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ACRYLAMIDE IN POTATO CHIPS, ITS FORMATION, REDUCTION AND IDENTIFICATION: A REVIEW

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ABSTRACT:

Acrylamide is a chemical compound that can be produced at high levels in carbohydrate and aspargine rich foods when treated at high temperatures. The risks of acrylamide to health and its toxic properties (neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity) were demonstrated by the Scientific Committee on Toxicity, Ecotoxicity and the Environment in 2001. Potato and bakery products account for around 50% and 20% of human exposure to acrylamide, respectively. Factors affecting acrylamide formation and degradation in foods are acrylamide precursors such as free amino acids (mainly asparagine), reducing sugars and processing conditions (i.e. baking time and temperature, moisture content of product). The aim of this article is to aware people and makes them familiarize with detoxifying methods to reduce the level of acrylamide in products.
Key Words:*Acrylamide, Detoxification methods, Toxicity, Formation in potato chips, Detection methods*

INTRODUCTION

Potato (*Solanumtuberosum*) is one of the world's major agricultural crops and it is consumed daily by millions of people from diverse cultural backgrounds. Potatoes are always cooked before consumption traditionally by frying and other cooking methods (Pedreschi et al., 2006). Deep fat frying is extensively used in food processing both industrially and at home and fried potato products are one of its largest applications (Pedreschi et al., 2007).

Acrylamide is a chemical compound that is formed from food components during heat treatment (frying, baking, roasting and extrusion) as a result of the Mailard's reaction between asparagine and reducing sugars (Pedreschi et al., 2004). It is also known as food processing contaminant as these are generated during the processing of foods (e.g., heating, fermentation). They are absent in the raw materials, and are formed by chemical reactions between natural and/or added food constituents during processing. The presence of these contaminants in processed foods cannot be entirely avoided. Technological processes can be adjusted and/or optimized, however, in order to reduce the levels of formation of processing contaminants. Examples are:nitrosamines, polycyclic Aromatic Hydrocarbons (PAH), Heterocyclic amines, Histamine, Acrylamide, Furan, Benzene, Trans fat, 3-MCPD, Semicarbazide, 4-hydroxynonenal (4-HNE), and Ethyl carbamate.

There is also the possibility of metal chips from the processing equipment contaminating food. These can be identified using metal detection equipment. In many conveyor lines, the line will be stopped, or when weighing the product with a Check weigher, the item can be rejected for being over- or underweight or because small pieces of metal are detected within it.

The highest acrylamide levels have been found in fried potato products, bread and bakery wares and coffee. The difference in the concentration of precursors (free asparagine and reducing sugars) in raw materials, pH, moisture content, frying oil, difference in food composition and in process conditions applied can easily explain the observed variability (Boon, de Mul, van der Voet, van Donkersgoed, Brette and van Klaveren, 2005, Ciesarova et al., 2006). Moreover, the actual acrylamide content of a food as it is eaten can largely vary according to domestic cooking conditions.

Children eat more acrylamide than adults probably because of their higher caloric intake relative to body weight as well as their higher consumption of certain acrylamide-rich foods, such as French fries and potato crisps (Dybing, et al., 2005) Heudorf, et al., (2009). Acrylamide starts to form at a temperature>100°C after an initial lag phase during which no acrylamide forms. Later on, the acrylamide concentration increases exponentially with time to a maximum concentration, after which it can decrease again because of the exhaustion of one of the reactants and/or by the elimination of acrylamide.

Acrylamide shows a variety of adverse effects in animals and humans. It is known to be neurotoxic (causing peripheral neuropathy) in humans and a reproductive toxic agent in rodents (Tritscher, 2004). Acrylamide is positive in a number of tests for genotoxicity, inducing chromosomal aberrations, micronuclei, sister chromatid exchange, polyploidy, aneuploidy and other mitotic disturbances in mammalian cells in the absence of metabolic activation (Tritscher, 2004).

The World Health Organization estimates a daily dietary intake of acrylamide in the range of **0.3–2.0 µg/kg body wt** for the general population and **up to 5.1 µg/kg body wt** for the 99th-percentile consumers **(WHO, 2005)**.

History:

In 2002, Swedish researchers have first reported the formation of acrylamide in foods processed at elevated temperatures (Tareke et al. 2002). Presence of acrylamide in common heated foods has been considered an important food safety issue by international authorities.

Acrylamide has been classified as probable carcinogenic to human by the International Agency for Research on Cancer (IARC 1994). Several researchers have established that the main pathway of acrylamide formation in foods is linked to the Maillard reaction and to the presence of free asparagine (ASN)(Mottram et al., 2002; Stadler et al., 2002; Stadler et al., 2004; Zyzak et al., 2003).

Chemistry of acrylamide:

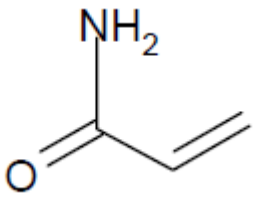
AA is a small hydrophilic small molecule (Anon, 1991). It is an odorless solid and its color ranges from colorless to white (Table 1). AA is generally formed from the hydration of acrylonitrile with sulphuric acid between 90-100 °C or by catalytic hydration using a copper catalyst. It is soluble in a number of polar solvents, e.g. acetone, acetonitrile and water. AA is susceptible to polymerisation during heating, which prevents the determination of boiling point at ambient pressure. At 3.34 kPa (25 mm Hg), it boils at 125 °C. It is regarded as a thermally unstable compound.

Physical parameters of acrylamide:

Parameter	Specification
Chemical formula	C3H5NO
Molecular weight	71.08 g /mol
Melting point	84 – 85 °C
Solubility	216g / 100 g water at 30 °C
Boiling point	125 °C at 3.34 kPa
Vapor pressure	0.007 mm Hg at 20°C
Vapor density	(Air = 1) 2.4 at 175 °C
Specific gravity	1.1222 kg/dm3 at 30 °C

AA possesses two functional groups, amide group and the electron-deficient vinylic double bond that makes it readily available for a wide range of reactions, including nucleophilic and Diel-Alder additions and radical reactions.

Structure:



Acrylamide in food:

AA is found in a wide variety of food products, with the major contribution originating from cereal-based products, potato-products and coffee. Among cereal products, the main contribution is from pastries and biscuits, processed cereal products and breads.

The contribution of each individual product varies between countries depending on food habits among many other factors. Coffee and green tea is among the products with a high AA content, as are cocoa products. Milk products, fish and seafood are examples of products that are found at the lower end of AA content range. The content in the different foods shows a wide range of variation. Consequently, the average exposure rate differs not only between countries but also among age groups.

Levels of acrylamide found in survey of 2002:

Acrylamide (µg/kg)					
Potato products		**Cereal products**		**Others**	
Raw/boiled	<30	Wheat flour	<30	Coffee (as drunk)	420
Baked	190	Bread	30	Dry roasted peanuts	30
Chips	310	Toast	75	Chicken nuggets	23
French fries	480	Breakfast cereal	180	Hamburgers	15
Crisps	1050	Popcorn	400		
		Rye crisp bread	2000		

*Coultate, T.P. (2009) Food. The Chemistry of its Components, RSC Publishing, Cambridge

Toxicity:

1. **Neurotoxicity:**

Neurotoxicity, characterized by ataxia, distal skeletal muscle weakness, and numbness of the hands and feet, is currently the only documented outcome in occupationally exposed human populations (Deng et al., 1993; Garland and Paterson, 1967; He et al., 1989; Spencer and Schaumburg, 1974a). In laboratory animal models, AA intoxication (10-50 mg/kg/d) produces neurological signs that, in many respects, resemble the neurotoxicity occurring in humans; i.e., ataxia (open field gait abnormalities), skeletal muscle weakness (decreased fore- and hindlimb grip strength) and hindlimb foot splay (Burek et al., 1980; Crofton et al., 1996; Edwards and Parker, 1977; LoPachin et al., 2002b; Moser et al., 1992; Shell et al., 1992; Tilson and Cabe, 1979). Studies of AA-exposed laboratory animals (primarily rodents) have also revealed an increased incidence of tumors in certain tissues (e.g., mammary gland fibroadenomas in female rats, tunica vaginalis mesotheliomas in male rats; e.g.. Bull et al., 1984a,b; Friedman et al., 1995; Johnson et al., 1986).

Nonetheless, the obvious health implications of food-borne AA has initiated substantial public and scientific concern (World Health Organization meeting June, 2002; US FDA meeting September, 2002) and has significantly increased interest in the toxic effects of AA. Since neurotoxicity has demonstrated relevance to human exposure, we thought it was important to discuss recent advances in related neurological and morphological research.

In particular, new evidence will be reviewed, which suggests that the **nerve terminal is a primary site of AA action and that inhibition of neurotransmission contributes significantly to the development of corresponding neurological deficits.** The work in this area suggests that **AA disrupts membrane fusion processes** that mediate Neurotransmission and membrane turnover in nerve terminals. AA is a neurotoxicant in both humans and laboratory animals.

2. **DNA alterations:**

AA is **biotransformed in vivo to its epoxide glycidamide**, which has been shown to be genotoxic in a variety of *in vitro* and *in vivo* test systems (Rice, 2005). The AA metabolite glycidamide has the ability to form DNA adducts, which account for a genotoxic and cancer risk increasing agent (Törnqvist, 2005). It has been demonstrated that AA is transformed to its metabolite glycidamide through the Hb adducts in both animals and humans. It is the glycidamide that gives rise to detectable DNA adduct levels in rodents exposed to AA.

The maximum tolerable level of AA is reported to be 0.5 µg AA/litre of water. AA is also found in tobacco smoke, resulting in smokers having higher exposure levels than non-smokers (Abramsson-Zetterberg, 2003; Bergmark, 1997; Jägerstad& Skog, 2005; WHO, 2003). An overall evaluation of the carcinogenicity of AA reported that 'AA and its metabolite glycidamide form covalent adducts with DNA in mice and rats; AA and glycidamide form covalent adducts with haemoglobin in exposed humans and rats; **AA induces gene mutation and chromosomal aberration in germ cells of mice and rats** and forms covalent adducts with protamines in germ cells of rodents *in vivo* (IARC, 1994).

3. Carcinogenicity:

In studies on the cancer risk for the large bowel, bladder or kidney, no excess cancer risk could be related to dietary AA (Dybing&Sanner, 2003; Mucci et al., 2003). On the other hand, an **increased risk of postmenopausal endometrial and ovarian cancer** has been observed with high levels of dietary AA, mainly among non-smokers, a kind of association not found with breast cancer (Hogervorst et al., 2007).

Acrylamide is classified by the International Agency for Research on Cancer (IARC) as category 2A "probably carcinogenic to humans ". Animal studies showed that acrylamide can induce an **increased incidence of cancer of the brain, the central nervous system, the thyroid and other endocrine glands as well as reproductive organs of mice**. A lifelong feeding of upto 3 mg/kg body weight/day to rats increased the incidence of tumors in several organs. The metabolite glycidamide was identified as the major carcinogen in rodents. Friedmann pointed out that it has to be elucidated if these carcinogenic manifestations can be transferred to humans. The research on the mechanisms of carcinogenesis showed that acrylamide and glycidamide are able to modify DNA both in vitro and in vivo. Acrylamide reacts with DNA, forming adenosine- and cytosine-adducts. **Concerning the reactivity with DNA, it was shown that glycidamide was 100 - 1000 times more reactive than acrylamide.**

4. Reproductive toxicity:

In **spermatides** of rats and mice **lethal mutations induced** by acrylamide were reported. Thus, acrylamide is classified as **mutagenic**. Furthermore, the feeding of water solutions of acrylamide to rats during breeding, gestation and lactation, led to disruptions in mating, interference with ejaculation, decreased food intake and body weight gain, decreased pup body weight at birth and weight gain during lactation. Feeding of neurotoxic doses to rats results in reproductive toxic effects: formation of abnormal sperm, decreased sperm count, reduced fertility rates and increased resorption of fetuses. The mechanisms of reproductive toxicity are assumed to be the alkylation of SH-groups in the sperm nucleus and tail, depletion of glutathione and DNA-damage in the testis.

Formation of Acrylamide:

1. Maillard Reaction:

The reaction between sugar and amino group leading to browning formation and later on indicated as Maillard Reaction is still at the very centre of the interest of scientists of different disciplines (Maillard, 1912). Among food scientists Maillard Reaction (MR) is important because of color and flavor formation in an enormous variety of processed foods; the major concern arising from heating processes come from the formation of hazardous compounds, the so-called food-borne toxicants i.e. compounds that are not naturally present in foods, but that may be developed during heating or preservation and that reveal harmful effects such as mutagenic, carcinogenic and citotoxic effects.

Acrylamide (AA) is a water-soluble, vinyl monomer that has multiple industrial applications: e.g., waste water management, ore processing. Given this toxic potential, significant concern was expressed when a Swedish research group recently (April 2002) announced preliminary findings of significant AA concentrations in certain potato or grain-based foods (e.g., 3500 |ig AA /kg potato chips) that had been prepared at high temperatures (> 160^^0). These early data were later confirmed by the Swedish group (Tareke et al., 2002) and by other researchers in Europe and America (e.g., Rosen and Hellenas, 2002; Sanders et al., 2002).

Shortly after its discovery in foods, it has been clearly established that the major pathway for acrylamide formation in foods is Maillard reaction with free asparagine as main precursor (Mottram, Wedzicha and Dodson, 2002; Stadler et al., 2002; Zyzak et al., 2003; Stadler et al., 2004). Asparagine can thermally decomposes by deamination and decarboxylation but when a carbonyl source is present the yield of acrylamide from asparagine is much higher explaining the high concentration of acrylamide detected in foods rich in reducing sugars and free asparagine such as fried potatoes and bakery products (Mottram, et al., 2002; Yaylayan, et al., 2003; Weisshaar and Gutsche, 2002).

2. Pyrolysis:

Recent evidence suggests that AA in food is generated from pyrolytic fragments of asparagine and that this reaction is facilitated by concomitant pyrolysis of Maillard-active dicarbonyl and hydroxycarbonyl precursors (Becalski et al., 2002; Sanders et al., 2002). Based on the AA content of various foods, it has been estimated that the average consumer is exposed to approximately 0.8 - 3 μg AA/kg BW/day (FAO/WHO report 2002).

However, it is important to emphasize that the toxicological risk of this daily AA intake has not been established in humans. The loss of thermo labile compounds such as vitamins as well as essential amino acids (lysine, triptophane) and/or the formation of undesired tastes and off-flavors are well established phenomena bringing about a loss in the nutritional value and sensorial quality of heated foods.

3. Other minor reactions:

Other minor reaction routes for acrylamide formation in foods have been postulated, from acrolein (Yasuhara, et al., 2003), from acrylic acid (Yasuhara, et al., 2003) and from wheat gluten (Claus, et al., 2006).

Finally, acrylamide can be generated by deamination of 3-aminopropionamide (3-APA) (Granvogl and Schiberle, 2006). 3-APA is an intermediate in MR, can also form by enzymatic decarboxylation of free asparagine and can yield acrylamide upon heating even in absence of a carbonyl source (Granvogl, et al., 2004; Granvogl and Schiberle, 2007).

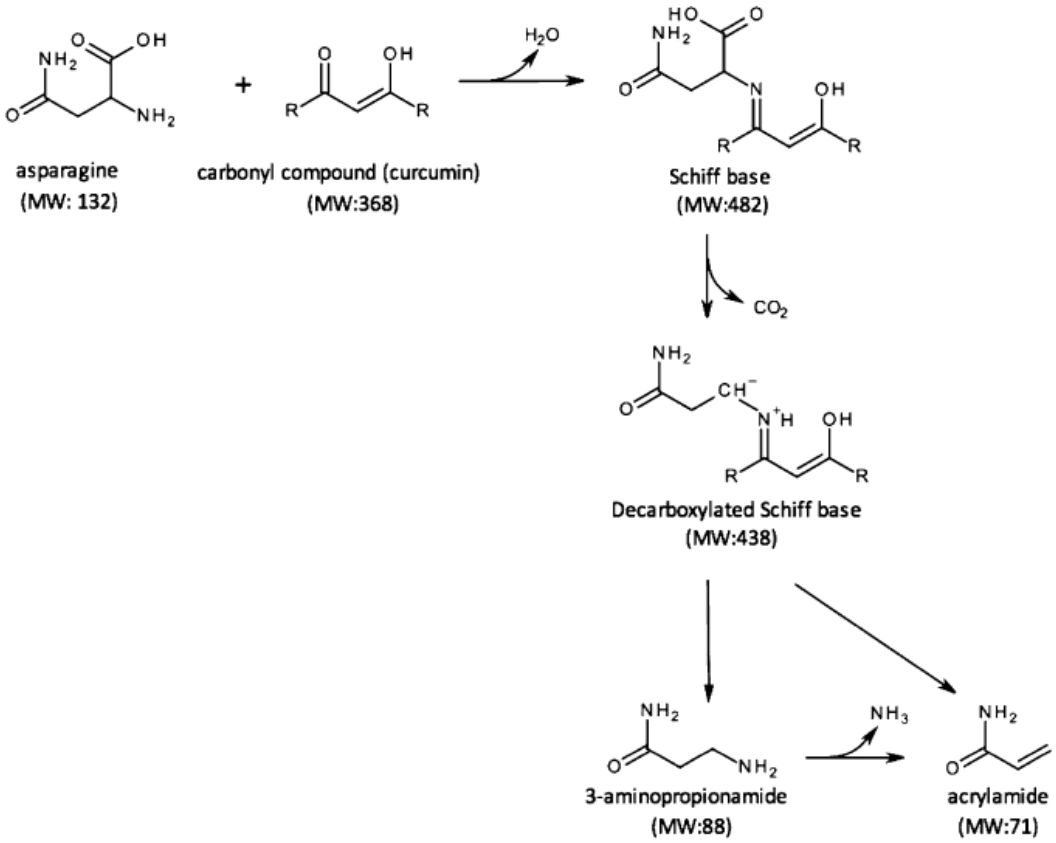


Figure: Proposed mechanism for the contribution of CUR on acrylamide formation from ASN

Regulations of Acrylamide according to WHO:

- **LD_{50} (Median Lethal Dose):** 150mg/kg body weight
- **NOAEL (No Observed Adverse Effect Level)** for chronic toxicity and carcinogenic studies: 0.5mg/kg body weight

Detoxification methods/Pre-treatments:

1. **Increasing peel removal may help reduce acrylamide(***Peeling***)**: Abrasive peeling is typically used in the potato chip industry. Peeling chipping potatoes more than what is typical for chip preparation can decrease acrylamide levels in the finished chips because reducing sugar levels may be higher near the peel (Refs. 26-27, 41). Trials of hot steam peeling as an alternative to abrasive peeling indicate that it may increase acrylamide levels in potato chips (Ref. 41).
2. **Washing or soaking potato chips before frying may help reduce acrylamide, but this may be helpful only for some chips(***Washing/Soaking/Blanching***)** Potato slices typically are washed in ambient temperature water before frying or they may be blanched optionally. Water can extract

acrylamide precursors from potatoes, thereby decreasing acrylamide production in fried slices. Prolonged soaking and higher temperature conditions lead to greater extraction of acrylamide precursors and decreased production of acrylamide. However, excessive soaking can affect organoleptic qualities (e.g., taste, mouth feel). The SFA concluded that washing potato slices with warm water reduces acrylamide levels to a modest extent, but also significantly affects quality and operations costs, while the Acrylamide Toolbox concluded that washing has had variable success in chips in laboratory and pilot trials, but that blanching is not a desirable mitigation technique because it leads to loss of flavor and increased oil uptake.

3. **Cutting fries in shapes with lower surface area to volume ratio and screening out small fragments may help reduce acrylamide(***Cutting):* Acrylamide formation is typically higher in the surface layer or crust of foods than in the inside; therefore, decreasing product surface area relative to volume may decrease acrylamide in cooked products. For frozen french fries, cutting thicker strips or shapes with lower surface area may reduce acrylamide. Cutting potato rings that avoid inner core material may also lower acrylamide compared to straight cut fries. Smaller or thinner potato pieces have a greater surface area to volume ratio than larger pieces, and produce more acrylamide on a volume basis. Screening to remove nubbins (short strips) and slivers (thin strips) may reduce acrylamide. Optimization of cutting practices may be useful in limiting production of small fragments.
4. **Cutting thicker potato chip slices may help reduce acrylamideL(***Slicing*): Cutting thicker slices for potato chips may result in lower acrylamide levels. Typical slice thickness may range from 0.04 to about 0.08 inches. One manufacturer reported success cutting flat slices from 0.068 to 0.072 inches and ridge-cut slices from 0.085 to 0.095 inches. Slicers allow control of slice variation to 0.010 inch.
5. **Changing blanching practices may help reduce acrylamide, although such changes may affect product quality(***Blanching):* Manufacturers of frozen french fries routinely blanch raw potato strips in hot water or steam before par-frying. Blanching can provide more uniform color after frying, inactivate enzymes, and form a layer of gelatinized starch that limits oil absorption and improves fry texture. Blanching also removes reducing sugars and asparagine from the potato surface, decreasing acrylamide in fries. However, excessive blanching can make potato products, especially potato chips, unacceptably light in color and cause flavor loss. Modifications to blanching processes, such as changes in time and temperature, may help reduce acrylamide, although such modifications may affect product quality. For example, one research study found that shorter blanching times at high temperatures were more effective at acrylamide reduction than longer low-temperature blanches .
6. **Using sugar dips to reduce variability may help reduce acrylamide, but using reducing sugars such as fructose in dips may increase acrylamide(***Dips and coatings):*For foodservice fries, blanching may be followed by dipping in a dextrose solution to replace sugars removed during blanching and provide uniform coloration. Initial recommendations called for avoiding the use of sugar solutions as a browning agent or a coating. However, french fries produced by blanching and dipping in a dextrose solution may show less variability in acrylamide levels and reduced acrylamide compared with fries that rely on naturally present sugars for color flavor and development. Less variability can simplify identifying treatments that mitigate acrylamide. Dipping with fructose should be avoided.
7. **Decreasing frying temperatures to no higher than 175 °C and targeting higher moisture endpoints may help reduce acrylamide, but it is important to determine if moisture endpoints provide acceptable product quality (***Thermal input*): Higher temperatures and longer cooking times will increase acrylamide formation in potato chips. In particular, acrylamide formation increases at the end of the frying process, as moisture content falls, e.g., below 3 percent.

   One approach to decreasing thermal input is to decrease frying temperatures, while increasing the amount of time chips are in the fryers. Some U.S. potato chip manufacturers have adopted recommendations to set fryer temperatures no higher than 175 °C (347 °F). Resulting chips have higher

moisture levels and lighter color than previous chips. Frying at lower temperatures (e.g., below 170 °C) may cause higher fat uptake and affect crispness.

Moisture levels in finished products are an important consideration. Targeting higher moisture endpoints (1.3 to 1.5 percent) can result in reduced acrylamide. Moisture levels that are higher (e.g., greater than 1.5 %) may affect flavor, texture, and shelf life, though some of these effects may be offset by other changes, such as in packaging. When evaluating proposed mitigation techniques, it is important to consider the moisture level of finished products. In some cases, mitigation techniques may appear to lower acrylamide, while actually raising product moisture to an unacceptable level (e.g., one that increases the rate at which products will become stale).

8. **Using alternative coloration methods may help reduce acrylamide by discouraging over-baking.** One approach used by manufacturers to reduce acrylamide formation in oven-baked frozen french fries is to add a food-grade coloring agent such as annatto. The darker color may improve product appearance and discourage over-baking by the consumer, which can increase acrylamide levels.
9. **Using SAPP may help reduce acrylamide, as may evaluating other dip or batter ingredients to determine if they contribute to acrylamide formation during frying.** Addition of sodium acid pyrophosphate (SAPP) during blanching or in a dip after blanching is standard industry practice to prevent darkening of uncooked potato fry strips. SAPP treatment also reduces acrylamide, presumably by acidifying the surface of potatoes. However, SAPP may cause off flavors at levels higher than current industry usage. Current industry usage is approximately 0.5 to 1.0 percent SAPP.

   Some french fries are dipped before frying in batter coatings containing flours, starches, hydrocolloids, or other ingredients to improve texture, flavor, structure, and heat retention. Some batters may reduce acrylamide formation, but Codex recommends examining batter ingredients to ensure there are no ingredients that can increase acrylamide formation in final fried products.
10. *Treatment with cations*4. Treatment of potato strips (french fries) with calcium salts or sodium chloride before frying decreased acrylamide significantly in laboratory studies, but treatment with calcium lactate gave poor results on an industrial scale. Calcium use may cause hard texture and off tastes in fries, and may not be compatible with SAPP use. Manufacturers may be reluctant to add sodium for nutritional reasons.
11. ***Treatment with acidulants*:**Acidulants (acetic acid, ascorbic acid, citric acid, monosodium citrate, sodium citrate, lactic acid, lactic acid bacteria) effectively suppress acrylamide formation in laboratory model systems and in fried potato products, including french fries. However, they can also cause a sour or tart taste, suppress development of other flavors, and potentially cause corrosive effects on production equipment. In a manufacturing trial, treatment with citric acid and acetic acid did not provide additional acrylamide reduction compared with standard practices.
12. ***Treatment with amino acids*:** Amino acids6 (e.g., glycine, lysine, cysteine, taurine) reduced acrylamide in potato model systems and in potato pieces and potato slices in laboratory studies. However, glycine reportedly caused excessive browning and bitter flavor in finished potato products and was ineffective when tested on french fries in laboratory studies. The amino acid cysteine reportedly caused unpleasant odors in potato model systems.
13. ***Other ingredients*:** Some studies have identified a number of other ingredients that reduced acrylamide formation in laboratory studies in french fries or other potato products, including plant extracts, hydrocolloids, vitamins, antioxidants, and spices. The efficacy of these compounds in finished food products is not clear. For new ingredients, it is important to consider such factors as impact on moisture content, sensorial quality, nutritional quality, regulatory status, and potential formation of byproducts.
14. **Adding calcium salts to potato doughs may help reduce acrylamide in fabricated potato products:** (e.g., 0.3 percent calcium lactate, 1 percent calcium chloride) to doughs is effective in reducing acrylamide in fabricated potato snacks. Reductions in acrylamide of 20 to 80 percent in

various potato-based snacks and fabricated potato chips have been reported. Calcium addition may be more effective at an acidic pH. Potential problems, particularly when calcium is in excess, include off flavors and changes in texture and color.

15. **Addition of Different Fresh Leaves with into Frying Sunflower Oil**
    Guava, rosemary, oregano, olive, cranberry and green tea leaves were added into sunflower oil during frying process at level (5%).
    Addition of fresh leaves into frying oil significantly influenced acrylamide formation.
    Oregano, rosemary, bamboo, guava and olive leaves caused the greatest reductions.
16. **Effect of spice extract on formation of acrylamide:**
    The extracts of spices (pimento, black pepper, marjoram, and oregano) were applied before the heat treatment to a model mixture simulating Addition of the extracts resulted in a different reduction of acrylamide contents (up to 75%)with pimento extract and 50% with black pepper extract in comparison with the control a fresh potato matrix.
17. **Reduction and comparison by using vitamins :**

Biotin, pyridoxine, pyridoxamine, and L-ascorbic acid exerted a potent inhibitory effect (>50%) on acrylamide formation in the chemical model system. Using the food model, it was shown that water-soluble vitamins are good inhibitors of acrylamide formation Effects of pyridoxal, nicotinic acid, and L-ascorbic acid were further examined using fried potato strips. Nicotinic acid and pyridoxal inhibited acrylamide formation in fried potato strips by 51% and 34%, respectively.

Detection/Quantification methods:

Identification and quantification of acrylamide by different analytical techniques:

1. HPLC-UV (High performance Liquid Chromatography-Ultra violet)
2. HPTLC-ESI//MS Conditions (High Performance Thin Layer Chromatography-
3. LC-MS/MS(Liquid Chromatography-Mass Spectrometry)
4. GC (Gas Chromatography)
5. HPLC with pre-column ultraviolet derivatization
6. RP-HPLC-DAD (Reverse Phase- High Performance Liquid Chromatography-Diode Array Detector)

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