, Y., Fu, J. & Fan, Q. (2020). Comparison of different rice flour- and wheat flour-based butter cookies for acrylamide formation. *Journal of Cereal Science*, 95, 103086. https://doi.org/10.1016/j.jcs.2020.103086

Chikkerur, J., Samanta, A.K., Kolte, A.P., Dhali, A. & Roy, S. (2020). Production of short chain fructo-oligosaccharides from inulin of chicory root using fungal endoinulinase. *Applied Biochemistry and Biotechnology*, 191 (2), 695–715. https://doi.org/10.1007/s12010-019-03215-7

Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food (n.d.). (OJ L 304, 21.11.2017, 24–44). http://data.europa.eu/eli/reg/2017/2158/oj

Curtis, T., Powers, s. J. & Halford, N. (2016). Effects of fungicide treatment on free amino acid concentration and acrylamide-forming potential in wheat. *Journal of Agricultural and Food Chemistry*, 64. https://doi.org/10.1021/acs.jafc.6b04520

Curtis, T.Y., Postles, J. & Halford, N.G. (2014). Reducing the potential for processing contaminant formation in cereal products. *Journal of Cereal Science*, 59 (3), 382–392. https://doi.org/10.1016/j.jcs.2013.11.002

Della Valle, G., Berzin, F. & Vergnes, B. (2011). *Modeling of Twin Screw Extrusion Process for Food Products Design and Process Optimization*. https://doi.org/10.1201/b11286-14

Elmore, J.S., Briddon, A., Dodson, A.T., Muttucumaru, N., Halford, N.G. & Mottram, D.S. (2015). Acrylamide in potato crisps prepared from 20 UK-grown varieties: Effects of variety and tuber storage time. *Food Chemistry*, 182, 1–8. https://doi.org/10.1016/j.foodchem.2015.02.103

Eriksson, S. (2005). *Acrylamide in food products: Identification, formation and analytical methodology*. (Doctoral thesis). Stockholm University. https://su.diva-portal.org/smash/get/diva2:197454/FULLTEXT01.pdf

Fabiani, A., Versari, A., Parpinello, G.P., Castellari, M. & Galassi, S. (2002). High-performance liquid chromatographic analysis of free amino acids in fruit juices using derivatization with 9-fluorenylmethyl-chloroformate. *Journal of Chromatographic Science*, 40 (1), 14–18. https://doi.org/10.1093/chromsci/40.1.14

FAO & WHO (2011). *Safety evaluation of certain contaminants in food*. Rome: Food and Agriculture Organization of the United Nations / Geneva: World Health Organization. (WHO food additives series; 63 / FAO JECFA Monographs 8)

Fellows, P.J. (2017). *Food Processing Technology*. 4. ed. Woodhead Publishing.

Fidler Mis, N., Braegger, C., Bronsky, J., Campoy, C., Domellöf, M., Embleton, N.D., Hojsak, I., Hulst, J., Indrio, F., Lapillonne, A., Mihatsch, W., Molgaard, C., Vora, R. & Fewtrell, M. (2017). Sugar in infants, children and adolescents: A position paper of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *Journal of Pediatric Gastroenterology & Nutrition*, 65 (6), 681–696. https://doi.org/10.1097/MPG.0000000000001733

Flores, A.C., Morlett, J.A. & Rodríguez, R. (2016). Inulin potential for enzymatic obtaining of prebiotic oligosaccharides. *Critical Reviews in Food Science and Nutrition*, 56 (11), 1893–1902. https://doi.org/10.1080/10408398.2013.807220

FoodDrinkEurope (2019). *Acrylamide Toolbox 2019*. https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf [2021-03-27]

Hagmar, L., Törnqvist, M., Nordander, C., Rosén, I., Bruze, M., Kautiainen, A., Magnusson, A.-L., Malmberg, B., Aprea, P., Granath, F. & Axmon, A. (2001). Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. *Scandinavian Journal of Work, Environment & Health*, 27 (4), 219–226. https://doi.org/10.5271/sjweh.608

Hellenäs, K.-E., Fohgelberg, P., Fäger, U., Busk, L., Zetterberg, L.A., Ionescu, C. & Färnstrand, J.S. (2013). *Acrylamide in Swedish food - targeted sampling 2011 and 2012*. (Rapport nr 25/2013). Uppsala: Swedish Food Agency. https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2013/2013_national_food_agency_25_acrylamide_in_swedish_food_targeted_sampling_2011_and_2012.pdf

IARC (2021). *List of Classifications - Agents classified by the IARC Monographs, Volumes 1–129*. https://monographs.iarc.who.int/list-of-classifications/ [2021-03-28]

Kristersson, M., Halldin Ankarberg, E., Lignell, S., Rosengren, Å., Lantz, C., Sjögren Bolin, Y. & Lagerberg Fogelberg, C. (2017). *Akrylamid och andra värmeinducerade ämnen i livsmedel, kunskapsöversikt*. (Rapport nr 11 del 1). Uppsala: Livsmedelsverket. https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2017/riskhanteringsrapport-akrylamid-och-andra-varmeinducerade-amnen-livsmedelsverkets-rapport-nr-11-2017-del-1.pdf

Kumar, C.G., Sripada, S. & Poornachandra, Y. (2018). Chapter 14 - Status and future prospects of fructooligosaccharides as nutraceuticals. In: Grumezescu, A.M. & Holban, A.M. (eds.) *Role of Materials Science in Food Bioengineering*. Academic Press, 451–503. https://doi.org/10.1016/B978-0-12-811448-3.00014-0

Liyanaarachchi, G.V.V., Mahanama, R., Somasiri, S., Punyasiri, N. & Kottawa-Arachchi, J. (2020). Total and free amino acid contents of popular rice varieties (Oryza sativa L.) consumed in the capital city of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, 48, 199–211. https://doi.org/10.4038/jnsfsr.v48i2.9565

Loaëc, G., Niquet-Léridon, C., Henry, N., Jacolot, P., Volpoet, G., Goudemand, E., Janssens, M., Hance, P., Cadalen, T., Hilbert, J.-L., Desprez, B. & Tessier, F.J. (2014). Effects of variety, agronomic factors, and drying on the amount of free asparagine and crude protein in chicory. Correlation with the acrylamide formation during roasting. *Food Research International*, 63, 299–305. https://doi.org/10.1016/j.foodres.2014.03.010

Ma, S., Neilson, A.P., Lahne, J., Peck, G.M., O'Keefe, S.F. & Stewart, A.C. (2018). Free amino acid composition of apple juices with potential for cider making as determined by UPLC-PDA. *Journal of the Institute of Brewing*, 124 (4), 467–476. https://doi.org/10.1002/jib.519

Mousavi Khaneghah, A., Fakhri, Y., Nematollahi, A., Seilani, F. & Vasseghian, Y. (2020). The concentration of acrylamide in different food products: A global systematic review, meta-analysis, and meta-regression. *Food Reviews International*. https://doi.org/10.1080/87559129.2020.1791175

Mustafa, A., Fink, M., Kamal-Eldin, A., Rosén, J., Andersson, R. & Åman, P. (2009). Interaction effects of fermentation time and added asparagine and glycine on acrylamide content in yeast-leavened bread. *Food Chemistry*, 112, 767–774. https://doi.org/10.1016/j.foodchem.2008.05.099

Mustatea, G., Popa, M.E., Negoita, M. & Roming, F.I. (2015). Asparagine and sweeteners – how they influence acrylamide formation in wheat flour biscuits? *Journal of Biotechnology*, 208, S81. https://doi.org/10.1016/j.jbiotec.2015.06.252

Pacetti, D., Gil, E., Frega, N.G., Álvarez, L., Dueñas, P., Garzón, A. & Lucci, P. (2015). Acrylamide levels in selected Colombian foods. *Food Additives & Contaminants: Part B*, 8 (2), 99–105. https://doi.org/10.1080/19393210.2014.995236

Raffan, S. & Halford, N.G. (2019). Acrylamide in food: Progress in and prospects for genetic and agronomic solutions. *Annals of Applied Biology*, 175 (3), 259–281. https://doi.org/10.1111/aab.12536

Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods (n.d.). . (OJ L 404, 30.12.2006, p. 9–25). https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32006R1924

Roberfroid, M.B. (2000). Prebiotics and probiotics: are they functional foods? *The American Journal of Clinical Nutrition*, 71 (6), 1682S-1687S. https://doi.org/10.1093/ajcn/71.6.1682S

Ruiz-Gutiérrez, M.G. & Quintero-Ramos, M.Á.S.-M. and A. (2017). *The extrusion cooking process for the development of functional foods*. *Extrusion of metals, polymers and food products* IntechOpen. https://doi.org/10.5772/intechopen.68741

Salehi, F. (2020). Physicochemical characteristics and rheological behaviour of some fruit juices and their concentrates. *Journal of Food Measurement and Characterization*, 14 (5), 2472–2488. https://doi.org/10.1007/s11694-020-00495-0

Singha, P., Muthukumarappan, K. & Krishnan, P. (2017). Influence of processing conditions on apparent viscosity and system parameters during extrusion of distiller's dried grains-based snacks. *Food Science & Nutrition*, 6 (1), 101–110. https://doi.org/10.1002/fsn3.534

Smith, O. (1976). Extrusion cooking. *New Protein Foods*. New York: Academic Press, p.87

Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A., Robert, M.-C. & Riediker, S. (2002). Acrylamide from Maillard reaction products. *Nature*, 419 (6906), 449–450. https://doi.org/10.1038/419449a

Stanhope, K.L. (2016). Sugar consumption, metabolic disease and obesity: The state of the controversy. *Critical Reviews in Clinical Laboratory Sciences*, 53 (1), 52–67. https://doi.org/10.3109/10408363.2015.1084990

Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenäs, K.-E., Lind, Y. & Rosén, J. (2003). Dietary intake of acrylamide in Sweden. *Food and Chemical Toxicology*, 41 (11), 1581–1586. https://doi.org/10.1016/S0278-6915(03)00188-1

Taeymans, D., Andersson, A., Ashby, P., Blank, I., Gondé, P., Eijck, P. van, Faivre, V., Lalljie, S.P.D., Lingnert, H., Lindblom, M., Matissek, R., Müller, D., Stadler, R.H., Studer, A., Silvani, D., Tallmadge, D., Thompson, G., Whitmore, T., Wood, J. & Zyzak, D. (2005). Acrylamide: update on selected research activities conducted by the European Food and Drink Industry. *Journal of AOAC INTERNATIONAL*, 88 (1), 234–241. https://doi.org/10.1093/jaoac/88.1.234

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. & Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 50 (17), 4998–5006. https://doi.org/10.1021/jf020302f

Tiefenbacher, K.F. (2017). Chapter Two - Technology of Main Ingredients - Water and Flours. In: Tiefenbacher, K.F. (ed.) *Wafer and waffle*. Academic Press, 15–121. https://doi.org/10.1016/B978-0-12-809438-9.00002-8

Versari, A., Parpinello, G.P., Mattioli, A.U. & Galassi, S. (2008). Characterisation of Italian commercial apricot juices by high-performance liquid chromatography analysis and multivariate analysis. *Food Chemistry*, 108 (1), 334–340. https://doi.org/10.1016/j.foodchem.2007.10.053

WHO (2021-03-03). *Cancer*. https://www.who.int/news-room/fact-sheets/detail/cancer [2021-04-22]

Williams, P.G. (2014). The benefits of breakfast cereal consumption: A systematic review of the evidence base. *Advances in Nutrition*, 5 (5), 636S-673S. https://doi.org/10.3945/an.114.006247

Xu, F., Oruna-Concha, M.-J. & Elmore, J.S. (2016). The use of asparaginase to reduce acrylamide levels in cooked food. *Food Chemistry*, 210, 163–171. https://doi.org/10.1016/j.foodchem.2016.04.105

Zeng, F., Ou, J., Huang, Y., Li, Q., Xu, G., Liu, Z. & Yang, S. (2015). Determination of 21 free amino acids in fruit juices by HPLC using a modification of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method. *Food Analytical Methods*, 8. https://doi.org/10.1007/s12161-014-9905-8

Zięć, G., Gambuś, H., Berski, W., Litwinek, D., Wyworcka-Gurgul, A. & Mickowska, B. (2015). Attempt to reduce acrylamide content in roasted chicory. *Journal of Microbiology, Biotechnology and Food Sciences*, 4, 188–191. https://doi.org/10.15414/jmbfs.2015.4.special3.188-191

Žilić, S., Dodig, D., Basić, Z., Vančetović, J., Titan, P., Đurić, N. & Tolimir, N. (2017). Free asparagine and sugars profile of cereal species: the potential of cereals for acrylamide formation in foods. *Food Additives &*

Contaminants: Part A, 34 (5), 705–713. https://doi.org/10.1080/19440049.2017.1290281

Özer, M., Kola, O., Altan, A., Duran, H. & Zorlugenç, B. (2012). Acrylamide content of some Turkish traditional desserts. *Journal of Food, Agriculture and Environment*, 1010, 74–77

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