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FACTORS INFLUENCE TO FORMATION OF ACRYLAMIDE IN FOOD

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Abstract

Acrylamide - AA is a chemical compound formed during the heat treatment of a wide variety of foods through the Maillard reaction and it is a concern for the snack food industry, because acrylamide has shown toxic effects on the nervous system and fertility, as well as carcinogenic effect. The major limiting factors responsible for the formation of acrylamide in potato and cereal products are reducing sugars (glucose and fructose) and free asparagine (amino acid) respectively. Acrylamide formation primarily takes place under conditions of high temperature (usually in excess of 120 ^{0}C) and low moisture.

Two sensitive and fast batch and flow-injection spectrophotometric methods for the determination of acrylamide are proposed. The methods were based on oxidation-reduction reaction of acrylamide with potassium permanganate. The calibration graphs are linear over ranges 1.0 - 0.8 and 2.0 - 13 µg/mL of acrylamide, with detection limits of 0.6 and 1.0 µg/ml, respectively. The methods are applied to the routine analysis of acrylamide in potato chip samples. Reduction of some cationic interference was carried out in the batch and flow injection analysis using cationic exchange. Because of gaps in the databases and the fact that results for different years are not always comparable, a reliable Europe-wide temporal trend analysis is not feasible. However, according to European food safety authority - EFSA, a dataset of manufacturers' measurements of AA levels in 40.455 samples of fresh sliced potato crisps from 20 European countries for the years 2002 to 2011 showed a substantial downward trend for mean levels of AA, from 763 ± 91.1 µg/kg in 2002 to 358 ± 2.5 µg/kg in 2011.

This indicates, that caution for reducing the factors that influence acrylamide formation need to be disclosed, at first glance, at the field, and to proceed continuously with storage and production processes.

***Key words**: Acrylamide, Potato chips, Spectrophotometric methods, Toxin.*

1. Introduction

The presence of acrylamide in some types of food cooked at high temperature was reported by the Swedish national food administration and researchers from Stockholm University. They found that acrylamide was formed in some foods cooked at high temperature (120 - 170 ^{0}C) by the reaction of the amino acid asparagine with a reducing sugars such as glucose [1].

Acrylamide - AA is a chemical compound formed during the heat treatment of a wide variety of foods through the Maillard reaction and it is a concern for the snack food industry, because acrylamide has shown toxic effects on the nervous system and fertility, as well as the carcinogenic effect [2].

According to European food safety authority (EFSA) [3], estimation of human exposure to AA revealed that infants, toddlers and other children were the most exposed groups. Depending on the survey and age group, chronic dietary exposure of children was estimated to be, on average, between 0.5 and 1.9 µg/kg body weight per day and the 95th percentile was between 1.4 and 3.4 µg/kg body weight per day. Chronic dietary exposure of adolescents, adults, elderly and very elderly was estimated to be on average between 0.4 and 0.9 µg/kg body weight per day, and the 95th percentile was between 0.6 and 2.0 µg/kg body weight per day depending on the survey and age group.

Since acrylamides revealing in 2002, numerous researchers and manufacturers are working on their mitigation and much has been learnt about the influence of diverse factors such as variety, storage conditions and different steps during the production process. Understanding of these involved mechanisms should help creating the food products that are safe for human public health and in compliance with as low as reasonably achievable (ALARA) concept.

2. Factors that influence acrylamide formation in food during processing

The major limiting factors responsible for the formation of acrylamide in potato and cereal products are reducing sugars (glucose and fructose) and free asparagine (amino acid) respectively [4]. In Food drink europe acrylamide toolbox [5], their composition varies between: crop cultivars, harvest season, climatic conditions, soil composition and agronomic practices. Properties also change with storage and initial processing, e.g. extent of milling. These differences and their impact on acrylamide formation are so far poorly understood and thus can't be consistently controlled. Seasonal and year-to-year variability of raw materials can have a greater impact on acrylamide levels than any of the interventions implemented, and must be taken into consideration.

Acrylamide formation primarily takes place under conditions of high temperature (usually in excess of 120 ^{0}C) and low moisture [6]. To detect its quantity and to understand its formation in food, as well as to assure quality of food products in compliance with the legoslation while working on AA mitigation, several analytical methods and instruments for detection have been employed in recent years. Instruments like: liquid chromatography/mass spectrometry (LC/MS), gas chromatography/mass spectrometry (GC/MS), GC/Nitrogen-phosphorous detector (NPD), or GC/Electron capture detector (ECD), Fourier transform infrared (FT-IR) and spectrophotometer are commonly used. The limit of detection depends on the nature and chemical complexity of samples (e.g. solid food samples such as bread vs. liquid samples such as coffee).

High-performance liquid chromatography (HPLC) based methods were reported for both ion trap systems and triple quadrupole systems after electrospray ionization (ESI), as well as atmospheric pressure chemical ionization (APCI). The HPLC separation is mostly performed on reverse phase columns or by ion exchange chromatography and the identification and quantification preferentially in MS/MS mode. In recent years the use of ultra-performance liquid chromatography (UPLC) became more and more popular. Because of the high sensitivity and selectivity without the need for derivatization, HPLC-MS/MS and UPLC-MS/MS methods have nowadays become the methods of choice for the determination of AA in food products [3]. According to Shibamoto [7], LC/MS has an advantage over GC/MS because LC/MS can analyze aqueous samples, while the other methods require the use of organic solvents (acrylamide is highly water soluble but less soluble in organic solvents).

FT-IR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". FT-IR is also the most useful method for identifying chemicals present in organic samples. When glucose/asparagine model reactions are performed, the facile formation of a decarboxylated asparagine product is detected by IR spectral analysis of a mixture of asparagine and glyceraldehyde in methanol [8].

Spectrophotometry based methods require specific sample preparation as well as wavelength detection adjustment, because acrylamide lacks a strong chromophore for UV detection. Two sensitive and fast batch and flow-injection spectrophotometric methods for the determination of acrylamide are proposed. The methods were based on oxidation-reduction reaction of acrylamide with potassium permanganate. The calibration graphs are linear over ranges 1.0 - 0.8 and 2.0 - 13 μg/mL of acrylamide, with detection limits of 0.6 and 1.0 μg/ml, respectively. Fakhre and Ibrahim [1], have shown that methods are applied to the routine analysis of acrylamide in potato chip samples. Reduction of some cationic interference was carried out in the batch and flow injection analysis using cationic exchange. However, it is important to note, that limit of quantification has mayor role, when the selection of appropriate analytical method for acrylamide detection need to be done.

Because of gaps in the databases and the fact that results for different years are not always comparable, a reliable Europe-wide temporal trend analysis is not feasible. However, a dataset of manufacturers' measurements of AA levels in 40.455 samples of fresh sliced potato crisps from 20 European countries for the years 2002 to 2011 (Figure 1) showed a substantial downward trend for mean levels of AA, from 763 ± 91.1 μg/kg in 2002 to 358 ± 2.5 μg/kg in 2011 [3].

2.1 Agronomy factors

Studies have shown that reducing sugars and asparagine are not the only ingredients that impact acrylamide

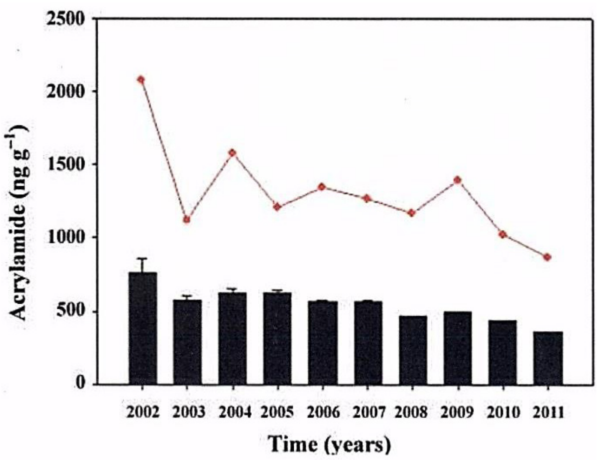

Figure 1. Overall mean acrylamide (AA) levels (ng/g) in 40,455 samples of fresh sliced potato crisps from 20 European countries over years from 2002 to 2011, with standard errors and with trend in 95 % (Q95)

formation. It is known that sucrose, after hydrolysis to individual monosaccharides can also form acrylamide and it is not reducing sugar. According to Rosen *et al.*, [9], the concentrations of sucrose and glucose in potato tubers are influenced both by genetic factors and environmental factors, including: growth conditions in the field, tuber storage conditions, and processing methods which result (as it is shown in Table 1) with different acrylamide levels in finished products.

Content of sugars and asparagine is in correlation with AA formation and used as an indicator for forming potential for many crops like: potato, cereals, coffee beans and almonds. From the other side, effects of nutrient availability during cultivation can influence free asparagine accumulation in many plant species by the multiplicity of stresses, including exposure to toxic metals such as cadmium, pathogen attack, and drought or salt stress. Free asparagine can accumulate when there is a plentiful supply of reduced nitrogen and low rates of protein synthesis [10].

Little is known about the influence of fertilization in potatoes over acrylamide formation after frying; some found a correlation between the nitrogen content of the soil and the amino acid content; however, some experiments have shown that nitrogen fertilizers have no effect on the levels of asparagine. The level of nitrogen fertilization in potatoes appears to have an influence on the formation of acrylamide after frying, when no fertilizers are added to soil [11]. Free asparagine concentration is the main determinant of acrylamide forming potential in wheat grain and accumulates to very high levels if wheat is grown under conditions of sulfur deficiency. This makes sulfur availability the most important factor affecting the acrylamide forming potential of wheat grain. The data showed a clear and significant effect of sulfur application in reducing the acrylamide forming potential of wheat in five of the six trials [12].

Climatic condition such as the harvest year has a significant impact on asparagine and reducing sugars in potatoes. The asparagine content was significantly lower in all the samples from the 2004 harvest as compared to 2003. This study concluded that an extremely hot summer will result in a lower acrylamide generation. Cold temperatures and senescent sweetening are the main causes of sugar accumulation in potatoes during storage. Higher temperature storage (more than 8 ^{0}C) which results senescent sweetening is also related to sprout formation in potatoes. Storing potatoes at low temperature (below 8° C) is found to be an effective technique/tool to inhibit sprouting; temperature below 4 - 6 ^{0}C has a major effect on reducing sugar accumulation [4].

2.2 Recipe factors

According to European Commission [14], the resulting acrylamide concentration depends on the concentrations of the precursors, as well as the processing conditions. To improve the taste of finished products, manufacturers use a big palette of additives which may impact important product attributes. Adding of: other amino acids, calcium salts, co-ingredients, pH, dilution and piece size and fermentation (e.g. usage of lower gassing yeast), are seen as powerful methods to reduce AA in products.

Amino acids, calcium salt addition and certain minor or co-ingredients have the potential to contain comparatively high levels of AA which could impact upon levels in the final product. There may be an impact with co-ingredients included to a composite product (e.g. pre-processed cereal pieces, vegetables, nuts, seeds) where they could be "cooked" several times over. This needs to be deliberately taken into account in product design, processing practices, and issues with processing equipment and performance. Replacing ammonium bicarbonate with other raising agents e.g. sodium hydrogen carbonate can significantly lower the levels of acrylamide formed in many cereal products [14]. Treatment of potato flakes with calcium salts during their production have demonstrated 30 - 40% reduction dependent on the product design and formulation. Too high levels can, however, generate undesirable product attributes [5].

Table 1. Acrylamide concentrations of French fries and chips from participating growers' potato plants

Growers	Variety	Preparation	Acrylamide Concentration* (ppb, fresh-weight basis)			
			0 Months	3 Months	6 Months	9 Months
K & O	Alpine Russet	Fry	409 +/- 205	619 +/- 34	633 +/- 395	1291 +/- 643
K & O	Russet Burbank	Fry	582 +/- 281	802 +/- 362	519 +/- 219	1306 +/- 249
Park Rapids Bliss	Russet Burbank	Fry	678 +/- 220	717 +/- 427	781 +/- 91	788 +/- 142
Park Rapids HCBE	Russet Burbank	Fry	493 +/- 67	838 +/- 321	681 +/- 171	834 +/- 116
Perham-Karsina	Dakota Trailblazer	Fry	554 +/- 49	371 +/- 243	584 +/- 121	908 +/- 363
Perham-RDO	Ivory Crisp	Chip	3510 +/- 859	2020 +/- 658	1056 +/- 119	1468 +/- 320
Goenner	Snowden	Chip	2197 +/- 608	1311 +/- 156	1908 +/- 994	5930 +/- 563

*Mean +/- S.D.

Lowering the pH using organic acids in the food system to reduce acrylamide generation may attribute to protonating the α-amino group of asparagine, which subsequently cannot engage in nucleophilic addition reactions with carbonyl sources. Lowering the pH of the cut potatoes (e.g., with citric acid 0.5 - 1.0% < 20 minutes) has been shown (Figure 2) to lower the levels of acrylamide formed. However, this approach can cause souring of flavor if a precise procedure is not followed and also the frying oil can become rancid. The correlation between pH decrease and acrylamide reduction varies among products due to multiple factors of different starting pH values of the products [10].

Importance of dilution and piece size should not be neglected as well. Slice/piece thickness can reduce AA through the surface area/volume effect when taking into account finished product moisture and fry temperature profile. With products that are fried to low moistures, reducing the surface to volume ratio (by producing a thicker cut crisp) can result in increased AA as it will require a higher thermal input for the same fry time or longer fry time at the same thermal input to reach the same moisture end. A thin cut potato crisp product would require less thermal input for the same fry time to reach the same moisture endpoint, so in practice would form less AA. Partial replacement with ingredients lower in key reactants can be effective too. For some pre-formed/reconstituted products/fabricated potato based products, partial replacement of potato components by ingredients lower in key reactants, reduces AA formation potential, e.g. use of cereals with lower asparagine amounts than a potato (e.g. wheat, rice, maize) in the recipe [5].

Fermentation of dough or potatoes favors kinetic control of the rate of acrylamide formation, controlling also, precursors consumption, as well as pH. Fermentation time has a strong impact on acrylamide levels and fermenting yeasts consumed high amounts of free asparagine, leading to a decay of 60% and 90% in the precursors of acrylamide in cereal products. Therefore, prolongation of the fermentation time to at least one hour was found to be appropriate for acrylamide reduction in bread making. Lactic acid fermentation of potatoes before deep frying lowered the acrylamide content from 48% to 78% in the end product. Combination of lactic fermentation with blanching would lead to an even higher reduction of acrylamide levels (79 - 94% less acrylamide) or the addition of 0.05 M glycine to the incubation medium (80% less acrylamide) [10].

2.3 Processing factors

<u>2.3.1 Asparaginase</u>

Asparaginase, an enzyme that converts the precursor (asparagine) into ammonia and aspartic acid, can reduce acrylamide formation in foods [4]. The use of enzyme asparaginase is a possible approach to interrupt the interaction of asparagine with reducing sugars, but further investigation is required. Novozymes A/S have introduced an enzyme solution of asparaginase, which can reduce acrylamide levels by up to 90% by converting asparagine into another common amino acid, aspartic acid, without altering the appearance or taste of the final product. The *Aspergillus oryzae* asparaginase has been cloned and expressed in commercial relevant yields in *A. oryzae*. The *A. oryzae* asparaginase has a pH optimum at pH 6 - 7 with good activity between pH 5 and 8, which may be the pH range for the production of potato products like French fries and crisps [11]. Though it is a promising strategy for acrylamide reduction, it is rather expansion compared with other strategies [4].

<u>2.3.2 Thermal input and moisture</u>

Gökmen *et al.*, [13], have shown that temperature and time have been significant factors affecting the amount of acrylamide formed in potatoes during frying. Since a temperature distribution in the product occurs due

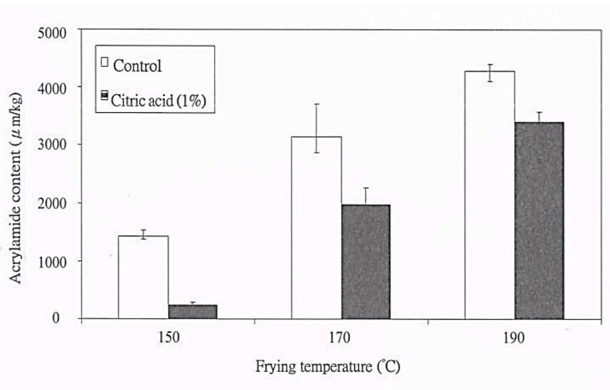

Figure 2. Acrylamide content of control and potato strips dipped in a citric acid solution of 10 g/L for 60 min. after being fried at 150 °C, 170 °C, and 190 °C. Control corresponds to potato strips dipped in distilled water for 60 min.

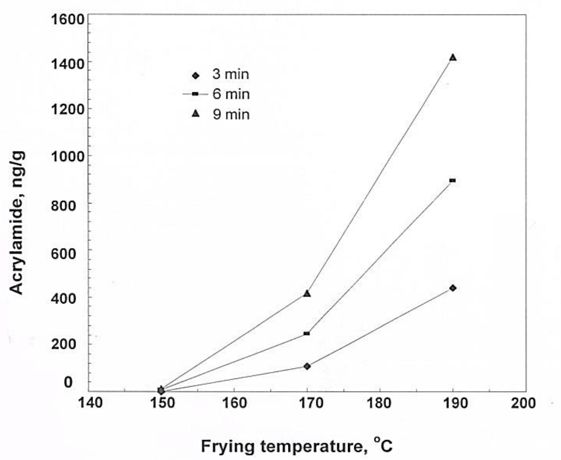

Figure 3. Effect of temperature on the amount of acrylamide formed in French fries in a fixed frying time

to conductive resistance to heat transfer and evaporation of water from the product, the rate of acrylamide formation will not be the same for layers of surface to core of the product. Acrylamide content after frying at 190 ^{0}C for 3 min. was about 40 times larger than that after 9 min. of frying at 150 ^{0}C when the acrylamide concentrations of whole potato strips are taken into account. In the Figure 3 is shown that the amount of acrylamide formed in French fries was about 800 ng/g after frying for 10 min. at 170 ^{0}C and increased to about 3700 ng/g after frying for 10 min. at 190 ^{0}C.

Cieasrova *et al*., [15], have shown that water content and the physical state of the food matrix can affect the mechanistic pathway to the acrylamide formation. Water impacts the chemical route (e.g. hydrolysis of the imine) as well as the molecular mobility of the chemical constituents which indirectly contributes to the formation of acrylamide (Figure 4). In low-moisture systems, however, the molecular mobility is the major driver of the acrylamide formation. Water management may be a key factor in controlling acrylamide levels in foods and warrants studies in both industrial processing and domestic cooking.

Reducing the baking temperatures can also help to achieve acrylamide reduction. For example, lighter baking of sweet biscuits has resulted in significantly lowering levels of acrylamide in some products. However, for some bakery products excess baking can have the same effect and also lead to lower levels of acrylamide. This is believed to result from a balance between formation and destruction of acrylamide at high temperatures [14].

2.3.3 Pre-treatment

Reduction of the sugar content by blanching could reduce the acrylamide concentration by about 60% depending on the raw material (potato variety and field site) and the production process variables (e.g., blanching conditions and frying temperatures). The longer blanching time the lower acrylamide formation after frying, and the lower glucose and asparagine content in potato strips before frying. Long time blanching treatments, such as that of 50 ^{0}C for 80 min. and 70 ^{0}C for 45 min., resulted in the lowest levels of acrylamide formation [11]. It is important to note that blanching process, as an option, can influence variety of products in a different ways. As it is shown in Food drink europe acrylamide toolbox [5], blanching of potato stick type products sliced from whole potatoes to remove sugars has been proven to reduce AA levels without the negative affect of flavor, texture and oil content, but blanching of sliced potato crisps is not desired as it results in loss of flavor, loss of texture and increased oil content due to the disruption of the potato cells on the surface of the slices, and is thus not a preferred mitigation tool.

Prolonged soaking and higher temperature conditions lead to greater extraction of acrylamide precursors and decreased production of acrylamide. However, excessive soaking can affect organoleptic qualities (e.g., taste, mouth feel).

2.3.4 Finished product color

In Food drink europe acrylamide toolbox [5], a study conducted by Ghent University on the main parameters (reducing sugar content and color evaluation) linked to acrylamide (AA) in the final product, revealed that the best correlation was achieved by color determination (Agtron process analyzer).

Dark colored chips come from individual potatoes that are very high in reducing sugars and can increase the AA level of a given sample by 25 - 50% and depending on the amount of dark colored crisps/products in the sample (EFSA [3]). The AA levels depending on color and cooking time of some pre-cooked French fries products for home cooking to illustrate the appearance of "French fries and potato fried" containing 656 μg/kg are shown in Figure 5. Elimination of dark

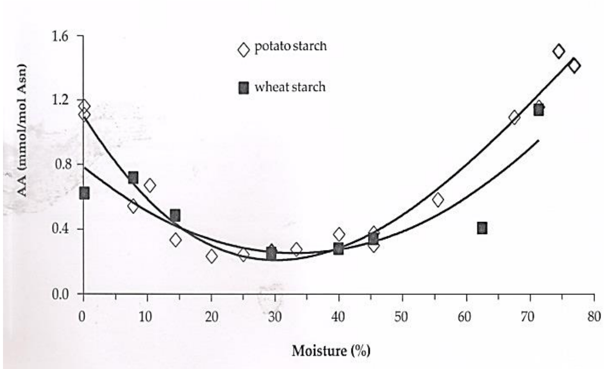

Figure 4. Effect of potato and wheat starch on the acrylamide yields in the model system consisting of starch and equimolar mixture of glucose and asparagine with the addition of water after 20 min. heat treatment at 180 ^{0}C

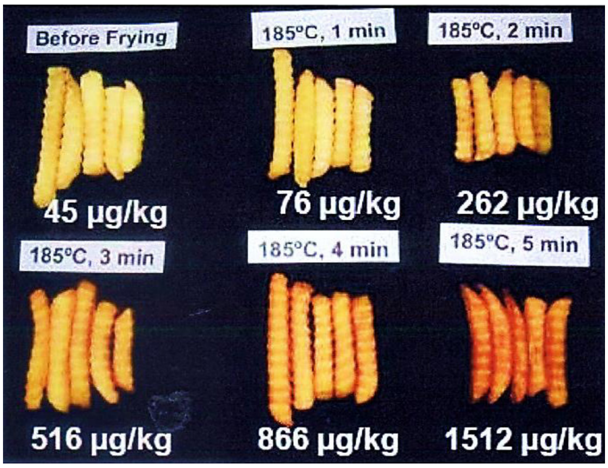

Figure 5. Level of AA according to color and cooking time of any pre-cooked French fries products for home cooking

colored crisps/products by in-line optical sorting has proven to be an effective measure to reduce AA. Continuous measurement of finished product color can be a reliable predictor of finished product AA levels [5].

3. Conclusions

- After numerous research studies, it is obvious that, AA concentration in prepared food cannot be reduced to "0", but only decreased to a certain level.

- Significant progress was achieved with a selection of varieties with low levels of reducing sugars and asparagine, as well as, with usage of precise fertilizers.

- Experimentation with storage conditions is only one piece of the big picture for AA reduction. Although many researchers have placed their studies directly on the field, there are others that believe in AA mitigation working with time-temperature and moisture effects during production. This indicates, that caution for reducing the factors that influence acrylamide formation need to be disclosed at first glance, at the field, and to proceed continuously with storage and production processes.

- Considering toxicity and carcinogenic effects on human health, scientific investigations on AA mitigation continue to feature, opening possibilities for practical, technological and analytical solutions, for both, industry and households.

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