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Acrylamide: Thermally Induced Toxicant in Foods and Its Control Measures

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Acrylamide is a thermally induced toxicant present in different processed foods in varying amount. Due to its detrimental effect on human health, it has become a major concern in public health and food safety. Various reports published recently have identified different processing techniques to reduce the level of this compound in the food. This paper aims to review and focuses on the mechanisms of acrylamide formation, the effects of different processing parameters such as pre-frying treatments, pH, temperature, time, types and the amount of raw materials, its toxicity level, and its detection methods in complex food systems. Toxicity levels of acrylamide have been found to be neurotoxic and carcinogenic. Food safety authorities including Codex Alimentarius Commission are in the process of reviewing their standards to fix the limit of acrylamide in processed foods.

Keywords: Acrylamide, Thermal processing, Toxicity, No-observed-effect-level, Reference dose

Introduction

Acrylamide is a highly reactive unsaturated amide with the chemical formula C_3H_5NO and named as prop-2-enamide in IUPAC system of nomenclature. It is a white odorless crystalline solid, highly soluble in water, ethanol, ether, and chloroform. Non-thermal decomposition of acrylamide produces ammonia whereas thermal decomposition produces carbon monoxide, carbon dioxide, and oxides of nitrogen (FSA, 2002).

Acrylamide is abundantly present in water and soil due to its heavy usage as sealant in many manufacturing works such as in tunnel fractures. The discovery of acrylamide was attributed to a tunnel leakage in Sweden in 1997. Due to large number of death of cattle during the period, effect and presence of acrylamide in the persons working in the tunnel were carried out. A high level of acrylamide was found in blood of both tunnel workers and control. The ubiquity of these acrylamide hemoglobin adducts led to a hypothesis that acrylamide might be ingested in the diet (Reynolds, 2002).

Despite of uncertainties due to acrylamide on actual human health effects at the level found in food, there is heightened public awareness about this compound because it has been proved as carcinogenic in experimental animals. Epidemiological studies conducted by the University of Mastricht indicated a positive association between dietary acrylamide and the risk of certain types of cancer (Hogervorst *et al.*, 2007).

In addition to certain cooked foods that can expose us to large amounts of acrylamide, there are many non-dietary sources exposure to this substance. These non-dietary sources include cigarette smoke and cosmetics (FSA, 2002). There is also airborne release of acrylamide during many different manufacturing processes, including the manufacturing of paper, asphalt, petroleum, photographic film, construction adhesives, varnishes, and dyes (GMF, 2011).

Thermal processing is an effective method of food preservation. Different varieties of food commodities are prepared by thermal processing. Frying in oils, baking and roasting are important steps in thermal processing and they need a high heat treatment. On the other hand, these thermally processed foods are consumed in significant amount in all parts of the world. The discovery of acrylamide in thermally processed foods and its detrimental effects in animal and human health has created a great concern about its safety aspect. It needs an immediate attention and urge in the development of simple and reliable methods for detection and quantification of the acrylamide contents and the methods for controlling its formation during food processing.

Mechanisms of acrylamide formation

The main ingredients that are responsible for acrylamide formation in foods are *carbohydrates* especially *reducing sugars* and *asparagine,* one of the non-essential amino acids. Maillard reaction has been considered as the major reaction pathways (Motram *et al.*, 2002, Stadler *et al.*, 2002). Studies to date have clearly showed that asparagine is mainly responsible for acrylamide formation in heated foods after condensation with reducing sugars or a carbonyl source (Gokmen and Palakzaglu, 2008).

Four major hypotheses are proposed to describe the mechanism of formation of acrylamide in foods. The first mechanism explains the direct formation of acrylamide from amino acids such as alanine, asparagine and glutamine and from methionine (Youssef *et al.*, 2004). The second mechanism involves the formation of acrylamide via the formation of acrolein. Acrolein, on the other hand, is produced from different compounds such as dehydration of glycerol,

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oxidation of monoacylglycerol, from starch and sugars as well as recombination of simple aldehydes. The third mechanism which describes the formation of acrylamide involves the formation of acrylic acid as an intermediate product. Some authors described the second and third mechanisms as a single one. It explains the role of oils and nitrogen containing compounds present in foods. It includes the formation of acrolein from the thermal degradation of glycerol (Umano and Shibamoto, 1987), oxidation of acrolein to acrylic acid and finally reaction of acrylic acid with ammonia leading to the formation of acrylamide (Figure 1b).

The most explained pathway for acrylamide formation is *via* Maillard reaction between reducing sugars and the amino acid asparagine. It involves the decarboxylation of the Schiff's base, rearrangement of the resulting Amadori product and subsequent â-elimination producing acrylamide (Yaylayan *et al.*, 2003) as shown in Fig. 1a. á- dicarbonyls are necessary co-reactants in the Strecker degradation reaction (Mottram *et al.*, 2002). The key mechanistic step is the decarboxylation of Schiff's base leading to Maillard intermediates that can directly release acrylamide (Stadler *et al.*, 2002).

A linear correlation was reported between total colour value and acrylamide formation in potato French fries suggesting a relationship between the acrylamide formation and the degree of non-enzymatic browning developed during frying (Pedreschi *et al.*, 2007). High temperature cooking such as frying, roasting, or baking is most likely to cause acrylamide formation. Boiling and steaming do not typically form acrylamide. Acrylamide is found in significant amount in plant based foods such as potato products, grain products or coffee. The amount of acrylamide found in dairy, meat, and fish products is negligible. The amount of acrylamide in different foods is listed in Table 1 (Ahn *et al.*, 2002, FDA, 2003; Takatsuki *et al.*, 2003).

Generally acrylamide is more likely to accumulate when cooking is done for longer periods or at higher temperatures. It is generally formed at temperatures higher than 120^{o}C and ncreases with increase in frying and baking temperatures. Temperature was observed to be a key factor for acrylamide formation since negligible amount is formed at temperatures lower than 115^{o}C.

The main factors responsible for acrylamide formation are summarized as in Figure 2. The carbonyl group (from carbohydrates) and the amine group (from L-asparagine) are the main precursors and the mixture of these two sources should be heated to a temperature more than 120^{0}C for the formation of acrylamide. The main principle for preventing or reducing its formation is based on removing any one of them.

$$\text{Asparagine+Glucose} \xrightarrow{\text{Heat}} \text{Schiff's base} \xrightarrow{\text{Decarboxylation}} \text{Amadori compound} \xrightarrow{\text{B-elimination}} \text{Acrylamide}$$

Fig1a. A pathway of Acrylamide formation from amino acids and reducing sugar (Yaylan et al, 2003)

$$\text{Triglyceride} \xrightarrow{\text{Heat, } O_2} \text{Acrolein} \xrightarrow{O_2} \text{Acrylic acid} \xrightarrow{\text{NH}} \text{Acrylamide}$$

Fig 1b. A pathway of Acrylamide formation from dietary fat (Umano and Shibamoto, 1987)

Table 1. Acrylamide in different foods

Food commodities	Maximum acrylamide content (ppm)	
	EU	US FDA
Breads	12-3200	<10-364
Crisp bread	<30-1670	
Crackers and biscuits	<30-2000	26-504
Toasted cereals	<30-2300	52-266
Potato chips	150-1280	117-2762
Other salty snacks	122-416	12-1168
French fries	85-1104	20-1325
Other potato products	<20-12400	
Other vegetable and	10-<50	<10-70
Meats	< 30-64	< 10-116
Fish seafood products	30-39	
Candy and dessert	< 20-110	< 10-909
Coffee and tea	170-700	175-351
Other non-alcoholic	< 30	
Beer, alcoholic	30	
Dairy products	10-100	< 10-43
Baby food and	40-120	< 10-130
Dry soup mixes		< 10-1184
Gravy and seasonings		38-54

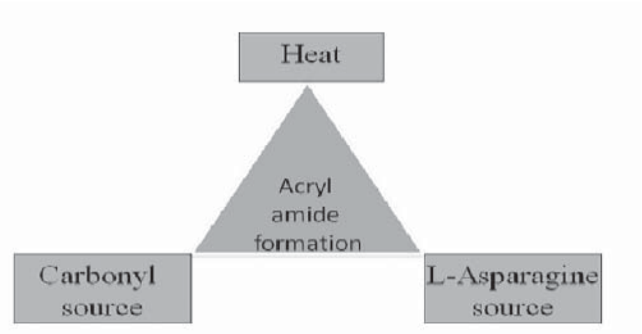


Figure 2. Factors affecting on acrylamide formation

Although different kinds of sugars can interact with different amino acids for acrylamide formation, one particular amino acid-called asparagine-has a far greater tendency to interact with sugars and to form acrylamide than other amino acids. The affinity of asparagine in the formation of acrylamide is shown in Table 2 (Petersen, 2002).

Table 2. Level of acrylamide formation by different amino acids

Amino acids	Level of acrylamide formation after combination with sugar and application of heat
Alanine	<50 ppb
Asparagine	9270 ppb
Aspartic Acid	<50 ppb
Cysteine	<50 ppb
Glutamine	156 ppb
Lysine	<50 ppb
Methionine	<50 ppb
Threonine	<50 ppb

One noteworthy example of acrylamide formation involves the conventional production of potato chips. There is small amount of asparagine present in raw potatoes before processing. During the frying process, fats used for frying can be oxidized and can become converted into acrolein and acrylic acid. Starches in the potato can also be broken down into sugars. This unique mixture of substances can interact in a way that results in unusual amounts of acrylamide formation. Potato chips can commonly contain more than 1,000 parts per billion (ppb) of acrylamide. One ounce snack-sized bag of potato chips can have 28 micrograms of acrylamide. This amount is just above the average total exposure to acrylamide experienced by U.S. adults, and about 20% of the maximum safe intake of dietary acrylamide (US-EPA, 1993).

The tendency of acrylamide to be formed from asparagine is far greater than any other commonly occurring amino acids. This proves a close relationship between the amount of asparagine found in food and its amount of acrylamide. While it is important for asparagine to be present for the formation of acrylamide, there are too many other requirements that must be met before acrylamide can be formed. For this reason, the amino acid asparagine is simply a necessary-but not sufficient-factor when it comes to the formation of acrylamide. The levels of asparagine and acrylamide in some commonly eaten food commodities are shown in Table 3 (GMF, 2011).

Table 3 shows that the food stuffs must definitely contain at least minimal amounts of the amino acid asparagine in order to form substantial amounts of acrylamide. However, the amount of acrylamide formed cannot be predicted based solely on the amount of asparagine found in a food. For example, the amount of asparagine found in asparagus can be relatively high, even though most studies show very low levels of acrylamide in this food (GMF, 2011).

Heating and cooking do not always result in significant acrylamide formation, even if a food contains significant amounts of the amino acid asparagine. However, when all factors for forming acrylamide are present, it takes approximately 121°C for appreciable formation of acrylamide in most foods. Interestingly, acrylamide formation may peak in temperature ranges commonly used for roasting (121-191°C). Some researches revealed the formation of acrylamide in green tea when roasted at these temperatures, and acrylamide formation in roasted coffee beans has also been shown to be substantial in this regard. The toasting of bread has also been shown to increase acrylamide formation. A temperature range of 163-191°C is also frequently used for the frying in oil of French fried potatoes and potato chips. It is always not

Table 3. Level of acrylamide and asparagines in different foods

Commodities	Asparagine level (mg/g product)	Acrylamide level (ppb)
Asparagus	41-820 (raw)	<10 (frozen, canned, grilled)
Carrots	3.2 (raw and dehydrated)	61 (grilled)
Onions	3-5 (raw)	70 (grilled)
Potatoes	6.6-16.0 (raw)	114 (roasted)
Broccoli	179-393 (raw)	235 (canned)
Plums and Prunes	16-116 (raw)	31-188
Coffee beans	0.33-0.97 (raw and dried)	179-351 (roasted)
Cocoa beans/Chocolate	9-31 (both raw powder and roasted beans)	24-909
Wheat/breads	20-215 (fresh wheat) 2-5 (fermented wheat dough)	27-364
Apple juice	220-720	Not detected

necessary that the heating of foods at temperatures between 121-191°C automatically mean that acrylamide is being formed in the food. It requires combination of asparagine together with a form of sugar, or the oxidation of fat into smaller carbon molecules, or both to result in substantial formation of acrylamide (Mottram *et al.*, 2002; Stadler *et al.*, 2002). Even though it has been shown that a temperature of 120 °C or higher is needed for the formation of acrylamide, there are reports confirming that this compound can be formed at temperatures below 100 °C, particularly in drying processes at 65–130°C (Eriksson, 2005). The amount of acrylamide formed is directly related to brown colour of the products and it varies with the variety of agricultural commodity used. Research has revealed the effect of variety and relationship of brown colour and acrylamide concentration in potato products as shown in Table 4.

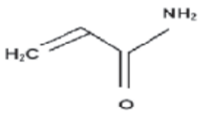
Table 4. Relationship between browning and acrylamide formation in potato chips

S.N.	Sample	Acrylamide concentration (μg/kg)		Remarks
		GC-MS	LC-MS-MS	
1	**Baking potatoes**			
a)	Raw	<10	ND	
b)	Boiled	<10	ND	AA formation varies with starting raw material
c)	Chipped and fried	310	350	
2	**King Edward Potatoes**			
a)	Raw	<10	ND	
b)	Boiled	<10	ND	
c)	Chipped and fried	2800	3500	
3	**Frozen frying chips**			
a)	As sold	200	100	
b)	Cooked	3500	3500	Acrylamide content increases with browning
c)	Overcooked	12800	12000	

Characteristics of acrylamide

Some major characteristics of acrylamide are listed in Table 5 (Jafari *et al.*, 2011).

Table 5. Major characteristics of acrylamide

Parameters	Values
Structure	
IUPAC name	prop-2-enamide
Molecular formula	C_3H_5NO ($CH_2CHCONH_2$)
Molar mass	71.08 g mol^{-1}
Density	1.13 g/cm^3
Melting point	84.5 °C
Boiling point	125 ^{0}C
Solubility in water	2.04 kg/L (at 25 °C)
Flash point	138 °C

Acrylamide mitigation strategies in food

After understanding the mechanism of acrylamide formation and the main role players in its formation, it is somehow easier to formulate strategies for reducing acrylamide level in food. The main variables that can affect acrylamide formation in fried or baked foods are: the concentration of glucose and asparagines and cooking time and temperatures. For food like potato chips and potato crisp made from potatoes alone, there is little chance to make change in the recipe formulation. However in breakfast cereals, replacement of cereals containing less asparagine in the recipe formulation can be useful. Depending on the type of food, single strategy may not work out. Improvement in parameters such as (1) potato variety, (2) potato storage temperature, (3) process control (thermal input, pre- processing) and (4) post preparation measures have contributed to a significant reduction in the average acrylamide content in French fries and potato crisps (Foot *et al.*, 2007).

There is need to reduce acrylamide content in fried potato products without adverse effects on its quality. To mitigate acrylamide formation in fried potatoes Hanley *et al.*, (2005) suggested as follows: (a) prevention of acrylamide formation by removal of the essential precursors (asparagine and a source of a carbonyl moiety- generally a reducing sugar); (b) interruption of the reaction by the addition of chemically reactive compounds that are able to react with intermediates in the Maillard reaction; (c) removal of acrylamide after it has been formed; and (d) minimization of acrylamide formation by changing frying conditions (frying temperature, pressure, time etc).

Zhang and Zhang (2007) reported that many additives were found to have the inhibitory effect in acrylamide formation in the Maillard reaction. Acrylamide formed in the Maillard reaction may also be reduced via the addition of exogenous chemical additives, which should comply with the following conditions: (i) the addition level should be properly controlled according to corresponding criteria of food or chemical additives; (ii) the selected additives should be regarded as no toxicity demonstrated by toxicity test from previous publication; (iii) the additives applied to the food systems cannot affect the connatural and sensory characteristics. Besides these, selection of raw materials containing low amount of precursors of acrylamide and proper storage of potato tubers (<10ºC) and soaking potato slices in water or acid solutions for at least 15min before frying can reduce acrylamide (Jackson and Al-Taher, 2005).

Possible acrylamide mitigation strategies in different food related to different stages of production are described below.

Raw materials: Halford *et al.*, (2007) suggested that increasing soil sulfur levels in wheat crops and reducing nitrogen availability in crops can decrease levels of asparagine, an acrylamide precursor. They also produced a new variety of potato through genetic modification that contains lower sugar levels than conventional potatoes and are targeting plant genes responsible for controlling asparagine levels in an effort to reduce acrylamide levels in food crops.

Use of selected agronomic methods: The use of genetic engineering in improving the nutritional properties of potatoes and cereals can help in acrylamide reduction in foods. Traditionally, plant breeders have worked to develop varieties of potatoes and wheat low in sugar and asparagine.

For potato based fried products, selection of proper cultivar of potato low in asparagines and reducing sugar is important factor. Asparagine concentrations in fresh potatoes are typically between 2 and 4 g/kg. Concentrations of reducing sugars range between 0.1 and 3g/kg after harvesting and may reach 20g/kg after currently used storage at low temperature (Amrein *et al.*, 2004). Seasonal growing conditions, agricultural conditions and regional factors have an impact on sugar levels in potato. Potato crisp manufacturers are advised to use potatoes having reducing sugar levels below 3g/kg on a fresh weight basis (CIAA, 2006).

The effect of variety on acrylamide content in potato products were studied by Mottram *et al.*, (2002) found that potato variety has significant effect on acrylamide content in the products made from them if treated under the same condition as shown in Table 4.

Influence of fertilizers: The nitrogen content and mineral content of soil also have effect on acrylamide content of wheat and potatoes. Use of low nitrogen fertilizers is proved to result in lower amount of asparagine in potatoes. Excess nitrogen in soil and deficiencies in potassium, sulphur, phosphorus or magnesium can cause asparagine levels to rise in wheat and potato and increase acrylamide risk (Halford, 2007).

Storage conditions: Storage of potatoes is generally done at low temperature to minimize losses due to spoilage and shrinkage. However, low temperature tend to increase sugar levels in potato, particularly if stored below 6ºC, through a process known as cold induced sweetening (CIS) (Sowokinos, 2001), resulting in dark-flavored and bitter-tasting fried products. The minimum storage temperature is generally 6ºC for long term storage of potatoes before processing. For short term storage of potatoes, hot temperature is desirable. However, storage of potatoes at higher temperature for longer period causes sprouting which can be controlled through the use of suppressants, such as chloropropham (CIPC). Reconditioning by warming the tubers to somewhat higher temperatures (e. g. 12º C) for up to a few weeks reduces the sugar levels, but does not restore initial levels (Foot *et al.*, 2007).

Pretreatment procedure

Cutting: Slicing or cutting potatoes to a specific surface area-to-volume ratio is particularly important because acrylamide formation typically occurs on the surface layer of the products. Coarse-cut potato French fries strips (14×14mm) with a surface area to volume ratio of 3.3 cm2-1cm3 resulted in significant lower ($p < 0.05$) amounts of acrylamide than fine-cut strips (8mm×8mm) (Matthäus *et al.*, 2004). The same applies to potato crisps, available from 1.2 to 1.75 mm. However, the organoleptic properties may change. The removal of fine cuts from the outer sphere of the tubers contributes to acrylamide reduction, because they tend to be overheated and the concentration of reducing sugars in the peripheral region of the tubers is higher (Foot *et al.*, 2007).

Soaking: Reducing sugar in fried potatoes can be lowered by soaking in water before frying. Pedreschi *et al.*, (2004) reported that glucose content in potato slices decreased slightly on increasing the soaking time due to leaching of glucose in water. The reduction in glucose content for 40min and 90min soaking was found to be 25% and 32% respectively as compared to control (no soaking). However, the asparagines content remained almost constant. The acrylamide content increased drastically when frying temperature increased from 150°C to 190°C for both control and soaked samples.

Blanching: Hot water blanching of sliced potatoes just before frying may decrease the asparagine and reducing sugar level in fried potatoes. Hasse *et al.*, (2003) reported that acrylamide formation reduced by about 60% when potato strips fried at three different frying temperatures. Blanching leads to leaching of reducing sugars and asparagines thereby forming less acrylamide in fried potatoes. Lowest level of acrylamide was detected in the samples which were blanched for 80 min at 500C and/ or 45 min at 700C and tested in three different frying temperatures. The colour of the blanched French fries was also found to be lighter than that of control (Pedreschi *et al.*, 2007).

Addition of functional components in food system

Antioxidants: Hedegaard *et al.*, (2008) reported that the addition of 1% rosemary extract with approximately 40 mg of gallic acid equivalent or comparable addition of rosemary oil or of dried rosemary leaves to wheat dough reduced the content of acrylamide in wheat buns by 62, 67 and 57% respectively. Immersion of potato crisps and French fries in an extract of bamboo leaves with antioxidant properties was reported to afford a 74.1 and 76.1% reduction of acrylamide, respectively. The relatively low amount of extract used (0.01-0.1% on a weight basis) apparently had no major impact on the sensorial properties of the products (Zhang and Zhang, 2007). Nearly 57.8 and 59.0% of acrylamide in fried chicken wings were reduced when the antioxidant from bamboo leaves addition ratios were 0.1 and 0.5% (w/w) respectively (Zhang *et al.*, 2007). Involvement of acrolein and inhibition of the reaction pathway to acrylamide by antioxidants was postulated. The inhibitory effect was less pronounced at higher levels of antioxidants. A possible explanation for the observed antioxidant is oxidation of the polyphenols to the corresponding quinones that can then react with 3-aminopropionamide (3-APA), thereby preventing deamination of 3-APA to acrylamide.

Amino acids and proteins: Addition of the amino acid glycine can reduce acrylamide levels either by competing with asparagine in the Maillard reaction or by reacting with the acrylamide formed. Bråthen *et al.*, (2005) reported a reduction of acrylamide by soaking potato slices in solutions of glycine or glutamine. Low *et al.*, (2006) reported on the effect of citric acid and glycine on the reduction of acrylamide in a potato model system. Both additives lowered levels of acrylamide in test samples cooked at 180°C for 10-60 min. The authors claimed that combining the two has an additive effect. They considered the effect to be due in part to the leaching out of the reducing sugars during dipping. Acrylamide formation can be reduced significantly by introducing other amino acids, such as cysteine, lysine or glycine, which would compete with asparagines for the carbonyl compounds in the Maillard reaction and/or enhance acrylamide elimination (Claeys *et al.*, 2005).

Salt: Acrylamide formation is dependent on water activity of the food. Addition of salt in soaking water for potato slices can reduce the water activity thereby reducing acrylamide formation. Pedreschi *et al.*, (2010) found that soaking of blanched potato slices (at 90°C for 5min) in the 1g/100g NaCl solution for 5 min at 25°C reduces acrylamide formation by 62% in potato chips after frying. However, only 27% of this effect was due to NaCl treatment and 35% due to heat treatment.

The addition of divalent ions like calcium chloride in wash solution to give a firm structure to final product is well known. Goken and Senyuva (2007) studied the impact of mono- and divalent ions on the acrylamide formation in asparaginase-fructose model system. They found that dipping potatoes into O.1M $CaCl_2$ solution for 60 min inhibited the formation of acrylamide by up to 95% during frying.

Asparaginase: The application of the enzyme asparaginase presents an efficient and simple way to decrease acrylamide formation in bakery products. This enzyme hydrolyses the key precursor asparagine to aspartic acid and ammonia and can be added to the dough during kneading. In general, no further adjustments of the recipe or the process have to be made. In this treatment the sensory properties of the product remain the same, which make this approach attractive and promising. The efficacy of asparaginase to limit acrylamide formation was first demonstrated in a potato model system (Zyzak *et al.*, 2003) and with heated wheat flour (Weisshaar, 2004). Shortly thereafter, the first results with real bakery products, such as gingerbread (Amrein *et al.*, 2004) and wheat crackers (Vass *et al.*, 2004) demonstrated the suitability of this enzyme for bakery. The acrylamide content of gingerbread was reduced by over 50% when asparaginase was added during preparation of the dough. Analysis of the enzyme-treated dough showed that about 25% of the initial amount of free asparagine was still present, which explains the still substantial formation of acrylamide in the enzyme-treated dough during baking. In the wheat cracker, the decrease of acrylamide concentration was almost 90%.

Complete elimination of free asparagine is hard to achieve because enzyme activity, the mobility of the enzyme and reactants and the availability of water are limited in dough. The limited availability and high cost of asparaginase is a constraint for this approach in limiting acrylamide formation. However, GMO-derived enzymes, which can be produced in larger quantities and more economically, will be released soon. Trials with such an enzyme were recently performed with a hazelnut biscuit on a pilot scale to check the influence of dosage and incubation time (Foot *et al.*, 2007).

Ciesarova *et al.*, (2006) evaluated the impact of L-asparaginase on the acrylamide content reduction after high heat treatment in a model system as well as in potato-based material. They found that an important mitigation (90-97%) was achieved also in products prepared from dried potato powder treated by L-asparaginase.

Organic acids: Lowering the pH by addition of organic acids in the food system to reduce acrylamide generation may attribute protonating of ?-amino group of asparagines, which subsequently cannot engage in nucleophilic addition reactions with carbonyl sources (Jung *et al.*, 2003). Potato slices dipped in citric acid solution (1-2%) for 1 hour resulted in a significant reduction (>70%) in acrylamide after frying (190°C, 6.5min). Dipping (soaking) in distilled water already provided a reduction (~25%), probably attributable to the leaching out of precursor from the disrupted cells on the surface layer.

Hydrogen carbonates: Graf *et al.*, (2006) showed that replacement of ammonium bicarbonate reduced acrylamide by 70% in semi-finished biscuits at an industrial scale. Amrein *et al.*, (2004) reported that ammonium bicarbonate strongly enhanced acrylamide formation in gingerbread and that its replacement with sodium hydrogen carbonate as a baking agent reduced acrylamide concentration by >60%.

Probiotics: Probiotics consists of specific microbial cultures and/or ingredients that stimulate gut flora capable of modifying the gastrointestinal environment which keeps the host healthy. Lactic acid bacteria and yeast are commonly used probiotics in food. Fermentation with yeast may be one possible way to reduce acrylamide content in bread by reducing free asparagines, an important precursor for acrylamide in cereal products (Konings *et al.*, 2007). Fermentation of dough by lactic acid bacteria decreases acrylamide formation in baked products by metabolizing the sugar available in dough. Huang *et al.*, (2008) reported that the addition of 0.8%yeast fermented for one hour could reduce the amount of acrylamide formed in the fried, twisted dough roll by 66.7%.

Process optimization during frying of food products

Process optimization involves control of critical processing parameters to reduce acrylamide formation. These parameters include heating temperature, heating time, temperature profile, oil type, pressure etc (Zhang and Zhang, 2007).

Temperature profile: Granda *et al.*, (2004) reported that high frying temperature significantly increase acrylamide formation in fried potatoes. Temperature below 170°C was suggested for initial frying and 150°C at final stage. However low frying temperature increases fat uptake which is not desirable. Low temperature vacuum frying and short frying time can be the alternative to reduce acrylamide formation.

Vacuum frying: Vacuum frying has advantage of using lower temperature (~130°C) for deep fat frying of foods than conventional method. Crisps with 97% acrylamide content were obtained when fried under reduced pressure of 0.079 bar (Granda *et al.*, 2004). However, texture and taste differ from the conventional crisps, at low temperatures approaching the taste of uncooked potato. Vacuum fryers have potential to produce crisps with less than 100μg/kg of acrylamide from potatoes with increased contents of reducing sugar. Par -drying and final cooking using high temperature dry steam is used by some processors to produce low fat crisps. It has been reported that this process also generates products with lower acrylamide (Foot *et al.*, 2007).

Frying time: Frying time affects the acrylamide formation in food products. Longer the frying time, higher the acrylamide content in food. The rate of acrylamide formation is not linear with increase in temperature (Pedreschi, 2009).

Moisture content in intermediate product: The water content of the semi-processed product to be fried determines the color development and acrylamide content in the final product. In the processing of potato chips, as the potato pieces dry, their moisture content decreases. However, color development and acrylamide formation only begin when sufficient drying has reached (Pedreschi, 2009).

Frying oil: The degree of saturation of fat affects acrylamide formation in fried product. Potato fried products from saturated fats tend to form less acrylamide than unsaturated ones. Becalski (2003), reported that acrylamide content in fried potato slices increased (>30%) in olive oil versus corn oil. Corn oil contains more saturated fatty acid than olive oil. The percentage of saturated fatty acid: palmitic and stearic are 10% and 4% in corn oil and 6% and 4% in olive oil.

Post- frying operation

Sorting of overheated items: There exists strong positive relationship between non-enzymatic browning and acrylamide formation in foods. So, use of color sorter in processing line reduces the acrylamide content in final product (Pedreschi, 2009).

Controlled degradation of acrylamide: Dark roasting of coffee can lead to the degradation of acrylamide but lead to the formation of undesirable products and affect taste/aroma of the product (Guenther *et al.*, 2007). Hence the methods to eliminate acrylamide formation are not advisable since they affect sensory properties of food.

Other alternatives

Microwave frying: Barutcu *et al.*, (2009) studied the effects of microwave frying on acrylamide formation in the coating part of chicken were investigated using various flour types (soy, chickpea and rice flour) in batter formulations. It was found that usage of all flour types except soy flour resulted in approximately the same moisture content and color development after 1.5 min of microwave frying. Acrylamide contents of batter parts of 1.5 min microwave fried samples having different flours were similar. Microwave frying provided lower acrylamide content and lighter color as compared to those fried conventionally for 5 min for all types of flours. This reduction in acrylamide level was the highest (34.5%) for rice flour containing batter. Pre-frying exposure to microwave resulted in a marked reduction in the formation of acrylamide at ratios of 53.77-71.88%, the AA content of fried potato strips decreased with increasing of microwave exposure time from 20 to 60 second.

Methods of detection and quantification of acrylamide in foods

Numerous methods have been used to analyze acrylamide in water and foods. The most commonly used measurement techniques for acrylamide in foodstuffs alongside other alternative methods have recently been reviewed (Wenzl *et al.*, 2003). Among the methods, gas chromatography (GC) using the 2, 3-dibromopropionamide (2, 3-BPA) derivatives and the selective and sensitive ECD are most suitable for trace level determination of acrylamide in environmental and biological samples. Most of the methods recently published are based on either GC-MS or LC-MS techniques with comparable distribution and performance. In many research LC-MS analytical methodology for simultaneous analysis of acrylamide and their precursors, such as asparagine and glucose, have been implemented with a detection limit for acrylamide of 20?g/kg, for French fries analysis (Nielsen *et al.*, 2006). Quantification is preferably based on labeled internal standards.

The addition of triethylamine makes 2, 3-DBPA completely converted to 2-BPA for determining acrylamide by GC/MS. The approach appeared to be suitable for analyzing acrylamide in food with confident recovery and a detection limit of 5?g/kg. The acrylamide contents in several Chinese foods including fried gluten, instant noodles, cup noodles, twisted cruller, old twisted cruller, and potato chips have been determined and compared with literature data (Yeh *et al.*, 2006).

The advantage of the LC-MS based methods is that acrylamide can be analyzed without prior derivatisation, which considerably simplifies and expedites the analysis. The greatest difference between the analytical methods is in the area of acrylamide extraction and cleanup, which is the crucial step. Online monitoring of acrylamide by proton transfer reaction without any sample preparation has also been reported. GC/MS and LC/MS/MS are two major detection methods for foods at detection level of 5 and 10?g/kg respectively. The LC/MS/MS is simpler and preferable due to the elimination of bromination. GC/MS has cheaper operation cost (US$ 150-200 per assay) than LC/MS/MS (US$ 200-300 per assay). Analysis using GC/MS, although not as simple and fast as that using LC/MS/MS, enables the analysis of difficult matrices, such as cocoa, soluble coffee, molasses or malt (Honeicke *et al.* 2004).

Initial difficulties with the establishment of reliable analytical methods have today in most cases been overcome, but challenges still remain in terms of the needs to develop simple and rapid test methods. The analysis of acrylamide in different foods showed that the compound has been lost during storage (Delatour *et al.*, 2004; Honeicke, 2005). Some of the data are given in Table 6.

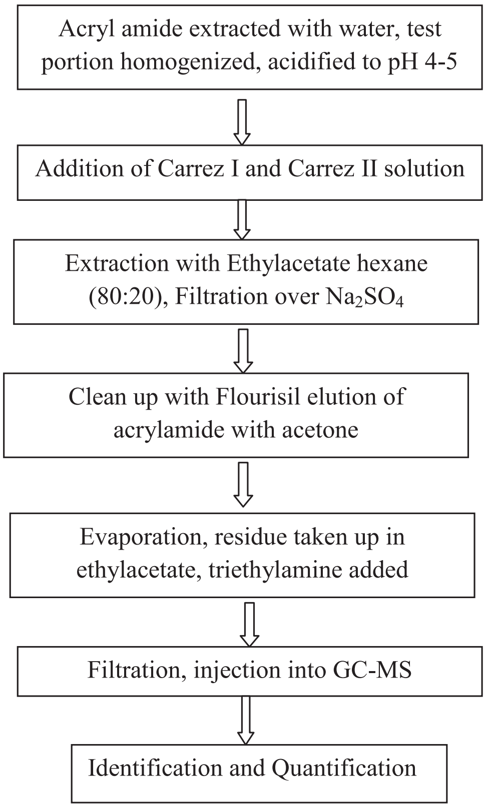


Figure 3. Steps in acrylamide analysis by GC-MS method

Toxicology of acrylamide

The dietary exposure of acrylamide varies in different countries. Urbanization and increased use of ready to serve foods has increased the use of deep fried foods due to their better taste. RfD (Reference Dose) for oral intake of acrylamide has been set. The reference dose are set on the basis of Toxicological Review of Acrylamide (AA) with comprehensive histological examinations of all major organs and tissues in the available chronic and sub chronic animal bioassays (U.S. EPA, 2010). The most sensitive observed adverse effect was identified as persistent microscopically-detected AA-induced degenerative nerve changes from lifetime exposures based on reproducible NOAELs of 0.5 mg/kg-day and LOAELs of 2 mg/

Table 6. Time-dependent stability of acrylamide in various foodstuf

Food product	Interval (month)	Acrylamide level (µg/kg)	
		Before storage	After storage
Breakfast cereal	12	238	238
Soluble coffee powder	12	771	256
Roasted barley	9	265	225
Roasted coffee	7	203	147
Dried chicory	5	214	174
Roasted chicory	5	4015	3395
Cocoa	3	180	177
Chocolate with almond	2	94	73
Soluble chocolate powder	1	54	41
Biscuit flour	3	45	50
Corn flakes	3	80	90
Crisp bread	3	760	770

kg-day in F344 male rats (Friedman *et al.*, 1995; Johnson *et al.*, 1986). There were no NOAELs for other exposure-related non neoplastic lesions that were below 5 mg/kg-day. Two chronic (2-year) drinking water studies (Friedman *et al.*, 1995; Johnson *et al.*, 1986) reported degenerative nerve changes in F344 rats, and were selected as co-principal studies to derive the RfD. Data from both studies were evaluated for dose-response characterization, and the final quantitative RfD values were established.

Acrylamide caused cancer in experimental animals where they were exposed to acrylamide at very high doses. Acrylamide causes nerve damage in people exposed to very high levels at work. FDA has not yet determined the exact public health impact, if any, of acrylamide from the much lower levels found in foods.

Acrylamide is capable of causing nerve damage in humans, including muscle weakness and impaired muscle coordination, particularly from industrial exposure to large levels of the chemical. Now, new laboratory studies suggest that chronic dietary exposure to the chemical is capable of damaging nerve cells in the brain and could potentially play a role in the development of neurodegenerative disease including Alzheimer's (LoPachin, 2008). It is noted that acrylamide is structurally similar to acrolein, a chemical found in increased levels in brains of patients with Alzheimer's and other neurodegenerative diseases.

The health effects of acrylamide were first published as the headlines of many newspapers in 2002, when scientists at the Swedish Food Administration reported that unexpectedly high levels of acrylamide, increased cancer incidence in laboratory rats. Epidemiological studies have since reported that everyday exposure to acrylamide from food substances is too low to be of carcinogenic concern, however, it is included in the list of substances of very high concern' (ECA, 2010) .

An oral reference dose (RfD) for acrylamide has been established to be 2x10-4 mg/kg/day based on a no-observed-effect-level (NOEL) of 0.2 mg/kg/day for nerve damage in male and female Fischer 344 rats exposed to acrylamide in the drinking water for 90 days (US-EPA, 2010). However, an inhalation reference concentration (RfC) has not been established yet. The International Agency for Research on Cancer (IARC) has classified acrylamide as a Group 2A carcinogen i.e. probable carcinogen to humans (IARC, 1994). The National Toxicology Program (NTP) has determined that acrylamide is reasonably anticipated to be a human carcinogen (NTP, 2005). EPA has classified acrylamide as a B2 carcinogen (probable human carcinogen). The classification is primarily based on the fact that most of the acrylamide research has been conducted on animals, and large-scale epidemiologic studies on humans are simply not available. The American Conference of Governmental Industrial Hygienists has classified acrylamide as an A3 carcinogen (ACGIH, 2008).

Codex Committee on Food Additives and Contaminants (CCFAC) has published a code of practice describing the different aspects of acrylamide. It mainly focus on how the formation of acrylamide in foods can be reduced (CAC, 2009)

How acrylamide is related to human metabolism?

Acrylamide is absorbed from all routes of exposure. Animal studies have shown that acrylamide and its epoxide metabolite glycidamide are widely distributed in all tissues of the body, including milk. The major metabolic pathway for acrylamide is qualitatively similar in humans and laboratory animals, however, quantitative differences must be considered in assessing risk for humans. Because metabolism and elimination involve pathways where there is genetic variability, there may be variation in the sensitivity of humans to the effects of ingested acrylamide. The elimination of acrylamide and glycidamide is about 2 hours in rats. Pharmacokinetic data in human are sparse (CAC, 2003).

Once ingested, acrylamide can be detoxified in the body if it is processed through our cytochrome P450 enzyme system and converted into glycidamide, or if it is hooked together with the sulfur-containing, antioxidant molecule called glutathione. Even though our metabolic pathways can help us detoxify acrylamide, however, we can still overload the detox capability of these pathways and put ourselves at health risk from excess exposure to this substance. The fact that we have detox capacity, however, makes it very likely that we can help lower our risk of problems from acrylamide if we have kept plenty of glutathione on hand in our metabolic reserves. One way to help support our glutathione supplies is to consume plenty of sulfur-containing foods (like onions, garlic, and cruciferous vegetables), and especially foods that contain significant amounts of the amino acid cysteine (Cysteine is one of the key components of glutathione.) Cruciferous vegetables like broccoli and Brussels sprouts, onions, garlic, and red peppers are plant foods that can provide higher-than-average amounts of this amino acid. Poultry, yogurt, and eggs are animal foods that have good concentrations of cysteine (GMF, 2011).

Conclusion and future trends

Acrylamide was detected in thermally processed carbohydrate-rich foods such as those prepared by grilling, roasting, baking, frying and deep-frying. The levels varied according to the food material and processing conditions. The levels of acrylamide in the commonly consumed foods such as rice, noodles, bakery and batter-based products were in general low, while higher levels were present in snack foods such as biscuits, chips and crisps.

The decreasing trend of production of rice and cereal grains since 2007 led many governments to appeal the reduction of rice intake and increase the potatoes instead. United Nations declared 2008 year as International Potato year. Potato products are loved by many people and are consumed in large quantities throughout the world. A balanced and varied diet having more fruits and vegetables should be included in daily diet to reduce the acrylamide exposure from processed foods.

The following points summarize the preventive measures of acrylamide in food and future trend for food safety.

- To minimize the risk of acrylamide in potato and cereal based food, cooking at excessively high temperature for longer time should be avoided whilst ensuring that food is fully cooked. Frying of potato chips and toasting of bread and related products should be done for shorter time to lightest color acceptable.
- Low temperature (<6°C) storage of potatoes intended for high temperature cooking should be avoided.
- Acrylamide formation in thermally processed foods is a major challenge for bakery, French fries and chip producing industries.
- Simple and rapid detection methods should be developed.
- Harmonization of reference dose in different countries should be established.

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