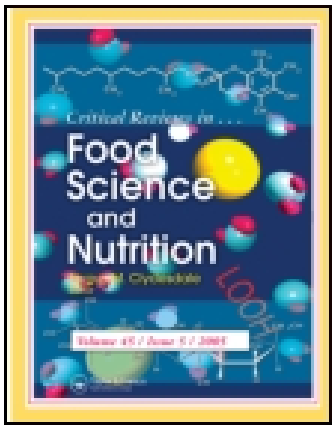

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Acrylamide: Formation, Occurrence in Food Products, Detection Methods, and Legislation

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Acrylamide: Formation, Occurrence in Food Products, Detection Methods, and Legislation

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This review aims at summarizing the most recent updates in the field of acrylamide (AA) formation (mechanism, conditions) and the determination of AA in a number of foods (fried or baked potatoes, chips, coffee, bread, etc). The methods applied for AA detection [Capillary Electrophoresis-Mass Spectrometry (CE-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Non-Aqueous Capillary Electrophoresis (NACE), High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS), Pressurized Fluid Extraction (PFE), Matrix Solid-Phase Dispersion (MSPD), Gas Chromatography-Mass Spectrometry (GC-MS), Solid-Phase MicroExtraction-Gas Chromatography (SPME-GC), Enzyme Linked Immunosorbent Assay (ELISA), and MicroEmulsion ElectroKinetic Chromatography (MEEKC) are presented and commented. Several informative figures and tables are included to show the effect of conditions (temperature, time) on the AA formation. A section is also included related to AA legislation in EU and US.

Keywords Acrylamide formation, acryamide in foods and water, acrylamide detection methods, legislation

1. INTRODUCTION

Acrylamide (AA) is an organic chemical recently determined to be present naturally in various food products. Among its many industrial applications, it has been used in the production of polyacrylamide gels, as a grouting agent in construction, as a papermaking additive, as a soil-conditioning agent, in ore, and sewage treatment, on several occasions as coagulant aid both for drinking water and waste water treatment (FSA, 2002; USFDA, 2004). AA has also been determined to be a cigarette smoke component.

AA (Fig. 1) is an odorless, white, crystalline organic solid. It readily forms cross-linked polymer, with polymerization, which is a known as polyacrylamide (Pac) gel. AA is biodegradable and does not constitute a burden to the environment. The highest environmental contamination caused by AA releases was due to the plastic industries (http://www.cfs.gov.hk/english/programme/programme_rafs/programme_rafs_fc_02_07.html).

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AA is a known cancerigenic in animals. Although scientists agree that certain doses of AA are toxic to the nervous system of both animals and humans there has not been a concensus regarding the "threshold" dose yet (Tareke et al., 2000). In April 2002, the Swedish National Food Authority reported the presence of high levels of AA in certain food which were processed at temperatures higher than 120°C. Since then, AA has been determined in numerous cooked and heat-processed foods in other countries, including The Netherlands, Norway, Switzerland, the UK, and the US (http://www.who.int/foodsafety/publications/chem/acrylamide_faqs/en/index.html).

AA naturally formed when several carbohydrate-rich foods are fried, baked, or roasted at high temperatures. AA forms when foods are cooked either domestically or in restaurants and when they are prepared commercially. The primary mechanism for AA formation in foods is the reaction of naturally occurring compounds in plants such as reducing sugars (RSs) (glucose, saccharose) with free asparagines (Asn) (and/ or other aminoacids), an amino acid occurring in a wide range of foods, during the browning reaction (http://www.acrylamidefacts.org/sitecore/content/Home/FAQs.aspx).

AA is not found in raw or boiled foods rich in carbohydrates, nor does occur in meat, fish, chicken, or infant formula. Furthermore, as more testing is conducted, the AA levels appear to

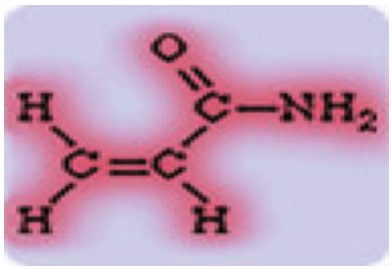

Figure 1 The chemical compound of acrylamide. (Color figure available online).

be highly variable across brands of the same food type and even within the same brand of food. For example, in a popular brand of potato chips, AA levels in 25 bags varied from 249 to 549 parts per billion (USFDA, 2004).

Another study was focused on people who consumed fried foods such as potato crisps in large quantities, which contain AA, in an attempt to determine whether these people were at higher risk of developing cancer compared to individuals who abstained from consumption of such foods. However, Mucci et al. (2003) believed that the levels of AA in food are too low to trigger any of the toxic effects of AA.

AA is quoted as toxic and the US EPA's "safe" level for AA-the concentration at which exposure presents a cancer risk to one in one million people is set at a low value of 0.00077 which is much lower than the allowed safe levels for other well-known toxins (http://www.grinningplanet.com/2006/02-21/acrylamide-in-food-article.htm): Benzene (0.12), Carbon tetrachloride (0.067), Chloroform (0.043), and Lead (0.013).

However, while no health authority has recommended consumers to modify their consumption behavior because of AA, there are several prudent measures to minimize the AA formation in many products. Food manufacturers and restaurants continue to explore and apply these potential solutions. Similarly, easy-to-follow advice is available for those consumers who wish to minimize the formation of AA domestically (http://www.acrylamidefacts.org/sitecore/content/Home/About-Acrylamide.aspx).

Foods should not be cooked excessively (for too long or at too high temperature), but they should be cooked thoroughly enough to kill the foodborne pathogens. Consumers adopt a balanced and varied diet that consists of plenty of fruits and vegetables, and must reduce drastically their consumption of fried foods (http://www.foodstandards.gov.au/newsroom/factsheets/factsheets2009/acrylamideinfoodfebr4211.cfm).

The most hazardous food groups are listed below roughly in descending order of AA content (http://www.grinningplanet.com/2006/02-21/acrylamide-in-food-article.htm): Potato Chips (Crisps), French Fries, Crackers, Toast, Bread Crisps, Cookies, Boxed Breakfast Cereal, Corn Chips (Crisps), Bakery Products, Coffee, Cocoa, and Bread.

Shortly after the discovery of AA an FAO/WHO Consultation was organized (FAO/WHO, 2002) covering this subject and the Scientific Committee on Foods expressed their view on the presence of AA in food (SCF, 2002). It is presently crystal clear that Asn, together with RSs, particularly fructose, are the precursors for the formation of AA in Maillard reactions (Mottram et al., 2002; Stadler et al., 2002).

These studies have resulted in recommendations of processing to reduce the formation of AA during processing, which were summarised in a report based on a workshop organised by the European Commission Health and Consumer Protection Directorate-General (EU DG SANCO) in 2003 (EU, 2003).

Another alternative is the selection of raw materials with low levels of free Asn and/or RSs such as fructose and glucose. In potato the concentration of RSs strongly determines AA formation on processing (Amrein et al., 2003). Selecting cultivars with low levels of RSs, the AA content could substantially be reduced in foods derived from potato. However, reduction of RS content in potatoes should not be at the cost of the quality (flavor and crispiness) of the processed product (Braethen and Knutsen, 2005).

Another potentially promising approach is the partial degradation of Asn by means of enzyme Asn, applicable to potato crisps (AA reduction of 97%) and French fries (AA reduction of 80%), retaining acceptable flavor and color of the product (Zyzak et al., 2003). To assess how promising new developments to reduce AA levels in foods will eventually affect the overall AA exposure, probabilistic modeling of exposure in combination with what-if scenario studies is a useful tool. Although up to now restricted to the fictional level the following what if scenarios stand for probable alternatives for AA reduction food in processing industries and/ or restaurants:

(1) lowering potato frying temperatures
(2) selection of potato varieties with less RSs
(3) application of new or altered processing practices by the food industry/restaurants
(4) follow up the advice of health education boards or food standard agencies (Jung et al., 2003; Rydberg et al., 2003; Shih et al., 2004)

1.1. Historical Overview

The AA scare began in 1997, when a herd of cows on the remote Bjäre peninsula in southern Sweden started showing alarming signs line staggering, collapsing and dying. Investigations revealed the cattle had been drinking from a stream containing AA, which had been leaching from drilling works on a nearby mountain tunnel where PAc was being used as crack sealant (http://www.newscientist.com/article/mg19025483.600-acrylamide-the-food-scare-the-world-forgot.html?full=true).

In April 2002, the Swedish National Food Administration (SNFA) and researchers from Stockholm University publicized their findings that AA, a toxic and potentially cancerigenic chemical, is formed in many types of food prepared/cooked at high temperatures. The SNFA informed regional and international authorities and organizations about their findings

in order to initiate international collaboration as a priority concern (FAO/WHO, 2002b).

1.2. AA Formation

AA formation in a food model during frying can be analyzed with regard to texture and time by means of a classical heat and mass transfer approach. A multi-objective SIMPLEX optimization has been finally employed for the overall model tuning, incorporating all phenomenological variations. The obtained numerical results confirmed that the AA formation is nonlinearly dependent on the applied thermal regime. In fact, an increase in processing temperature by 10% led to approximately double the average levels (Carrieri et al., 2009). A mechanism of AA formation in the presence of asparagine and carbonyl is shown in Figure 2.

The lowest temperature AA can be formed is 120°C. At this temperature, drying of the food matrix often occurs (Biedermann et al., 2002; Mottram et al., 2002; Tareke et al., 2002;). However, it is noteworthy that AA formation was also claimed at lower temperatures in specific products and models (Biedermann et al., 2003, Amrein et al., 2004; Granvogl et al., 2004).

For more that 10 years, there has been an enhanced interest in determination of AA in Turkish foodstuffs. It was known that both protein-rich and carbohydrates-rich foods thermally treated at high-temperatures can trigger AA formation (Yaylayan et al., 2003). Carbohydrates-rich foods such as potato crisps/chips and biscuits have been among the most often investigated foods compared with protein-rich foods such as meat dairy and fish/seafood. The obtained results revealed that AA contents varied considerably depending on the a type of food itself, that is, the range content of AA in fried chicken was from 164 up to 2.25 mg/kg, whereas in fried minced beef amounted to only 0.017 mg/kg (Kaplan et al., 2009).

The Maillard reaction has been recognized for over 60 years as a major route to flavor and browning in cooked foods (Kawamura, 1983). This extremely complex reaction between amino compounds (principally amino acids) and RSs has been the subject of much research by food scientists seeking to identify compounds that provide the flavor and color characteristics

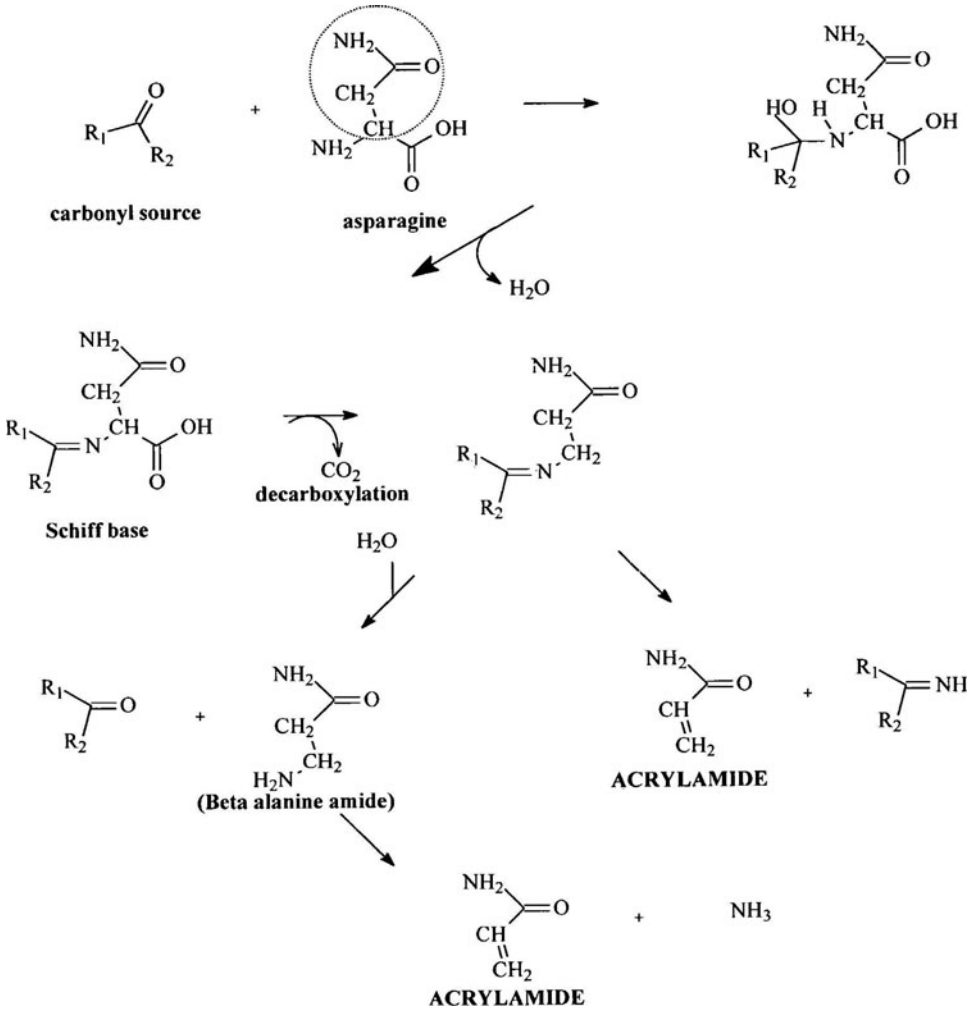

Figure 2 AA formation from asparagine and carbonyl compound (www.freepatensonline.com/7189422.html).

of heated foods (Hodge, 1967; Nursten, 1980, 2005; Mauron, 1981; Hurrell, 1982; Mottram, 1994).

The potential of 15 vitamins in minimizing the formation of AA were investigated. Biotin, pyridoxine, pyridoxamine, and L-ascorbic acid displayed a potent inhibitory effect (>50%) on AA formation in the under study model system. Application of the food model system clearly showed that water-soluble vitamins are very strong/effective inhibitors of AA formation. However, fact-soluble vitamins displayed rather minor inhibitory effects. To be more specific, both nicotinic acid and pyridoxal inhibited AA formation in fried potato strips by 51% and 34%, respectively (Zeng et al., 2009).

Romani et al. (2009) investigated the effect of processing frying conditions, simulating as much as possible domestic and catering practices, in order to optimize the process to obtain a good final product limiting the AA formation. Experiments were carried out by using commercially frozen pre-fried potato strips, a fixed initial frying temperature of 180°C, two different fryers for domestic and catering use, and two potato-to-oil ratios (1/4 and 1/8 w/v).

It has been suggested that the vacuum treatment could strongly enhance the AA formation by inducing the decarboxylation of the Schiff base, one of the main intermediates of AA formation. Although a more in depth research is required to determine per food category the oprimal process conditions for (a) removing the AA content and (b) lessening its formation this technology could potentially be a promising and alternative strategy to mitigation interventions aimed at reducing AA levels in foods (Anese et al., 2010).

The currently applied kinetic modeling approaches (both single- and multiresponse) to describe heat-induced AA formation and elimination during processing of foods have been thoroughly described by De Vleeschouwer et al. (2009).

Three empirical models were applied to describe the formation of AA in crisps of three different coldsweetened potato genotypes, fried under the same conditions. The predictive capacity of the "Logistic-Exponential" model was tested, as this model displayed a strong correlation between parameter and the RS content of the raw potato. Although the application of the "Logistic-Exponential" model as a tool to predict AA in potato crisps appears to have some potential, a more thorough investigation is required (Knol et al., 2009).

Powdered samples of freeze-dried potato samples from the varieties Saturna and Peik subjected to different agronomic conditions and storage and of the same growth year were mixed with oil and heated an oven to investigate the potential for AA formation. For baked samples originating from unstored potato a linear relationship was established between Asn and RS content and AA formation (Knutsen et al., 2009).

Barutcu et al. (2009) investigated the effects of microwave frying on AA formation in the coating part of chicken. They also aimed at determining the effects of various flour types (soy, chickpea and rice flour) in batter formulations on the AA formation and the color of fried chicken. Utilization of all flour types except for soy flour led to approximately the same moisture content and color development after 1.5 min of microwave frying. Microwave frying resulted in lesser AA content and lighter color than those fried conventionally for 5 min for all types of flours. The highest reduction in AA level (34.5%) was reported for rice flour containing batter.

The formation of AA in potato crisps was fitted with empirical mathematical models by Knol et al. (2008). The temperature developments in the surrounding oil and outer cell layer of the potato slices were monitored, providing more insight in the frying process and facilitating the comparisons among studies. Apart from making easier the comparisons of different AA formation patterns in the future, there is also the possibility to predict the AA formation in potato crisps.

1.3. Foods Where AA is Formed

The health impact of dietary Maillard products (MPs) can be realistically investigated by means of clinical studies only with proper design of nutritional diets corresponding to high and low levels of MPs. Some suggestions in this direction include the following: replacement of the cooking fat with similar raw fat as seasoning in the low-MP diet, the high caloric density due to from water loss in the high-MP diet can be balanced with higher food quantities offered in the low-MP diet, and the vitamin loss in fruit and vegetables because of high temperatures in the high-MP diet can be overcome by augmenting the corresponding portion size (Pouillart et al., 2008).

Although AA has been established to be a toxic compound, its effect and potential implications to public health based on the amounts occurring in food have not been elucidated yet. For the time being, one can benefit from progress already made and knowledge acquired on AA formation in food and on ways promising approaches toward lowering the amounts present (Slayne and Lineback, 2005).

The processing method is the main parameter affecting the formation of AA, because application of high temperatures enhances substantially AA levels formed. Therefore, lowering the processing temperature below 120°C will definitely the quality of the affect negatively product because of higher fat uptake and potential adverse effect on texture (Gertz and Klostermann, 2003). The couple of lower temperature with extended heating time should be preferably linked with other treatments such as vacuum frying. The latter is a promising technique quality criteria must be monitored to identify the optimal frying parameters. Blanching cut potatoes is another effective approach toward reducing the amount of AA precursors (Granda et al., 2004).

1.3.1. Potatoes

The production of French fries of a very low AA content (pending on RSs drop) was suggested by Grob et al. (2003). Following careful selection of potato tubers (cultivar, storage conditions), thin strips from the tubers' outer part were cut. The latter were removed because they tend to reach the optimum

frying temperature more rapidly than the rest of the potato thereby turning brown and have high AA concentration. This is accentuated because the sugar content in the peripheral layers of the potato is particularly high (Grob et al., 2003). The cut potatoes were immersed in water for several minutes to reduce the amount of precursors in the surface layer. Finally, the fryer was loaded with no more than 100 g potato per litre of oil. To improve the golden-yellow color of the finished product, French fries are sometimes dipped in glucose or sugar solution before par frying. At glucose contents above 0.5%, AA concentrations increased more substantially (Taeymans et al., 2004). Therefore, the use of sugar dips should be reconsidered.

The content of AA increased with increase in both temperature and frying time. Although the amount of AA remained comparatively low at temperatures up to 175°C, there was a pronounced increase at temperature higher than 180°C (Matthäus et al., 2004). The increase of AA formation at constant temperature is linear function, with greater slope at higher temperatures. Varying temperature at constant frying time resulted in an exponential shift of the AA concentration in the product, with disproportionate AA concentrations at higher temperatures (Matthäus et al., 2004; Taeymans et al., 2004).

Figure 3 shows the content of AA in duration of temperature.

It is noteworthy that frying at low temperatures are closely related to versus frying dutarion at various temperatures are negative quality effects, such as higher fat uptake and poor texture (Taeymans et al., 2004). It is also important to monitor the final moisture content of the French fries, so that they are not too dry (lower than 38%) or too soft and wet (higher than 45%).

The capacity of 15 vitamins to reduce the formation of AA was investigated by Zeng et al. (2009). It was found that in the food model only water-soluble vitamins are good inhibitors of AA formation whereas fat-soluble vitamins displayed had a very weak in inhibitory action. When pyridoxal, nicotinic acid, and L-ascorbic acid were applied on potato strips, it was reported that the presence of nicotinic acid and pyridoxal inhibited AA formation in fried potato strips by 51% and 34%, respectively.

Commercial biscuits and potato chips were subjected to vacuum treatments in conjunction with different combinations of pressure, temperature, and time. It was found that AA removal was feasible only in samples previously hydrated at $a_w > 0.83$. In fact, the maximal AA removal was reached between 5 and 15 min of vacuum treatment at 6.67 Pa and 60°C. Under these processing conditions, the AA percentage removal from biscuits and potato chips amounted to 43% and 18% AA, respectively (Anese et al., 2010).

An optimized method was used for the determination of AA levels in Turkish grilled meat and chicken samples, potato chips, coffee, and biscuit. The determined concentrations for all studied foods were in the range of 0.02–0.25 μg/kg (Kaplan et al., 2009).

Three empirical models were applied in an attempt to identify the best fit the formation of AA in crisps of three different coldsweetened potato genotypes. The "Logistic-Exponential" model revealed a strong correlation between the parameter a* and the RS content of the raw potato (Knol et al., 2009).

Baked powder from freeze-dried potato varies samples originating from unstored potato revealed a linear relationship between Asn and RS content and AA formation. However, the RSs is not always the limiting substrate for AA formation because of the north European extreme conditions (Knutsen et al., 2009).

Gökmen and his coworkers (2009) investigated the effects of single- and multiple-stage extraction on the extraction yield of AA for various cereal- and potato-based thermally processed foods. It was found that a single-stage extraction greatly underestimated the concentration of AA in foods by a factor of up to 50% pending on the extraction solvent used. The extractability is an exponential function which can be effectively used to optimize the multiple extraction conditions in the analysis of foods for AA.

Although the quality of potato tubers has always been an important topic in terms of food and industrial processing, the

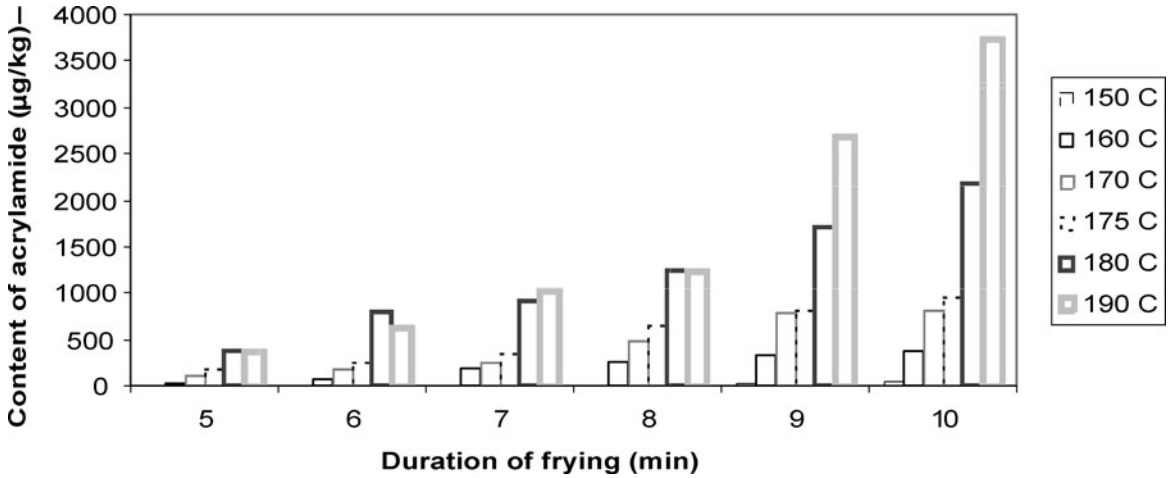

Figure 3 The content of acrylamide versus frying time of par-fried French fries at various temperatures (Matthäus et al., 2004).

effects of future atmospheric carbon dioxide (CO_2) enrichment on related attributes have not been clarified yet. Significant negative relationships between CO_2 treatments and concentrations of leucine, phenylalanine and methionine, and as a trend for di-tyrosine, histidine, and aspartic acid, were also found. This finding may eventually deteriorate the nutrition quality of potatoes because of the reduction in physiologically valuable amino acids. As regards to the organic acids, the CO_2-related alterations were restricted to lower concentrations of citric acid. He latter may degrade the processing quality of potato tuber, because there is high discoloration risk and the parameters related to taste must be improved (Högy and Fangmeier, 2009).

AA contents and color shifts in dry potato granules during direct heating in hot oil were investigated and the results modeled. Oil temperatures in the range 117°C and 173°C were applied and samples were analyzed after heating for 10, 20, 30, 40, and 60s. For all temperatures, after only 20s the AA contents raised significantly. All determined AA values spreading about three magnitudes were well fitted by this model. Furthermore, a color shift (browning) of the powder is well described with a first order model (Franke et al., 2009).

According to Anese et al. (2009), potato cubes before deep frying, were subjected to lactic acid fermentation in the presence or in the absence of glycine, as well as to immersion in aqueous solution of glycine alone. Determination of AA contents revealed that deep-fried potatoes exposed to the above-mentioned pretreatments had 35% and 50% less AA content than the water-dipped ones. Lactic acid fermentation in glycine presence was shown to be the most effective in decreasing AA formation up to 70%. It is noteworthy that this pretreatment had no effect on the sensory properties such as browning, flavor, sourness, and crispness of the deepfried potatoes.

The effect of blanching time and temperature on the extraction of RSs from potato strips and slices (French fries and crisps) was investigated by means of a central composite design. However, the extraction efficiency of RSs was more than 10% lower when the potato cuts were blanched in water (already used for blanching) thereby resulting in higher than 10% drop in the determined AA content (Mestdagh et al., 2008).

Concerns about the potential health issues associated with the dietary intake of this reactive compound led researches to suggest the reduction of the accumulation of Asn, in the tubers of potato (*Solanum tuberosum*). Both French fries and potato chips accumulated as little as 5% of the AA present in wild-type controls. In view of the crucial role of processed potato products in the modern Western diet, a replacement of current varieties with intragenic potatoes could substantially reduce the daily intake of AA by approximately 33% (Rommens et al., 2008).

AA formation in French fries was investigated in relation to blanching and asparaginase soaking treatments before final frying. Control or blanched strips were then dried at 85°C for 10 min and immediately partially fried at 175°C for 1 min. Finally, frozen par-fried potatoes were fried at 175°C for 3 min to prepare French fries. Soaking of blanched potato strips (75°C, 10 min) in an 10,000 ASNU/l asparaginase solution at 40°C for 20 min is an effective way to reduce AA formation after frying by reducing the amount of one of its important precursors such as Asn (Pedreschi et al., 2008).

Cummins and his coworkers (2008) carried out a farm-tofork human exposure assessment model for AA in fried potato crisps for Irish consumers. The average Irish consumer exposure to AA in potato crisps was estimated to be 0.052 and 0.064 μg/kg bw/day for males and females, respectively. Moreover, the initial level of RSs was shown to be the most critical parameter (correlation coefficient 0.58 and 0.57 for glucose and fructose, respectively), thereby pinpointing the importance of selecting cultivars with low RS contents for crisp production.

The effect of frying time on quality and AA content of French fried potatoes, obtained simulating home-cooking practices, was investigated in order to identify the optimal conditions to minimize the concentration of produced toxicant but maintaining at the same time satisfactory sensory quality. After around 4 min of frying, when the temperature of potato surface and the oil bath reached 120 and 140°C, respectively the further frying time became the dominant factor in terms of the quantity of AA and its formation rate. The best sensorial product with regard to color, oil content and AA concentration was obtained after 5 min of frying (Romani et al., 2008).

Pan-frying of boiled potato cubes resulted in higher levels of AA (0.53–1.100 mg/kg) than in the wedges (0.14–0.25 mg/kg). Blanching combined with a shorter roasting time was shown to be an effective way of lowering the AA content in roasted potato wedges, especially in the experiments conducted after long-term storage, where the AA content was reduced from 0.11–0.26 down to 0.05–0.14 mg/kg (Skog et al., 2008).

It has been reported that lowering pH in a potato model system by means of sodium acid pyrophosphate, citric, acetic, and L-lactic acid substantially decreased the final AA content. Addition of aminoacids such as free glycine, L-lysine, and L-cysteine also reduced AA, without having any effect on the mitial pH. A usage of acetic acid in conjuction with aminoacids induced an antagonistic AA lowering effect. The presence of calcium and magnesium ions induced a supplementary AA reduction in addition to a lower pH of the food matrix (Mestdagh et al., 2008b).

After having monitored for 11 months both RSs and Asn content in three varieties of Irish ware potatoes, samples of them were used for French fries preparation similarly to the domestic procedure. A negative relationship was established between Hunter L^* values and AA content of the French fries thereby suggesting that L^* values could effectively serve as a convenient and reliable indicator of AA levels in French fries (Brunton et al., 2007).

The highest AA contents were recorded in potato tubers grown with high N and insufficient K supply, which also had the highest contents of precursors. The results obtained clearly revealed that nutrient supply had considerable effect on the contents of AA precursors and thereby for the AA formation over frying (Gerendás et al., 2007).

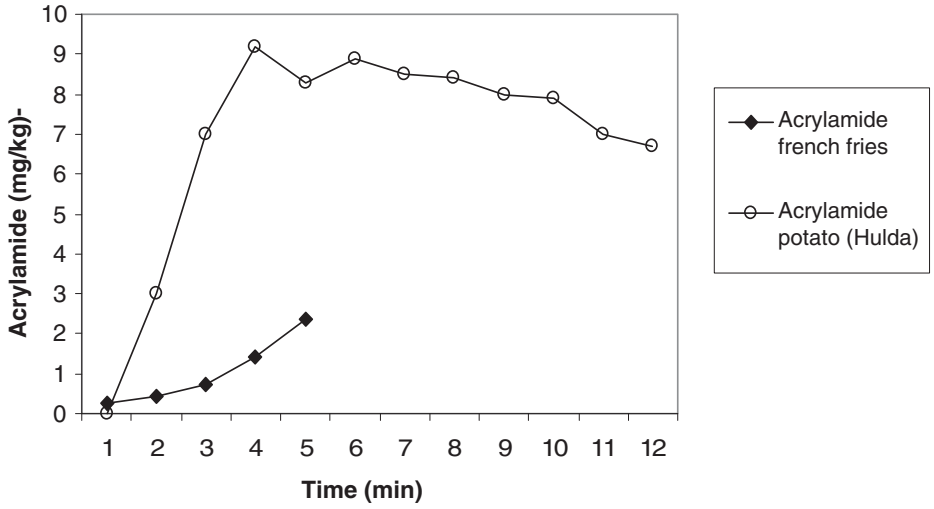

Figure 4 Effect of frying time on acrylamide formation in French fries (Foot et al., 2007) and potato *(Hulda)* (Knol et al., 2009).

Alternatively to water extraction for AA analysis in potato and crisp bread products is the accelerated solvent extraction (Cavalli et al., 2003) and methanol, propanol, and ethanol/dichloromethane mixtures (Owen et al., 2005).

Figure 4 displays the effect of time on AA formation in French fries and potato *(Hulda)*.

AA formation conditions and its determination in potatoes is summarized synoptically in Table 1.

1.3.2. Cereals

Breakfast cereals are significant contributors to the daily intake of food-derived AA in Western countries. AA content ranged from a 62–803 lg/kg (average 292 lg/kg, and median 258 lg/kg, with an estimated AA intake from breakfast cereals of 2.68 lg AA/person/day. Wheat-based cereals contained significantly higher levels of AA, as did samples with higher fibre or protein content. In addition, puffed breakfast cereals also contained significantly higher levels of AA. There was no significant correlation between AA levels and contents of 5-hydroxymethylfurfural, furosine, or cereal browning (Rufián-Henares et al., 2006).

Dry starch systems, containing varying amounts of Asn and glucose, freeze-dried rye-based flat bread doughs, flat bread, and bread, were baked at varying temperatures and times according to central composite designs. In the starch system, freeze-dried flat bread doughs and flat breads, the amount of AA formation went through a maximum at approximately 200°C, depending on the system and the baking time. The amount of AA was reduced at long baking times (Bråthen et al., 2005).

In a recent review, an AA Claus et al. (2008) summarized the results of approximately 5 years studies of academic and industrial research on AA content in cereal products. This review gives recommendations on how to minimize the AA formation in foods (suitable raw materials, optimization).

Data from an industrial case study of breakfast cereal production indicated that the generated amounts of AA are greatly dependent upon the combined effects of temperature and heating time in a roasting step process. The correlations obtained for predicting AA generation in the case study present a useful tool for food processing industry to minimize AA generation. It was possible by lowering process temperature and prolonging residence time to achieve an approximately 80% reduction in AA content while maintaining the desired product quality (Jensen et al., 2008).

The aim of the work was to compare the impact of different salts such as monovalent and divalent chlorides, hydrogencarbonates, phosphates, and lactate on AA formation in cereal model system during baking at 190°C for 9 min. Sodium acid pyrophosphate, sodium as well as potassium dihydrogen phosphate were also very effective and brought about 75% AA content decrease, followed by calcium lactate, sodium chloride, and potassium chloride causing 40–45% of AA elimination and finally sodium and potassium hydrogen carbonates that achieved 30% reduction of AA (Kukurová et al., 2009).

Cereal products, and in particular gingerbreads are greatly affected by AA formation up to 1.000 mg/kg and more when asparaginase was applied prior to baking the drop of AA content reached more than 97% whereas the sensory quality of end products remained unaltered. Addition of sodium raising agents led to further decrease in AA content, without affecting the physical characteristics of gingerbread (Ciesarovát et al., 2009).

Plant breeders and farmers are advised to take advantages of the varietal differences in AA content risk and to adopt and supervise the application of Good Agronomic Practice (GAP) toward AA reduction (Halford et al., 2007).

Although several bakery products consumed in the UK (crumpets, batch bread, and Naan) may be anticipated to contain high levels of AA because they of their having strong Maillard colors and flavors. In fact, the higher AA contents were recorded for thereby produced biscuits (Sadd and Hamlet, 2005).

Table 1 Acrylamide formation conditions and its determination in potatoes

Potatoes	Frying/baking conditions/MW	Pretreatment	AA analysis determination method	AA content	References
Potatoes	Frying at 170°C for 10 min with an electric fryer	Prior to frying drained for 2 min	HPLC, LC-MS/MS	–	Zeng et al., 2009
Potatoes	60°C, for 30 min, under magnetic stirring	Vacuum treatment from 2.67 to 6.67 Pa at 60°C for 1 h	HPLC-MS	200 lg/kg	Anese et al., 2010
Potatoes	0.3 ml/min for flow rate of mobile phase, 70V for fragmentor potential	–	HPLC-MS	1.376 mg/kg	Kaplan et al., 2009
Cold-sweetened potato genotypes	Frying at 160°C for 2, 2.5, 3, and 4 min	Washed and cut into 1.5 mm slices	LC–MS/MS	50 lg/kg dry matter (dm).	Knol et al., 2009
Freeze-dried potato	Baking at 150°C for 12 min in a baking oven	–	LC-HRMS	–	Knutsen et al., 2009
Potato powders	Frying above 120°C for 10, 20, 30, 40, and 60 s	–	HPLC	Acrylamide content increased after heat treatment in hot oil	Franke et al., 2009
Deep-fried potatoes	Frying at 180°C for 90 s with an electric fryer	Dipping in a vacuum oven (1.32 kPa) at 75°C	HPLC-MS/MS	990 lg/kg	Anese et al., 2009
French fries and potato crisps	Frying at 180°C for 5 min in a 5L semiprofessional deep-fryer (French fries) Potato slices were fried at 170°C for 1.5 min in above-mentioned deep-fryer (potato crisps)	–	LC-MS/MS	After frying AA was 254 mg/kg in French fries (Blanched in tap water) 232 mg/kg in Potato crisps (Blanched in tap water)	Mestdagh et al., 2008
French fries and potato chips	Frying for 4 min	–	HPLC	4.318 ± 1.138 mg/kg (French fries) Chips from lines 1256-27 and 1256-83 accumulated much lower levels of acrylamide (0.861 and 1.153 mg/kg, respectively)	Rommens et al., 2008
French fries	Frying at 170°C for 1 min	Drying of raw potato strips	LC	French fry acrylamide content of 2.075 mg/kg	Pedreschi et al., 2008
Potato crisps	Frying at 165°C for 2 to 3 min	Blanching and soaking of potatoes	Monte Carlo simulation techniques	0.720 mg/kg	Cummins et al., 2008
French fries	Frying at 180°C in different times (3, 4, 5, 6, 7, 8, 9 min)	–	GC-MS	0.830 mg/kg	Romani et al. 2008
French fries	Frying at 190°C for 6 min (using a Tefal Pro-fry deep fat fryer)	–	LC-MS/MS	–	Brunton et al., 2007
French fries	Frying at 175°C for 7 min using silicon-free, semiliquid, pure vegetable oil	Prefrying at 125°C for 2 min	–	–	Gerendás et al., 2007

Among the various upproaches toward AA content reduction in breakfast cereals, the most promising and effective way is to minimize the free Asn contents (CIAA, 2004).

However, Asn also needs a RS to react with in order to form AA, therefore, the sugar content and type and starch degradation are also of crucial importance. The maximum contend of reducing sugents, the cereal flours can have, amounts to 16.000 mg (Elmore et al., 2005). According to Haase et al. (2003), the cereal cultivar in conjuction with the applied processing (extraction) are the two most important parameters greatly affecting the amount of free Asn. In this instance, it is noteworthy to report that addition of glucose or fructose hardly affected the AA formation in bread (Surdyk et al., 2004; Mustafa et al., 2005).

The content of AA versus temperature frying is displayed in Fig. 5.

Table 2 provides an overview of the contents of free Asn in different cereal grains and fractions.

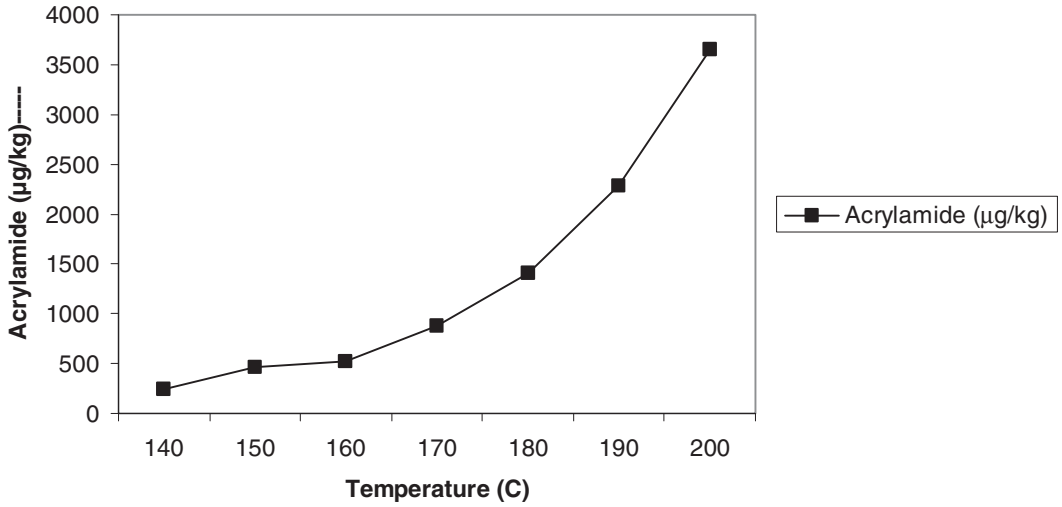

Figure 5 The content of acrylamide in potato crisps after temperature (Haase et al., 2003).

1.3.3. Flours

Capuano and his co-workers (2009) investigated the effect of flour type (wheat, rye, and whole-wheat flours) and processing conditions in a bread crisp model system consisting of flour, water, and yeast. The bread was toasted at different temperatures for different times. According asparaginase dropped AA formation up to 88% it hardly had any effect on browning and antioxidant activity. When green tea antioxidant compounds were added no effect was recorded on AA formation probably due to the low fat content.

Asn concentration in conjunction with the potential of AA formation of winter wheat was examined in a 2-year field experiment. Although the grain yields varied considerably between 61 und 104 dt/ha DM depending on cultivar (cv), fertilization, and year, the quality requirements regarding crude protein concentration and sedimentation value were met. Application of CAN, CAN+S, urea, or a combination of liquid manure and CAN showed that the Asn concentrations in flours varied from 2.6 to 13.6 mg per 100 g flour DM depending on cultivar, fertilization, and year (Weber et al., 2008).

The influence of cooking at 180°C on AA levels in wheat cake and rye is exhibited in Fig. 6.

Table 2 Overview of the amount of free asparagine in different cereal grains and fractions

Commodity	Fraction	Free Asparagine (g/kg)*	References
Wheat	Germ	55.5–57.4	Fredriksson et al., 2004
	Bran	1.12	Nestle Research Centre**
	Bran	1.48	Fredriksson et al., 2004
	Whole wheat flour	0.17	Elmore et al., 2005
	Flour	0.18–0.19	Nestle Research Centre
	Flour	0.14–0.17	Fredriksson et al., 2004
	Flour	0.15–0.4	Noti et al., 2003
Oats	Bran	0.71	Nestle Research Centre
	Flour	0.5	Nestle Research Centre
Rye	Whole grain	1.07	Fredriksson et al., 2004
	Flour	0.53–0.68	Fredriksson et al., 2004
	Flour	0.63	Elmore et al., 2005
	Flour	0.26	Nestle Research Centre
Freeze-dried rye	Wheat starch	100–3000 μg/g	Bråthen et al., 2005
	Flour	2000 mg/g	Bråthen et al., 2005
Maize	Flour	0.596–1.07	Wang et al., 2001
	Semolina	0.22	Nestle Research Centre
	Corn starch	<0.01	Biedermann and Grob, 2003
Rice	Germ	0.23	Friedman, 2003
	Bran	0.28	Friedman, 2003
	Flour	0.07	Nestle Research Centre
Flour	Wheat	0.15–0.02	Capuano et al., 2009
	Whole-wheat	0.45–0.02	
	Rye	0.55–0.03	

**Personal communication, Drs Campos, E. and Benet, T.

1.3.4. Bread

Mustafa and his co-workers (2009) studied the interaction effects of fermentation time in the presence of Asn and glycine on AA precursors (Asn and RSs) in dough and content of AA in yeast-leavened wheat bread. Two experiments, with low and high levels of added Asn (0–0.044 and 0.071–0.476 g/100 g flour, respectively), were carried out. Glycine was found to enhance considerably, the color intensity and decreased the AA in bread. The latter was dependent on the Asn content.

The urgent need for a certified matrix reference material (CRM) of an AA in toasted bread was repeatedly emphasized by the competent authorities as a tool to (a) improve comparability, (b) ensure accuracy, and (c) traceability of analytical results. The bread slices were initially crushed by means of a PTFE pestle, then pulverized in an impact mill at a speed of 10,000 rpm, to avoid over-heating of the mill and the material. The powdered bread was sieved first through a 500-μm and than through a 63-μm sieve (Dabrio et al., 2008).

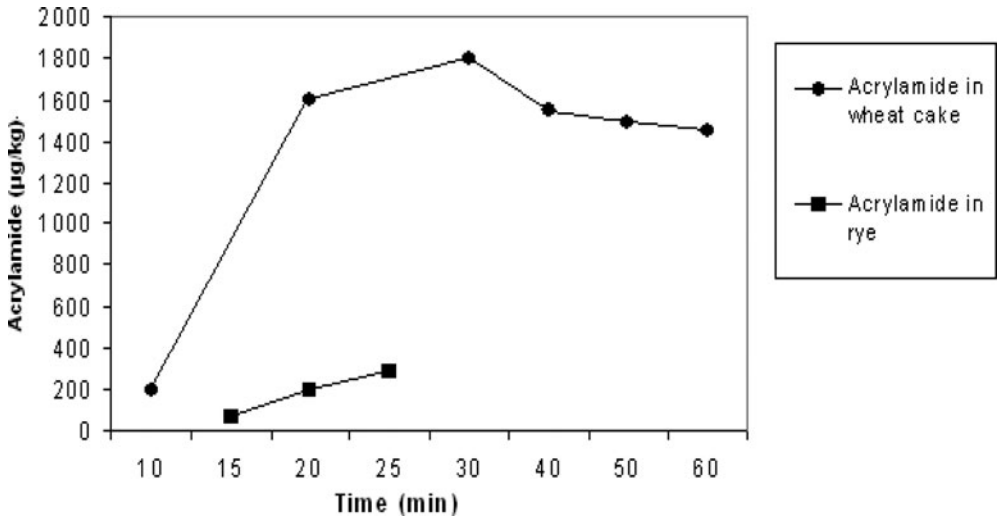

Figure 6 Effect of cooking time at 180°C on acrylamide levels in wheat cake (Claus et al., 2008) and rye (Capuano et al., 2009).

Hedegaard et al. (2008) added 1% aqueous rosemary extract with approximately 40 mg of gallic acid equivalents or (or equivalent concentration of rosemary oil or of dried rosemary leaves) to wheat dough. They found that the content of AA in wheat buns decreased by 62, 67, and 57%, respectively, compared to control samples (wheat buns without rosemary). Greater addition of aqueous rosemary extract to 10% had no further effect on the AA content compared to a 1% extract.

Along the same line were the results reported by Eriksson and Karlsson (2006) who demonstrated that enzymatic treatment had no effect on AA content extracted at alkaline conditions. It was found that the pH of extractions of AA from whole grain bread was the dominant parameter reaching up to 5 times higher content (1.000 mg/kg for pH >12 compared to 0.20 mg/kg for pH = 2–80.

AA is found in all baked goods and the most important products are bread, crispbread, gingerbread, crackers, cookies, and biscuits. Nevertheless, bread was calculated to contribute about 10% to the total dietary exposure of AA because of its frequent and large consumption (Swiss Federal Office of Public Health, 2004; Boon et al., 2005). In total, about 30% of the dietary exposure originates from bakery products (Boon et al., 2005).

Springer et al. (2003) reported that the AA content of crispbread greatly depended on the location of the product on the oven belt. The quantity of free Asp in the dough prior to baking affects the AA formation and it can be effectively correlated with the AA content of the baked good. Such correlations were reported for gingerbreads where free Asn was added on purpose to the flour, (Amrein et al., 2004) in bread, (Springer et al., 2003; Surdyk et al., 2004; Mustafa et al., 2005) and in several food model systems (Biedermann et al., 2003; Springer et al., 2003).

The presence of baking agents $NaHCO_3$ and Na_2CO_3 do not only affect favorably the AA formation as in NH_4HCO_3 case in various products (Biedermann-Brem et al., 2003; Amrein et al., 2004; Vass et al., 2004; Weisshaar, 2004; Graf et al., 2005; Grothe et al., 2005; Levine and Smith, 2005) but rather promote the elimination of AA (Levine and Smith, 2005). Therefore, baking temperature and time obviously greatly affect the AA formation.

It has been repeatedly shown that addition of glycine decreased considerably the AA contents and made the browning more pronounced (Amrein et al., 2004; Bråthen et al., 2005; Kim et al., 2005). A typical example is the effect of glycine on the AA of breadcrust although corresponding results.

Similar effects were reported for gingerbread as well. The addition of 10 g glycine per kg dough reduced the AA by more than 60% and the browning increased while the pH was not altered (Amrein et al., 2004). The browning is anticipated to be positively correlated with the AA concentration. Addition of 1 g of cysteine to 150 g flour decreased the AA content of a cracker model approximately by 50% whereas the recovery of deuterated AA was markedly suppressed (Levine and Smith, 2005).

The content of AA versus frying/baking temperature in gingerbreads and bread crisps is shown in Fig. 7.

1.3.5. Almonds

Amrein and his coworkers (2005a) investigated the effect of composition and roasting conditions on AA formation in almonds and hazelnuts Samples of almonds from US and Europe were analyzed for sugars and free amino acids. Furthermore, the AA formed over roasting was determined as well. It was shown that the low content of free Aspn in the raw material resulted in very low AA content. On the other hand, the RSs content was not a crucial parameter for AA formation.

The influence of roasting conditions on the AA content and on the color of roasted almonds of three cultivars was studied by Lukac et al. (2007). The four parameters measured at various roasting temperatures and times ware: interior temperature of almond kernel, the water content, the color, and the AA content. AA started to get formed only when the kernel temperature

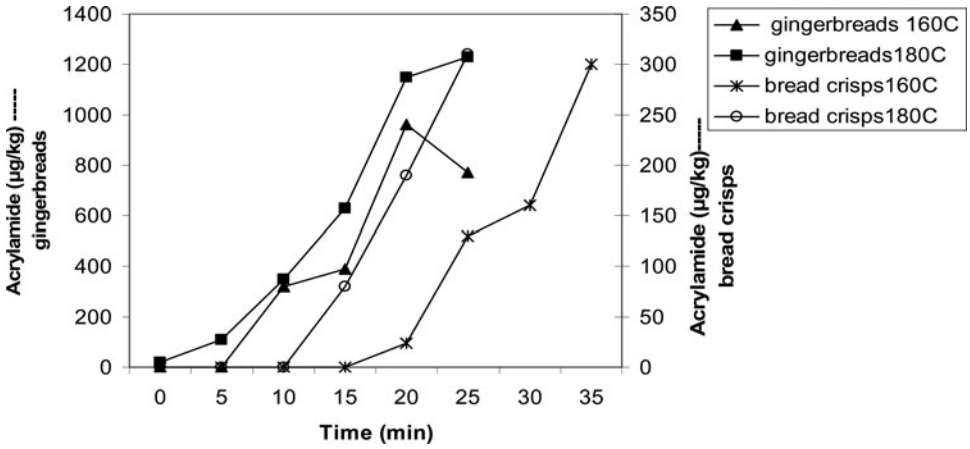

Figure 7 The content of acrylamide after temperature in gingerbreads (Amrein et al., 2004) and bread crisps (Capuano et al., 2009).

went above 130°C. The activation energy for the AA formation during the roasting of almonds was 123 kJ/mol. A good correlation was established between the degree of brightness and the AA content as AA content increased with increasing darkness. At constant roasting conditions, it was found that almonds with greater initial moisture content produced less AA after roasting, which could be attributed to the development of lower temperature over roasting because of the moisture presence.

AA was determined in a wide range of almond products (86), starting with raw and roasted almonds, almond-containing bakery products, and marzipan. The greatest AA contents were reported in dark (brown) roasted almonds, whereas only moderate AA contents were measured in bakery products. It was found that the effect of roasting temperature on AA formation was much stronger than the effect of roasting time (Amrein et al., 2005).

Significant correlations were established between the roasting temperature and the AA concentration of almonds after their roasting for 10 min (Amrein et al., 2005). Insofar, the almonds were roasted at 120 and 140°C for 10 min they were only slightly roasted and did not acquire the anticipated roasting character. However, when the roasting temperature reached 190 and 200°C the almonds were over roasted and their tasted turned into bitter and burnt. The optimal quality reached in the range 140 to 180°C. The obtained data from extreme process conditions with products containing a few mg AA per kg may result in confusing conclusions, i.e., AA content in bread may be proportional to $-t^2$ or $-T^2$ (Bråthen et al., 2005).

It is interesting to note that olives and dried fruits may contain AA because high of AA can be formed upon heating these products, a phenomenon requiring further study (Amrein et al., 2007).

The dependence of AA levels versus frying temperature in French fries and almonds is displayed in Fig. 8.

1.3.6. Meat

The content AA formation was related to the degree of unsaturation of the oils and animal fats. The decreasing order of AA formation from dietary oils or animal fats with Asn was sardine oil (642 μg/g Asn) > cod liver oil (435.4 μg/g) > soybean oil (135.8 μg/g) > corn oil (80.7 μg/g) > olive oil (73.6 μg/g)

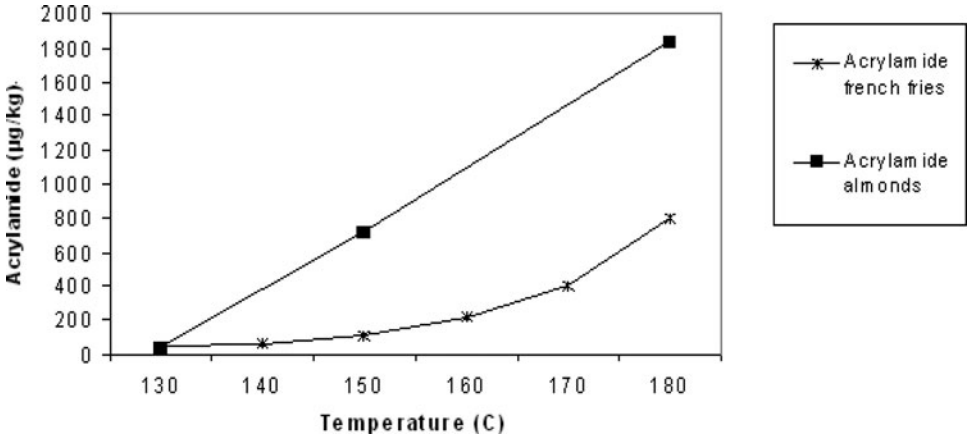

Figure 8 Dependence of acrylamide levels in French fries (Romani et al., 2008) and almonds (Amrein et al., 2005).

> canola oil (70.7 μg/g) > corn oil (62.1 μg/g) > beef fat (59.3 μg/g) > lard (36.0 μg/g). The obtained results confirmed that AA is formed in Asn-rich foods over deep fat frying even in the absence RSs (Ehling et al., 2005).

AA was determined with GC-MS in the diet as well as in body tissue and eggs. After three weeks, the AA concentration in the eggs remained constant (max. 0.0172 mg/kg egg). A considerable difference was reported for the carry-over rates for AA from the diet to the egg. The rates were 0.022 and 0.010 for the experimental and central hens, respectively (Halle et al., 2006). The highest content of AA determined in the kidneys of the experimental hens (0.0277 mg/kg) shows that the contribution of poultry products to the total AA intake by humans is still quite low.

Zhang et al. (2007a) reported on the efficiency of antioxidant from bamboo leaves on the reduction of AA over thermal processing and optimization of levels of addition of antioxidant from bamboo leaves applied to fried chicken wings. The results revealed that approximately 57.8 and 59.0% of AA in fried chicken wings decreased when the antioxidant from bamboo leaves addition ratios amounted to 0.1 and 0.5% (w/w), respectively. The sensory analysis results clearly indicated that the odor and flavor of fried chicken wings with antioxidant from bamboo leaves treatments did not have any significant difference compared with normal food matrixes when the antioxidant from bamboo leaves addition ratio was <0.5% (w/w). The color acceptability within the frame of sensory evaluation found to be in good correspondence with color formation of fried chicken wings.

Paleologos and Kontominas (2007) determined over the generation of AA frying and cold storage of 28 commercial breaded chicken products. Initial AA concentrations were in the range 0.00091–0.00097 mg/kg and attributed to the synergistic effect of batter and meat. In all cases, AA concentrations enhanced over storage, reaching a maximum (0.00136 to 0.00180 mg/kg) between day 15 and day 19.

The analysis and formation of AA in French fries and chicken legs during frying were studied by Chuang et al. (2006). According to the three authors "French fries and the outer flour portion of chicken legs fried at 180°C generated a higher level of AA than at 160°C. Compared to soybean oil and palm oil, a lower amount of AA was produced in French fries and the outer flour portion of chicken legs fried in lard. However, it should be noted that no AA was detected in the inner meat portion of fried chicken legs".

AA formation conditions and its determination in foods is summarized in Table 3.

1.3.7. Coffee

AA is a carcinogenic and mutagenic compound found in many industrially processed foods and beverages, including coffee. Guenther and his coworkers (2007) claimed that the "loss" of AA during storage of roast and ground coffee is warranted, and studies in this direction have already been initiated. Finally, risk/benefit analysis must be addressed in a complex food such as coffee, known to harbor numerous health beneficial/chemoprotective compounds with antioxidant and antimutagenic properties".

The content of AA in coffee was shown to attain a peak early in the roasting process, thereby confirming the occurrence of both formation and decomposition of AA over roasting. Glucose and moisture in green coffee hardly exhibit any significant correlation with AA in roasted coffee. The four main factors which substantially affect the sensorial characteristics of the brew and as modifications of the process have to comply with the consumer-accepted boundaries of taste profiles. In this case, only small effects on the AA level are expected to be achievable (Lantz et al., 2006).

Espresso coffees were analyzed for AA contents by matrix solid-phase dispersion (MSPD) and GC–MS. Espressos from commercial blends were determined to have an average AA level of 1.26 ± 0.28 lg/L. Which is much greater than the rest coffee beverages. However, since the espresso volume per cup is quite small, its contribution to AA ingestion is minimal (Alves et al., 2009).

The analysis of carbohydrates and amino acids in green coffee is of the utmost importance because both of these two classes are potential precursors of the Maillard reaction which is responsible for color and aroma formation. The two aminoacids with highest concentrations are alanine (Al) and Asn. The content of Al and Asn are 1200 and 680 μg/g for robusta and 800 and 360 μg/g for arabica, respectively (Murkonic and Derler, 2006).

Baum and his coworkers (2008) carried out experiments on determining the AA contents in roasted coffee versus storage time and temperature. An attempt was made to monitor the lost AA* by means of ^{14}C labeled. No formation of volatile 14C-AA-related compounds was detected during storage and coffee brewing. It is noteworthy that approximately 90% of the radiolabel in the filter residue (i.e., spent R&G coffee) was firmly bound to the matrix, despite the repetitive extractions with various extraction solvents such as aqueous ammonia, ethyl acetate, chloroform, and hexane, occasionally in conjuction with enzymatic digestion (Baum et al., 2008).

Mass spectrometry and an enzymatic test kit were used for determination of AA content in four Italian coffees. Comparison of average values using the two methods permitted allowed the validation of results obtained with the kit and the kit itself thereby confirming that AA is present in low amount (Gianni et al., 2007).

Coffee samples from the Robusta and Arabica varieties were roasted at 236°C over different time periods to obtain very light, light, medium, and dark roast. It was found that an increase in the roasting degree resulted in a drop in AA concentration in conjuction with radical scavenging capacity (Summa et al., 2007).

The effects of heating on color generation (measured as CIE color space parameters of L*a*b*) and AA formation were

*over storage of roasted and ground coffee (R&G).

Table 3 Acrylamide formation conditions and its determination in foods except for potatoes

Foods	Frying/baking conditions/MW	Pretreatment	AA analysis determination method	AA content	References
Breakfast cereals	–	–	LC-MS	292 μg/kg	Rufián-Henares et al., 2006
Breakfast cereals	Fluidizing the pieces in hot air (air lift) for a few seconds (19 ms, 385°C at the inlet and 330°C at the outlet)	–	LC-MS/MS	600-800 μg/kg	Jensen et al., 2008
Cereals	Baking at 190°C for 9 min	–	–	Inorganic salts declined the AA content	Kukurová et al., 2009
Flour type (wheat, rye, and whole-wheat flours)	Toasting at 180°C for 22 min	–	LC-MS/MS	A final content of 262.3, 291 and 301 μg/kg after 25 min of toasting in wheat, whole-wheat and rye containing samples, respectively	Capuano et al., 2009
Winter wheat	Drying of about 5 g flour at 105°C for 24 h	–	GC-MS	–	Weber et al., 2008
Bread	Baking at 180–280°C for 15–45 min in a fan oven	One half-baked immediately and the other freeze-dried prior to baking using the same temperature and time	HPLC	–	Bråthen et al., 2005
Almonds and hazelnuts	Roasting at 165°C	–	LC–MS/MS, high resolution GC–MS	Roasted hazelnuts contained very little AA	Amrein et al., 2005a
Roasted almonds of 3 cultivars	Roasting	–	–	Almonds with higher initial moisture content contained less AA after roasting AA content increased with increasing darkness	Lukac et al., 2007
Roasted almonds, almond-containing bakery products (such as biscuits, cookies, cakes, bars, breads, pastry), raw almonds, and marzipan	Roasting at 150°C for 15–22 min (roasted almonds)	–	GC-MS	Highest AA concentrations were found in roasted almonds, with most of the values being around 400 μg/kg	Amrein et al., 2005b
	Roasting at 160°C for 10 min (raw almonds)			For the bakery products the values being 200 μg/kg	
Roasted almonds	Roasting at 165°C	–	GC-MS	An elevated roasting temperature is not a suitable measure to decrease AA levels in roasted almonds	Amrein et al., 2007
Hens	Heating at 150°C for 2 h	–	GC-MS	The highest content of AA was found in the kidneys of the experimental hens (27.7 μg/kg)	Halle et al., 2006
Fried chicken wings	Frying in palm oil, as 50–60 g batches, for exactly at 170°C for 4 min in a 10-l electric frying pan (Rui'an, Zhejiang, China)	–	LC-MS/MS	214.3 μg/kg	Zhang et al., 2007
Breaded chicken products	Baking in an oven at 250°C for 1 h or fried in olive oil ($T = 180$°C) for 25 min	All samples were sliced in round pieces and either baked	NP-HPLC with UV detection	AA concentrations increased during storage, attaining a maximum (1.36 to 1.80 μg/kg) between day 15 and day 19	Paleologos and Kontominas, 2007
Chicken legs	Frying at 180°C for 1 min	–	GC-MS	No AA detection in the inner meat portion of fried chicken legs	Chuang et al., 2006

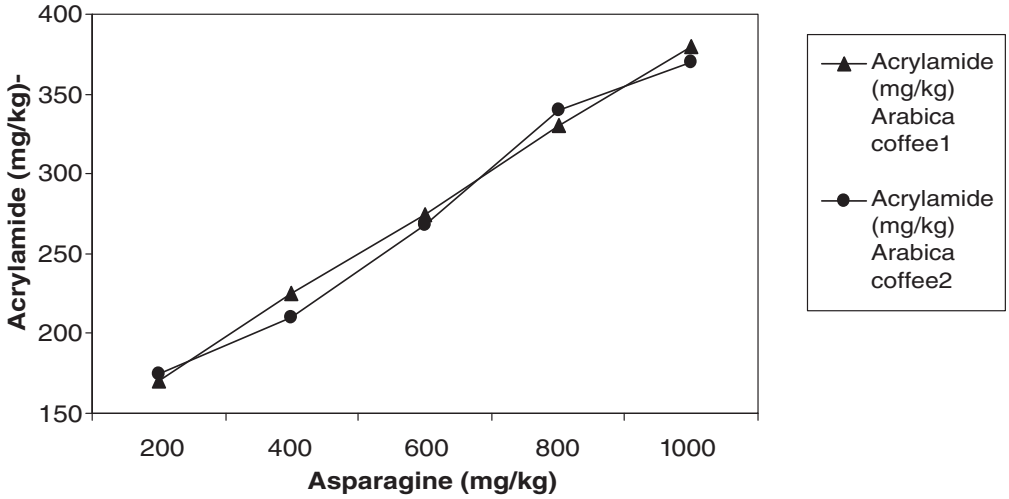

Figure 9 Acrylamide levels versus asparagine levels in Arabica coffee [(Guenther et al., 2007) and (Lantz et al., 2006)].

investigated in various food matrices including green coffee, wheat flour, and potato chips at various temperatures. The correlation of AA with redness parameter a^* over heating revealed that color could be a reliable indicator of AA levels in thermally processed foods (Gökmen et al., 2006).

Pardo and his co-workers (2007) developed and validated a selective and sensitive procedure for the determination of AA in complex matrices, like coffee and chocolate. The limit of quantitation (LOQ) of the method (LC) was 0.001 mg/kg for coffee and 0.0006 mg/kg for chocolate. Application of optimized method, to 20 coffee and 15 chocolate samples from Valencian (Spain) supermarkets, for AA, resulted in median levels of 0.146 and 0.102 mg/kg for coffee and chocolate, respectively.

An improved GC-MS method to determine AA in a wide range of coffee and coffee products was developed by Soares et al. (2006). The levels of AA in Z6 coffee samples were in the range 11.4–36.2 μg/l for "espresso coffee" and 200.8–229.4 μg/l for coffee blends with cereals. The obtained results suggest that the presence of cereals significantly increased considerably the AA contents.

The formation of AA content versus Asn levels in Arabica coffee is shown in Fig. 9.

AA contents and extractability in espresso coffees are exhibited in Fig. 10.

1.3.8. Tea

Minimization of AA formation during the Maillard reaction has been attempted in several studies. In this study, the effects of 35 crude aqueous extracts from dietary plants (spices, fruits, tea, beans, and herbs) and 11 phenolic compounds on the mitigation of AA in an Asn/glucose model system were investigated. Zhu et al. (2009) developed a simple method using reversed-phase high-performance liquid chromatography (RP/HPLC) with 100% water as mobile phase was developed for AA quantification.

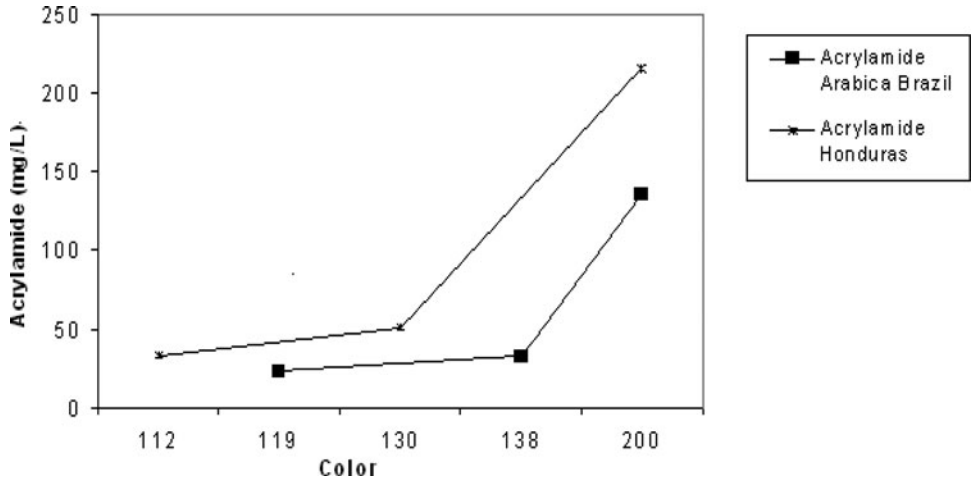

Figure 10 Acrylamide contents and extractability in espresso coffees (adapted from Alves et al., 2009).

Mizukami et al. (2008) optimized the roasted green tea (Houjicha) processing by applying roasting treatments to reach AA reduction without affecting the quality. The two most important odorants were separated and identified with aroma extract dilution analysis were 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine. The AA contents in tea infusions amounted to 2.0 and 4.0 μg/l by roasting at 160°C for 30 min and at 180°C for 15 min, respectively. It was found out that degradation of tea catechins was minimized insofar the roasting temperature did not exceed 160°C.

Although it is debatable whether AA formation occurs higher than 120 or 150°C, it is unanimously accepted that thermal treatment of foods and drinks at 180°C or higher results in formation the highest AA levels. To be more specific, when tea samples were roasted at 180°C for 10 min the greatest AA amounts were formed. Application of higher temperatures and prolonged processing times initiated a drop in the AA content. Furthermore, an analysis of 82 tea samples revealed that the Asn content in tea leaves was a more important factor thatn the RS contents as regards the AA formation in roasted products (Mizukami et al., 2006).

1.3.9. Water

Bowyer et al. (2009) undertook study to demonstrate whether changes in the gene expression or histological signs of neurotoxicity in some parts of the forebrain might AA exposure because of drinking water. The obtained results could not be correlated, even at maximally tolerable levels, with changes in gene expression or neurotoxicity in the central nervous system.

AA formation conditions and its determination in beverages is summarized in Table 4.

Table 4 Acrylamide formation conditions and its determination in beverages

Beverages	Roasting/baking conditions/Mw	Pretreatment	AA analysis determination method	AA content	References
Roasted Robusta coffee	Roasting conditions at temperatures ranging from 150 to 240°C for 5–15 min	–	–	0.258 mg/kg	Guenther et al., 2007
Roasted coffee	Roasting	–	LC-MS/MS	0.312 mg/kg	Lantz et al., 2006
Espresso coffee	The oven temperature was initially programmed at 85°C for 1 min, increasing at 15°C/min to 280°C (10 min hold), and the transfer line set at 280°C	–	GC-MS	1.240 and 2.190 mg/kg, for arabica and robusta coffee, respectively	Alves et al., 2009
Green coffee	Roasting range from 240°C for 6 min to 270°C for 3 min	–	HPLC	–	Murkonic and Derler, 2006
Italian coffee	Stirring for about 20 min at 50°C	–	HPLC-MS	0.10 mg/kg	Gianni et al., 2007
Roasted and ground coffee	Roasting at 236°C over different time periods to obtain very light, light, medium, and dark roast	–	EPR	Increasing the roasting degree led to a decrease in AA concentration	Summa et al., 2007
Coffee and chocolate	–	–	LC-MS	174 mg/kg in coffee 200 mg/kg in chocolate	Pardo et al., 2007
Coffee and coffee products (roasted beans, instant, and coffee blends)	–	–	GC-MS	AAmax was 36.2 μg/l in espresso AAmax was 95.2 μg/l in soluble coffee AAmax was 229.4 μg/l in coffee blends with cereals	Soares et al., 2006
Tea, spices, fruits, beans, and herbs	–	–	HPLC	Of the phenolic compounds, p-coumaric acid caused the greatest reduction (53%), whereas hesperetin increased acrylamide content by 9%	Zhu et al., 2009
Roasted green tea *(Houjicha)*	Roasting at 160°C for 30 min and at 180°C for 15 min	–	–	AA amounts in tea infusions were 2.0 and 4.0 μg/l by roasting at 160°C for 30 min and at 180°C for 15 min, respectively	Mizukami et al., 2008
Tea	Roasting at 180°C for 10 min	–	GC-MS	Higher temperatures and longer processing times caused a decrease in the AA content	Mizukami et al., 2006

2. AA METHODS

2.1. Capillary Electrophoresis-Mass Spectrometry (CE-MS)

Trace analysis by CE-MS of analytes such as low molecular mass amines, nitroaromatics, alkylphosphonic acids, azo dyes, antidepressants, and antibiotic drugs, among others, in air, sediment and water samples have been extensively reviewed (Castle et al., 1991). The application of CE-MS analysis of pesticides (triazolopyrimidine sulphoanilides), antibiotics (sulphonamides, b-lactones, quinolones and tetracyclines) and other extraneous compounds such as AA and toxic peptides in food matrices has also been reviewed (Robledo and Smyth, 2009).

Simo and his coworkers (2004) applied successfully a new polymeric coating on CE-MS to investigate the basic proteins of several foods. In another article the applications of CE coupled to MS detection (CE-MS) for the analysis of organic contaminants in food were also reviewed by Font et al. (2008). This review contains a great deal of useful analytical information related to sample foods studied. Specific sections are included on pending on pesticides, drug residues, and toxicants formed in food processing.

The applicability of field amplified sample injection (FASI) in reversed polarity was evaluated in order to decrease detection limits for AA analysis in foodstuffs. The developed FASI–CE–MS/MS method provided a detection limit of 0.008 mg/kg satisfactory linearity ($r = 0.999$) and precision (day-to-day lower than 15%) (Bermudo et al., 2007).

According to Bermudo et al. (2006a) "two inline preconcentration capillary zone electrophoresis (CZE) methods (FASI and stacking with sample matrix removal (LVSS) were evaluated for the analysis of AA in foodstuffs. For both FASI and LVSS methods, linear calibration curves over the range studied (10–1000 μg/l and 25–1000 μg/l, respectively), limit of detection (LOD) on standards (1 μg/l for FASI and 7 μg/l for LVSS), LOD on samples (0.003 mg/kg for FASI and 0.02 mg/kg for LVSS) and both run-to-run (up to 14% for concentration and 0.8% for time values) and day-to-day precisions (up to 16% and 5% for concentration and time values, respectively) were established. "However, due to the low detection limits obtained with the FASI–CZE, this particular method was applied to the analysis of AA in biscuits, cereals, crisp bread, snacks, and coffee."

Linear calibration curves over the range studied (0.3–100 μg/ml), the LOD (0.07 μg/ml), and both run-to-run (RSD values of 5.8 and 2.2% (for concentration at low and medium concentration levels, respectively) and day-to-day precisions (up to 11.2 and 6.7% at low and medium concentration levels, respectively) were established. The potential applications of the CZE proposed methodology was demonstrated by analyzing AA contents in French fries, breakfast cereals, and biscuits (Bermudo et al., 2006b).

2.2. Liquid Chromatography-Mass Spectrometry (LC-MS)

The amin advantage of LC-MS analysis over the LC technique in reversed-phase mode lies in its compatibility with an aqueous solvent that is best suited for extraction of AA from foods. Extraction solvent for AA in foods is water and this extract is directly compatible with reversed-phase LC using an aqueous mobile phase with a small amount of organic modifier. In many cases, the SPE clean-up step has been combined with a molecular size cut-off filter (3 to 5 kDa) to remove larger molecules that would otherwise give problems in the analysis (Rosén and Hellenäs, 2002; Tareke et al., 2002).

Although triple-quadrupole mass spectrometers for LC-MS/MS are rather expensive, they have the required sensitivity for conducting AA analysis in water extracts of foods whereas single stage instruments are of low sensitivity for AA analysis. The main ions observed for AA are m/z 72 (protonated molecular ion), 55 (loss of amino), and 27 (subsequent loss of CO). Storage Resource Management (SRM) traces neets the criteria prescribed in the Commission Decision 2002/657/EC (Riediker and Stadler, 2003).

The only drawback, common in both LC-MS and GC-MS methods is the not good (simply satisfactory) precision of measurements taken either within or among laboratories. The current state of the art of LC-MS applications was summarized by Hoenicke et al. (2004).

LC-MS was applied to Swedish baby food products, that is, breast milk substitute (infant formula), gruel, porridge, and canned baby food (Fohgelberg et al., 2005). The LoQ was determined at 0.0005 mg/kg for liquids and 0.002 mg/kg for other foods.

Gökmen et al. (2006) used analysis of digital color images of fried potato chips in conjuction with LC-MS based analysis of AA to develop a rapid tool for the assessment of AA during processing. Interpretation of potato chips images revealed, a linear correlation ($r = 0.989$) between AA level and NA2 value. The results obtained corroborated the capability of computer vision system to provide concrete and comprehensive description with regard to inspection and assessment of potato chips.

The ratio potato/NaCl solution is critical during extraction where the optimum ratio is 0.125 g/ml NaCl 2M solution Rufián-Henares and his coworkers (2006) the validated method (LC-MS) performance for LOD (0.0232 mg/kg) and quantitation (0.0918 mg/kg), linearity ($r > 0.999$, 25–1000 lg/kg), and recovery (98.8%). The method was successfully applied on commercial potato chips where the intra-day repeatability was set at 3.9% and values were corrected with a labeled internal standard ($^{13}C_3$-AA). It is noteworthy that no significant differences were reported on the AA content between industrial and domestic-scale processed potato chips.

A generic sample preparation method for the determination of AA in foods was developed by using chromatographic separation performed on ODS-3 column by means of an isocratic mixture of 0.01mM acetic acid in 0.2% aqueous solution of methonoic acid at a flow rate of 0.6 ml/min at 25°C. It was found that the recoveries of AA from potato chips, biscuits and coffee ranged between 92.8 and 101.5% with relative standard deviations of 4.1% or less. The LOD and the LOQ were

0.002 mg/kg and 0.006 mg/kg in the basis of signal to noise ratios of 3:1 and 9:1, respectively (Gökmen et al., 2006).

AA was determined with in LC–MS/MS several of the most frequently consumed carbohydrate-rich foodstuffs commercialized in Spain such as potato crisps and chips, biscuits, crisp breads, pastry, and "churros" (typical Spanish pastry), dried fruits, chocolates, and coffee. Different levels of AA were obtained and pastry and dried fruits showed the lower levels (<0.020 mg/kg). The highest levels of AA were recorded for potato chips and French fries (0.50–9.250 mg/kg) whereas the lowest for pastry and dried fruits (<0.02 mg/kg) (Bermudo et al., 2006).

Fohgelberg and his coworkers (2005) analyzed the AA levels in breast milk and the main categories of Swedish baby food products, that is, breast milk substitute (infant formula), gruel, porridge, and canned baby food. According to these researchers "the average levels found for gruel, porridge, and canned baby food, all ready to eat, were 1.4, 26, and 7.8 lg/kg, respectively. For all breast milk samples except one the AA level was below the limit of quantification (0.0005 mg/kg). Assuming an AA level of 0.00025 mg/kg in breast milk, the mean AA intake during the first six months for children who were exclusively breast-fed was estimated to be 0.00004 mg/kg b.w./day. The mean AA intake from breast milk and commercially made baby food during the whole first year varies due to the length of breast-feeding and the choice of baby food. The intake level range was estimated to be 0.00004–0.0012 mg/kg b.w/day."

An improved analytical method for the determination of AA in coffee with LC-MS was described by Şenyuva and Gökmen (2005). Recoveries for the spiking levels of 50, 100, 250, and 500 mg/kg ranged between 99 and 100% with relative standard deviations of less than 2%. The impacts of roasting on the formation of AA and color development were also studied at 150, 200, and 225°C. Change in a* color value demonstrated a good correlation with the change in AA.

Fried potato snack food, baked breakfast food, bread, coffee, and tea drinks were analyzed for AA with an improved LC/MS/MS. It was reported that the LOD was 0.003 mg/kg; mean recoveries ranged from 95 to 113%; coefficients of variation ranged from 1.3 to 10.0% for repeatability test and 3.3 to 6.9% for reproducibility test. It was shown that brown sugar with high AA contents results in high AA content in some foods even through they may have not been cooked at high temperatures (Cheng et al., 2009).

The detection limit of AA in various mainly starchy foods (roasted potato, potato chips, fried pasta, crispbread, fried rice, cereal breakfast, butter cookies) by means of an LC-MS/MS method was as low as 0.030 mg/kg. The highest value of AA (8.974 mg/kg) was determined in mashed-roasted potato, and the lowest value in butter cookies (0.151 mg/kg). The calculated average daily intake amounted to 34.03 μg AA/person/day. Which divided by the average person weight (i.e., 70 kg) can lead to 0.00049 mg/kg body weight/day (Tawfik and El-Ziney, 2008).

Analysis of AA levels in approximately 350 food products (onginating from Chinese market) with LC-MS/MS was carried out by Chen et al. (2008). All samples were found to contain AA apart from drinking water and tea. Potato products had the highest AA level, with an average level of 1.467 mg/kg. It is noteworthy that the average dietary exposure of AA amounted to 0.00038 mg/kg body weight per day, comparatively low with the result obtained by the Food and Agricultural Organization/World Health Organization (FAO/WHO). Another issue is the margin of exposure for neurotoxicity, reproductive toxicity, and carcinogenicity of AA determined to be 1318, 5250, and 787, respectively.

Zhao and his co-workers (2005) proposed an isotope dilution LC-MS method for AA determination in heated starchy food. This method includes the usage of a C_{18} analytical column and methanol-water containing 0.1% formic acid (2:98, v/v) as mobile phases. The detection limit of the method was 0.006 mg/kg, and the limit of quantification was 0.20 mg/kg, the average recoveries were higher than 96%, and the relative standard derivations were lower than 10%.

Application of Liquid Chromatography-Diode Array Detector (LC–DAD) for AA quantification and the LOD in extract of starch-based foods were shown to be 30 mg/kg. The determination of AA was carried out in spiked food samples without native AA yielding recoveries in the range 92.5 to 104.0% (Geng et al., 2008).

The urinary excretion of metabolites of 53 subjects was recorded with solid phase extraction and liquid chromatography (SPE-LC) connected with positive electrospray MS/MS detection. According to Bjellaas et al. (2007), "the median dietary exposure to AA was estimated to be 0.47 (range 0.00017–0.00116) mg/kg body weight per day. In a multiple linear regression analysis, the urinary excretion of AA metabolites correlated statistically significant with intake of aspartic acid, protein, starch, and coffee. Consumption of citrus fruits was negatively correlated with excretion of AA metabolites."

Application of tandem mass spectrometry (MS/MS) showed to be a useful and a time saving analytical tool, with many applications of direct detection of target molecules in food samples. Kotretsouand Koutsodimou (2006) reviewed the use of MS/MS in the determination of several food contaminants such as alkaloids, pesticides, marine toxins, mycotoxins, arsenosugars, antibiotics, dioxins, polychlorinated biphenyls, and AA.

2.3. Non-Aqueous Capillary Electrophoresis (NACE)

Field amplified sample stacking (FASS) techniques in the NACE were introduced for the online concentration of the AA to improve AA detection at 210 nm with DAD. Choosing 30 mmol/l $HClO_4$, 20 mmol/l $NaClO_4$, 218 mmol/l CH_3COOH in Cellulose Acetate Nitrate (CAN) as the separation electrolyte and employing sample stacking methods, the LOD value of AA was decreased to 2.6 ng/ml with electrokinetic injection and 4.4 ng/ml with hydrodynamic injection (Tezcan and Erim, 2008).

The detection limit of a developed NACE method for quantitive determination of AA in processed food was shown to be as 0.041 mg/l using UV detection at 200 nm. The run-to-run and day-to-day precisions for the corrected peak areas were calculated as 1.65 and 3.90%, respectively. The applicability and potential of the method was effectively demonstrated by carrying out analysis of AA in potato chips and French fries. The advantages of the developed NACE method are its simplicity rapidity low cost and applicability in a wide range of foods (Başkan and Erim, 2007).

2.4. High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)

AA recovery rates from spiked Chinese style foods with various spiking levels (50–1.000 mg/kg were in the range of 79–93% for the gas chromatography-micro-electron capture detector (GC-MECD) including derivatization and 84–97% for the HPLC-MS/MS method. The quantification limits of the HPLC-MS/MS method were 0.004 mg/kg for AA. AA contaminant was determined in all samples at the concentration up to 0.7711 and 0.7345 mg/kg detected by the GC and HPLC method, respectively (Zhang et al., 2007b).

Employment of HPLC with LC-MS/MS was found to be a reliable method the detection limit of which was sensitive enough both for foods (0.038 mg/kg) and drinks (5 μg/l). The main types of food analysed were potato and cereal-based foods, and processed foods (beef, chicken, ham, bacon) and coffee. AA was detected at levels, ranging from nondetectable to 1.480 mg/kg level in solid food, with crisp bread exhibiting the highest levels. As regards drinks, the highest value (29 μg/l) was recorded in regular coffee drinks (Eerola et al., 2007).

To increase the performance in terms of sensitivity Govaert et al. (2006) introduced a preconcentration to small volume before HPLC coupled to MS/MS analysis on a μ-Bondapak C_{18} column with d_3-AA as internal standard. This alteration in the method proved to be advantages because parameter like LOD, LOQ, and mean recoveries amounted to 0.01 mg/kg, 0.02 mg/kg, and 100–115%, respectively. Moreover, the coefficients of variation for repeatability were quite low in the range 1.36 to 8.06%.

Another method using normal phase (NP) HPLC with UV detection was developed for the analysis of AA and methacrylamide. The detection limits obtained for both analytes were 10 μg/l. When the method was applied for the determination of AA in spiked food samples the recoveries were in the range 95 and 103%. The applicability, of this method to AA was tested in the analysis of commercial samples of french and roasted fries, cookies, cocoa, and coffee (Paleologos and Kontominas, 2005).

2.5. Pressurized Fluid Extraction (PFE)

Pardo and his coworkers (2007) proposed the application of PFE with acetonitrile, florisil clean-up purification inside the PFE extraction cell, and detection with LC coupled to atmospheric pressure chemical ionisation in positive mode (APCI–MS/MS). According to the authors "the LOQ of the method was 0.001 mg/kg for coffee and 0.00006 mg/kg for chocolate. The recoveries ranged between 81–105% for coffee and 87–102% for chocolate. Employment of the optimized method to 20 coffee and 15 chocolate samples from Valencian (Spain) supermarkets, the recorded median values of AA amounted to 0.146 mg/kg in coffee and 0.102 mg/kg in chocolate.

In another application of PFE to foods (potatochips, snacks, biscuits, breakfast cereals, and crisp bread) with acetonitrile and precipitation with Carrez reagents for AA determination, the final extract was analyzed with LC coupled to electrospray ionization (ESI) MS-MS. The LOQ and the recoveries range determined were 0.005 mg/kg and 93–101%, respectively (Yusa et al., 2006).

2.6. Matrix Solid-Phase Dispersion (MSPD)

A sample preparation technique based on MSPD was applied to the determination of AA in potato chips. The obtained AA values for LOD, LOQ after employment of this method amounted to 0.0128 and 0.0388 mg/kg, respectively. In order to evaluate the performance of the MSPD-GC/MS developed method. Analysis of 17 potato chips samples were simultaneously treated with MSPD and hot water (as alternative method) revealed a good correlation [correlation factor (r) of 0.9985] (Fernades and Soares, 2007).

Soares and his coworkers (2009) described the development of an optimized MSPD procedure for AA analysis in processed foods such as bread, toasts, breakfast cereals, snacks, cookies, biscuits, chocolates, and baby foods. The LOD, LOQ, and precision range of this method were 0.0052 mg/kg, 0.0157 mg/kg, and 1–7%, respectively.

2.7. Gas Chromatography-Mass Spectrometry (GC-MS)

The occurrence of AA in drinking and discharge-waters motivated the scientists to develop the first analysis for AA by using bromination and GC (Habermann, 1991; Bologna et al., 1999).

The GC analysis applied makes use of an alkali flame-ionization detector or an electron capture detector (ECD). The majority of laboratories employ GC in conjuction with MS to the extra selectivity and improve selectivity and confidence (dueto MS) further enhanced by the usage of isotopically labeled internal standard (Nemoto et al., 2002).

The satisfactory agreement between results obtained with bromination-GC-MS and underivatised LC-MS on AA content of various foods confirmed that both methods are equally effective (Ahn et al., 2002; Ono et al., 2003). Similarly, in check-sample exercises, there has been no evidence of any bias from Br-GC-MS methods compared with other methods of test used

(Klaffke et al., 2005; Owen et al., 2005; Wenzl and Anklam, 2005).

Although AA is not the best compound for direct GC-MS analysis because of its high polarity, low volatility, low molecular weight, several laboratories have undertaken the arduous task to develop a direct GC method for AA determination (Biedermann et al., 2002).

Several researchers reported a bias of the obtained results with GC-MS without derivatization (Klaffke et al., 2005; Owen et al., 2005; Wenzl and Anklam, 2005). The currently employment protocol for underivatized GC-MS analysis is based on Honicke et al. (2004) approach. The latter proved to be very effective in analysis of complex matrices such as cocoa and molasses since the lowest AA detection limit was 0.005 mg/kg.

Pittet et al. (2004) developed a quantitative method for the determination of trace levels (<0.050 mg/kg) of AA in cereal-based foods. The LOD and LOQ were estimated at 0.002 and 0.005 mg/kg respectively, and recoveries of AA from spiked samples (levels of 0.005–0.5 mg/kg) were in the range 93–104%. The performance of the method was shown to be particularly good when comparative tests were carried out by two independent laboratories at very low AA contents.

Zhang and his coworkers (2006) coupled GC with ECD and successfully applied for the rapid determination of AA in conventionally fried foods, such as potato crisps, potato chips, and fried chicken wings. According to these authors the LOD was found to be 0.00001 mg/kg on the basis of ECD technique. The AA recoveries from conventional samples spiked at levels of 0.15, 0.5, and 1.000 mg/kg ranged between 87 and 97% with low relative standard deviations (RSD) (<4%).

In another piblication GC-ECD was used for extracting AA with water, filtration, defatting with n-hexane, derivatization with hydrobromic acid and saturated bromine-water, and liquid–liquid extraction with ethyl acetate. GC-MS analysis clearly shaved that 2,3-DBPA was converted to 2-BPA almost completely on the polar capillary column. Quantitative determination of AA content in food was possible by applying a four-point standard addition protocol. Accordinng to the ECD technique the LOD amounted to 0.0006 mg/kg (Zhu et al., 2008).

The determination of AA in fried starchy foods was carried out with isotope dilution GC-MS. The limit of quantitative detection, the recovery range and the standard deviation were 0.005 mg/kg, 90–105%, and 6.3%, respectively. The French fries AA content amounted to 0.278–0.518 mg/kg AA which is 10,000 times greater than the drinking water guidelines of WHO (Zhong et al., 2005).

2.8. Solid-Phase MicroExtraction-Gas Chromatography (SPME-GC)

Lee and his coworkers (2007) optimized the SPME experimental procedures to extract AA in water solutions, by means of a carbowax/divinylbenzene (CW/DVB)-coated fiber at pH 7, extraction time of 20 min, and analyte desorption at 210°C for 3 min. The detection limit and the relative standard deviation were 0.1 μg/l and 10.64%, respectively. Determination of AA trace with SPME-GC for French fries and potato crisps resulted in 1.2 and 2.2 μg/g, respectively.

According to Granby and Fagt (2004) "the analysis of prepared coffee includes a comprehensive clean-up using multimode SPE by automatic SPE equipment and detection by LC-MS/MS using electrospray. The recoveries of AA in ready-to-drink coffee spiked with 5 and 10 μg/l were 96 $\pm$ 14% and 100 $\pm$ 8%, respectively. Coffee samples prepared twice by coffee machines and twice by a French Press Cafetière coffee maker contained 8 $\pm$ 3 μg/l and 9 $\pm$ 3 μg/l AA, respectively. Hence, the results did not reveal any significant differences in the AA contents in ready-to-drink coffee prepared with coffee machine, French Press or from instant coffee. It is noteworthy that medium roasted coffee contained more AA ($\sim$10 μg/l) than dark roasted coffee ($\sim$5 μg/l)".

2.9. Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA was recently found to have a high specificity and good sensitivity for AA, with LOD in water samples of 0.0657 mg/kg, thereby covering a wide range of food commodities This was one of the first reports of an immunoassay capable of detecting AA because the small size of the latter requires analytical detection via high cost, comparatively slow techniques such as GC or LC coupled to MS (Preston et al., 2008).

2.10. MicroEmulsion ElectroKinetic Chromatography (MEEKC)

The effect of MEEKC operating conditions, such as the type of water-immiscible alcohol, aqueous phosphate buffer concentration, pH, on AA migration was investigated by Bermudo et al. (2004). According to these authors the "linear calibration curves over the range studied (1.25–125 μg/ml), the detection limit (0.70 μg/ml), and both run-to-run (up to 3.4% for concentration and 1.6% for time values) and day-to-day precision (lower than 11.6% for concentration) were determined".

The detection methods of AA in foods and beverages are summarized in Table 5.

3. LEGISLATION

In Commission Recommendation 2007/331/EC, the Commission stated that it is necessary to collect reliable data on AA levels in food over at least a three year time span across the Community in order to get an insight in the levels of AA in foodstuffs known to contain high AA levels and/or contribute significantly to the dietary intake of the whole population and of specific vulnerable groups, such as infants and young children.

Table 5 Detection methods of acrylamide in foods and beverages

Methods	Conditions	Foods	References
Capillary Electrophoresis- Mass Spectrometry (CE-MS)	–	Water samples, soft beverages, and fruit juices	Font et al., 2008
CE-MS	Heated capillary temperature 175°C Overimposed pressure of 3.5 kPa on the CE inlet vial and −3 kV as electrospray needle voltage	Foodstuffs (potato crisps, biscuits, crisp bread, breakfast cereals, and coffee)	Bermudo et al., 2007
CE	Temperature was held at 25°C Direct detection was carried out at 210 nm Samples were loaded by hydrodynamic injection pressure assisted (3.5 kPa) during 5 s	Home-made french fries, breakfast cereals and biscuits	Bermudo et al., 2006
CE-MS	–	Pesticides such as triazolopyrimidine sulphoanilides, different types of antibiotics, and other exogenous compounds such as AA and toxic oligopeptides in food samples	Robledo and Smyth, 2009
Liquid Chromatography-Mass Spectrometry (LC-MS)	Frying temperature was adjusted to 150 (3, 5, 8, 10, and 15 min), 170 (3, 5, and 8 min), and 190°C (1 and 3 min) to prepare 10 pieces of training samples Fried chips were drained over a wire screen for 5 min to remove excess oil and then the samples were photographed	Fried potato chips	Gökmen et al., 2006
LC-MS	A portion of the sample (1.0 g) was weighed with a precision of 0.1 mg and suspended with 8 ml of sodium chloride 2 M in polypropylene 15 ml centrifugal tubes AA extraction was performed by incubation in a water bath at 60°C for 30 min, and 10 s shaking every 10 min	A series of commercial potato chips and fried potatoes	Rufián-Henares et al., 2006
LC-MS	Heating at 65°C for 30 min Drying gas temperatures 325°C Capillary voltage of 4 kV	Foods (potatoes and cereals)	Gökmen et al., 2006
LC-MS	Heat capillary temperature, 150°C	Potato chips, french fries, pastry, crisp breads and crackers, breakfast cereals and dried fruits, chocolate, and coffee	Bermudo et al., 2006
LC-MS	Powdered gruel (75 g) was mixed with water (4.5 dl) and heated in a saucepan on a regular stove to 37°C For the breast milk substitute, water (4.5 dl) was boiled and cooled to 60°C (brand A) or 40°C (brand B) before the powder (65 g) was added	Breast milk substitute (infant formula), gruel, porridge, and canned baby food	Fohgelberg et al., 2005
LC-MS	Drying gas temperatures 325°C Capillary voltage of 4 kV	Coffee	Şenyuva and Gökmen, 2005; Gokmen and Senyuva, 2006
LC-MS/MS	–	Fried potato snack food, baked breakfast food, bread, coffee, and tea drinks	Cheng et al., 2009
LC-MS/MS	–	Mashed-roasted potato, fried pasta, soluble coffee, biscuits, potato chips, cocoa powder, crisp bread, fired rice, roasted Turkish coffee, cereal breakfast (corn), and butter cookies	Tawfik and El-Ziney, 2008
LC	The column temperature was set at 50°C Flow rate was maintained at 0.6 ml/min while detection was performed at 200 nm Raw potatoes were peeled, sliced into pieces, blanched in 100°C water for 1 min and dried in an oven at 60°C	Fried potato chips, biscuits, and Chinese fried/baked foods	Geng et al., 2008
Nonaqueous Capillary Electrophoresis (NACE)	The wavelength was set at 210 nm The separation was performed at 25 kV The temperature was set at 25°C	Processed food (potato chips and almond extracts)	Tezcan and Erim, 2008

(Continued on next page)

Table 5 Detection methods of acrylamide in foods and beverages *(Continued)*

Methods	Conditions	Foods	References
NACE	After being activated for 2 h at 150°C under vacuum The sample of 5 ml of French fries were kept in 0.5 g of sieves for 14–16 h and then were extracted Fried potato samples were kept in a refrigerator at –4°C	Potato chips and French fries	Başkan and Erim, 2007
High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)	Capillary voltage was 3.5 kV Source temperature was 100°C Desolvation gas temperature was 350°C	Chinese traditional carbohydrate-rich foods such as fried bread stick, clay oven rolls, hemp flowers, glutinous rice sesame balls, and steamed buns	Zhang et al., 2007a
HPLC-MS	Potato-ham casseroles were heated in an oven (RK210W Rosenlew, Vantaa, Finland) at 180°C for 40 min or in a microwave oven (Grilli Mikro automatic 992D, Rosenlew) Instant coffeewas prepared by weighing instant coffee powder (1 g), boiling water was added (100°C, 200 ml) Two tea bags were brewed in hot water (100°C, 200 ml) for 3 min and bulk tea leafs (2 g)	Potato and cereal-based foods, processed foods (pizza, minced beef meat, meat balls, chicken nuggets, potato-ham casserole, and fried bacon) and coffee	Eerola et al., 2007
HPLC-MS	Source temperature was 120°C Capillary voltage was 3.7 kV	Potato and cereal products	Govaert et al., 2006
HPLC	Potatoes were sliced in round pieces and either baked in an oven at 250°C for 1 h or fried in olive oil ($T = 180$°C)	Commercial samples of french and roasted fries, cookies, cocoa, and coffee	Paleologos and Kontominas, 2005
Pressurised Fluid Extraction (PFE)	Temperature of 35°C with 5 min heat-up period under a pressure of 1500 psi	Coffee and chocolate	Pardo et al., 2007
PFE	Capillary temperature was 200°C	Potato chips, snacks, biscuits, breakfast cereals, and crisp bread	Yusa et al., 2006
Matrix Solid-Phase Dispersion (MSPD) and GC-MS	Injector temperature: 280°C Oven temperature: 85°C (1 min), 15°C/min to 280°C, hold 10 min (total of 24 min) Transfer line: 240°C	Potato chips	Fernades and Soares, 2007
Gas Chromatography-Mass Spectrometry (GC-MS)	Injection temperature: 260°C Transfer line was held at 280°C Ions monitored were m/z 70, 149, and 151 for 2-bromopropenamide	Cereal-based foods	Pittet et al., 2004
GC-MS	Injector interface temperature and detector interface temperature were both held at 250°C	Potato crisps, potato chips, and fried chicken wings	Zhang et al., 2006
GC-MS	–	Heat-processed foods	Zhu et al., 2008
GC-MS	–	Fried starchy foods	Zhong et al., 2005
Solid-Phase Microextraction-Gas Chromatography (SPME-GC)	The oven temperature was initially set for 80°C (0 min hold) Injector temperature of 210°C Linear temperature gradient of 15°C/min to 220°C, and held for 2 min	French fries and potato crisps	Lee et al., 2007
SPE	The source temperature was set at 120°C Capillary set at 3.0 kV Desolvation gas (flow 150 l/h)	Coffee	Granby and Fagt, 2004
Enzyme Linked Immunosorbent Assay (ELISA)	–	Bread and other bakery products, crisps, chips, breakfast cereals, and coffee	Preston et al., 2008
Microemulsion Electrokinetic Chromatography (MEEKC)	–	Home-made French fries	Bermudo et al., 2004

It therefore recommended that Member States conduct yaerly in 2007, 2008, and 2009, in accordance with Annex 1 of Recommendation 2007/331/EC the monitoring of AA levels in the foodstuffs mentioned to in that Annex. It is recommended that Member States provide by 1 June each year the monitoring data of the previous year to EFSA with the information and in the format as set out in Annex II for compilation purposes into one database. (http://www.fsai.ie/legislation/food_legislation/contamination_of_foodstuffs/acrylamide.html).

International efforts to develop approaches to AA mitigation are getting gradually successful. Moreover, FDA is aware that at least some manufacturers in the US are exploring methodologies

ways to reduce AA in their products. Therefore, FDA is considering issuing guidance for industry on reduction of AA levels in food products (http://www.foodqualitynews.com/Legislation/FDA-considering-industry-guidelines-for-acrylamide).

Since 2002, the Food and Agricultural Organization and WHO have been involved in the risk assessment of AA in foods. They held a special consultation to review available data on AA in 2002, and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) held a meeting on the topic in February 2005 (http://pubs.acs.org/cen/government/84/8433gov1.html).

4. CONCLUSIONS

AA is a chemical used in a variety of industrial applications, including in the production of polyacrylamide plastics and other materials that may contain low levels of residual AA. AA is also present in tobacco smoke. The toxic effects of AA on the nervous system in humans following high occupational and accidental exposures are well documented (INFOSAN, 2005).

Although the calculated risk assessment is quite conservative, it does provide a rough indication of the magnitude of a risk for cancer related to AA in foods. Any risk of neurotoxic or reproductive toxic effects associated with AA in foods is judged to be very small (Dybing and Sanner, 2003).

AA is widely used worldwide in industry and it can also be produced by the cooking and processing of foods. It is harmful to human beings, and human brain and creatine kinases (HBCK) have been suggested to be one of the important targets of AA. The effects of AA on CK were proposed to be isoenzyme- and species-specific, and the underlying molecular mechanisms (Sheng et al., 2009).

Since there is still ongoing research on AA, it is anticipated that the current legislation may become eventually stricter based on the findings and their correlation with toxicity risk in humans.

ABBREVIATIONS

AA	Acrylamide
Al	Alanine
APCI	Atmospheric Pressure Chemical Ionisation
Asn	Asparagine
CAN	Cellulose Acetate Nitrate
CE	Capillary Electrophoresis
CIAA	Central Intercollegiate Athletic Association
CIE	Commission Internationale de l'Eclairage
CO_2	Carbon Dioxide
CRM	Certified Matrix Reference Material
cv	cultivar
CW	Carbowax
CZE	Capillary Zone Electrophoresis
DAD	Diode Array Detection
DVB	Divinylbenzene
ECD	Electron Capture Detector
EFSA	European Food Safety Administration
ELISA	Enzyme Linked Immunosorbent Assay
EPA's	Environmental Protection Agency
ESI	Electrospray Ionization
FAO	Food and Agricultural Organization
FASI	Field Amplified Sample Injection
FASS	Field Amplified Sample Stacking
FDA	Food and Drug Administration
FSA	Food Standards Agency
GC	Gas Chromatography
Hb	Hemoglobin
HBCK	Human Brain and Creatine Kinases
HPLC	High Performance Liquid Chromatography
INFOSAN	International Food Safety Authorities Network
JECFA	Joint Expert Committee on Food Additives
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantitation
LVSS	Large Volume Sample Stacking
MECD	Micro-Electron Capture Detector
MEEKC	Microemulsion Electrokinetic Chromatography
MPs	Maillard Products
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MSPD	Matrix Solid-Phase Dispersion
NACE	Nonaqueous Capillary Electrophoresis
NFA	National Food Administration
NP	Normal Phase
PFE	Pressurised Fluid Extraction
R&G	Roasted and Ground
RP/HPLC	Reversed-Phase High-Performance Liquid Chromatography
RS	Reducing Sugar
RSD	Relative Standard Deviations
SPME	Solid-Phase Microextraction
SRM	Storage Resource Management
SWCNTs	Single-Walled Carbon Nanotubes
WHO	World Health Organization

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