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Review Article
Protective Role of Natural Antioxidants Against the Formation and Harmful Effects of Acrylamide in Food

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Abstract
Acrylamide has been listed in the list of substances harmful to human health transmitted by processed foods to the body since 2002, the Swedish National Food Service has found an appropriate amount of acrylamide in many thermo-treatment, carbohydrate-rich foods such as potato chips, coffee and bread, after that many studies investigated the causes of acrylamide formation in foods and the critical levels that the consumer should not be exposed. The formation of acrylamide in food depends on the product type, ingredients and cooking method. Acrylamide was found fried potato, hamburger, fried chicken and many other foods. Acrylamide is known as neurotoxic and carcinogenic compound. Many studies investigated the effect of natural antioxidants and lactic acid bacteria (LAB) in reduce the formation of acrylamide during food processing. Lactic acid bacteria were reduced the level of acrylamide in potato chips by fermentation the sugar on the surface of potato before frying. The natural antioxidant also plays an important role in eliminating the harmful effect of acrylamide in the body by increasing the activity of antioxidant enzymes and reduction of free radical level. Therefore, this study focused on acrylamide formation, how to reduce its formation during food preparation and how to face its negative effects on the body by consuming nutritious foods.

Key words: Acrylamide, natural antioxidants, fried foods, lactic acid bacteria

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INTRODUCTION

Food is important for the individual's life as it provides energy and several essential nutrients. Any edible food is a complex mixture of compounds, most of them are beneficial, protective and essential for life. Some are harmful components, which may interact with each other or with other food constituents. These harmful compounds are introduced to such edible food because of pollution by some environmental elements or as a result of heat treatment of foods to produce such compounds. Parts of them are important for the sensory properties such as color, flavor and aroma of the heated products due to maillard reaction which is a complex reaction between amino compounds (principally amino acids) and reducing sugars. Some of these compounds may not be beneficial or may be even toxic to humans like acrylamide. Acrylamide is traditionally known as an industrial chemical substance whose major use is to produce poly acrylamide, a coagulant used in drinking water and waste water treatment[1]. Concern about the health of the tunnel workers encouraged specialists to conduct studies which led to find that acrylamide was carcinogenic in rats and a neurotoxin in occupationally-exposed workers. These findings led a group of scientists to develop a test that measured the presence of an acrylamide protein adduct in blood. The investigators not only found this adduct in the exposed tunnel workers, but also among members of the general population that had not known occupational exposure to acrylamide[2]. Acrylamide has been classified as a group 2A carcinogen by the International Agency for Research on Cancer and a Category 2 carcinogen and Category 2 mutagen by the European Union. This finding has caused worldwide concern[3]. In 14 April, 2002 the Swedish National Food Administration (NFA) and researchers from Stockholm University reported that acrylamide is formed in many types of foods prepared or cooked at high temperatures. The highest levels were found in potato and cereal-based products subjected to heat processing such as frying, grilling or baking. Long-term exposure to acrylamide might cause damage to the nervous system both in humans and animals to a certain extent and acrylamide was also considered as a potential genetic and reproductive toxin[4-6]. Since the subject of the study has a great concern at local and international levels. The aim of this study emerged through the expected identify the average of acrylamide intake among individuals around the world. Also, increase awareness of acrylamide dangers on health and how to reduce its formation in foods, improving food and nutritional concepts and behaviors in consumers.

DISCOVERY OF ACRYLAMIDE IN FOODS

In April, 2002, the Swedish National Food Administration (SNFA) and the University of Stockholm together reported that processed carbohydrate-rich foods that are fried or baked at relatively high temperature may contain considerable levels of acrylamide[7,8]. Potato products (such as; potato chips, French fries and hash browns), bread, biscuits and cereal were among the food items containing the highest amounts of acrylamide[7]. Acrylamide levels rise very strongly with time duration of heat processing as the extent of cooking time affects the degree of formation as 10-20 fold increase in acrylamide levels which have been reported between cooked and over-cooked fried potatoes. In contrast, acrylamide formation has not been demonstrated at temperatures below 120°C as a temperature >100°C is required for its formation[9,10]. This formation of acrylamide is primarily the result of the Maillard reaction between the amino acid asparagines and sugars such as; glucose and fructose[11]. Foods that are rich in these two elements are considered precursors, which become major sources of acrylamide when cooked. They are derived from products of vegetable origin, such as potato, cereals, peanuts, lentils and asparagus, but apparently not from products of animal origin[12].

Acrylamide levels in food: Potato products such as French fries, chips, roasted and baked potatoes are characterized by high levels of acrylamide. In particular, french fries which may contain more than 2000 μg kg^{-1} a level which is considered human carcinogen compound. Also, acrylamide contents in the same food differ according to cooking methods[13,14].

The European Food Safety Agency reported that acrylamide is formed during frying, roasting and baking but low contents are found in boiled or microwave foods[15]. The highest acrylamide levels have been found in fried potato products, bread, bakery wares, coffee, hazelnuts and almonds. On the other hand, acrylamide is found in foods not subjected to severe heating like olive and also dried fruits e.g., plums, pears and apricots[16,17]. Animal derived heat treated foods such as; meat and fish, generally exhibit low or negligible levels of acrylamide[15].

Ingredients play an important role in acrylamide formation as different ingredients have various amounts of free asparagine and reducing sugars available for the formation reaction. White wheat flour has a low content of free asparagine, but certain ingredients used in baking, such as germ and bran contain significant amounts[18,19]. Therefore, different levels of acrylamide may be present in a wide range, depending on the product type and ingredients. However, in other dishes fried potato of containing breaded fish, hamburger or fried chicken may not reach such values of acrylamide. A possible explanation for this result may be the use of different potato varieties. The boiled rice contained less than 50 μg kg^{-1} but was increased to 113 μg kg^{-1}, when various ingredients were added to produce risotto. Also values of less than 50 μg kg^{-1} were reported for simple tomato sauce but it was increased to 124 μg kg^{-1}, when other ingredients such as olives and capers were added[20].

Possible mechanisms for acrylamide formation in foods: A number of theories have been proposed for the mechanism by which acrylamide (AA) is formed in food. Asparagine needs a carbonyl compound to be converted to acrylamide. The carbonyl can come from multiple sources[21].

Due to the diversity of antioxidants in structure, properties and complexity of the reactions, different antioxidants are involved in different reactions during the process of Maillard reaction thus causing different effects towards acrylamide formation. It seems that whether the carbonyl is available in both A and B pathways depends on the functional group in the β-position to the nitrogen atom. The presence of a hydroxyl group favors the re-arrangement from azomethine yield to the decarboxylated Amadori product to afford acrylamide (pathway A). The α, β, γ, δ-diunsaturated carbonyl group and α-dicarbonyl groups may play a key role in pathway B. As for the carbonyl group, aldehydes are more reactive than ketones. Also, a pathway named acrolein pathway (pathway C) stating that acrolein and asparagine as well as acrylic acid (oxidized acrolein) and ammonia produced a significant amount of acrylamide. This pathway might be marginal, since the nitrogen atom of acrylamide comes from asparagine and other carbonyl compounds such as reducing sugars are way more reactive with asparagine that it's hard to spare nitrogen atom to acrolein in foodstuffs[22].

Major acrylamide (AA) formation pathway: The major pathway for acrylamide formation in food is known as the asparagine route via Maillard reaction[11]. This non-enzymatic browning reaction influences several aspects of food quality such as flavor, color and aroma formation. In this N-glucosides route, asparagine is converted to acrylamide through thermal decarboxylation and deamination, which necessarily needs the presence of a carboxyl compound (such as; a reducing sugar)[23]. Fructose, which contained two α-hydroxycarbonyl groups increases acrylamide formation by about 2-fold compared with other reducing sugars such as glucose[24-26]. Asparagine has been proved to provide the backbone chain of acrylamide by mass spectral studies demonstrated that all the three carbon atoms and the nitrogen atom are from asparagine. Although asparagines alone may release acrylamide by thermally initiated decarboxylation and deamination in the presence of reducing sugars, acrylamide formation from asparagine is significantly increased. Asparagine alone produced acrylamide (0.99 μg g^{-1} of asparagine) upon thermal degradation, while glutamine produced 0.17 μg g^{-1} under the same conditions. When asparagine was heated at 180°C with glucose, a large amount of acrylamide (1200 μg g^{-1} of asparagine) was formed. The potential of generating acrylamide from suitable precursors has mainly been attributed to the concentration of asparagine, which directly provides the backbone of the acrylamide molecule[27]. The type of sugar or in general the carbonyl compound may significantly affect the final amount of acrylamide generated through the Maillard reaction[11]. For example that fructose was, by a factor of about 3, more efficient compared to glucose under the same pyrolytic conditions[28]. The acrylamide formation was enhanced when the ratio of glucose to asparagine was greater than or equal[29] to 1.

Minor acrylamide formation pathway: Other minor reaction routes have been proposed for acrylamide formation, such as from ammonia and acrolein in the absence of asparagines, a pathway that was suggested to play a role in lipid rich foods[22]. Asparagine and glutamine are found abundantly in wheat, corn and oats contribute to non-enzymatic browning of these grains by release of ammonia. Both of these amino acids are abundant in potatoes[30,31]. Acrolein can be formed by oxidative lipid degradation or from glycerol, leading to acrylic acid. Acrylic acid can react with ammonia to form acrylamide[32]. However, this pathway does not seem to be involved in acrylamide formation in fried potatoes[10]. It was also reported that oil degradation products such as glycerol, mono and diacylglycerols had no significant impact on acrylamide formation in a potato model system and French fries[33]. Acrylamide formation has also been proposed from protein pyrolysis in dry heated wheat gluten[34].

SOURCES OF HUMAN EXPOSURE

Exposures to acrylamide may occur via the inhalation, oral or dermal routes. The potential routes of human exposure to acrylamide are ingestion, dermal contact and inhalation[35]. Acrylamide can be absorbed through unbroken skin, mucous membranes, lungs and the gastrointestinal tract[36].

Human dietary intake of acrylamide: In the USA, most of dietary AA comes from potato chips, french fries, breads, cereals, biscuits/cookies, fried pastries and other salty snacks[37]. In European countries, more than half of AA exposure comes from consumption of bread, crisp bread, rusks, coffee and potatoes[38]. The resulting intake estimates ranged from 0.8 µg kg^{-1} b.wt./day for the average consumer to 3 µg kg^{-1} b.wt./day for the 95th percentile consumer and 6.0 µg kg^{-1} b.wt./day for the 98th percentile consumer[9]. The general agreement of the several methods used to estimate exposure using well described food consumption data from Australia and Norway. The Netherlands, Sweden, USA and from the IARC EPIC study indicated a lower bound estimate of typical exposures in the range of 0.3-0.8 µg kg^{-1} b.wt./day depending upon whether the average or median exposure is estimated and which age groups were evaluated. According to exposure assessments, the daily intake of AA of diet is approximately 0.4 µg kg^{-1} b.wt., with a 90th percentile of 0.95 µg kg^{-1} b.wt.[39]. Median intakes in children and adolescents are higher due to lower body weight and different consumption patterns and worst-case intake scenarios of up to 6.9 µg kg^{-1} b.wt./day have been discussed[40]. It was found that the simulated daily exposure to acrylamide for males and females with mean exposures which were 0.052 and 0.064 µg kg^{-1} b.wt./day, respectively[41]. In this scenario, females are exposed to slightly greater amounts of acrylamide. This may be due to the averaging of total acrylamide intake over respective body weights (i.e., males, in general, weigh more than females). The exposure level is significantly lower than that estimated when eating commercially produced French fries (0.27 and 0.20 µg kg^{-1} b.wt./day for males and females, respectively as given in Cummins[42]. This is mainly due to the greater exposure levels and portion size for potato French fries compared to potato crisps. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated the mean dietary acrylamide intake for general population including children, between 1 and 4 µg kg^{-1} b.wt./day. It was noted that children have dietary acrylamide exposures at least twice as high as for adult consumers when expressed on a body weight basis[43]. The EFSA also recently performed an exposure assessment based on acrylamide monitoring results from 2007-2008. The 95th percentile of acrylamide intake for adults (>18 years) and children (3-10 years) were estimated to range between 0.6-2.3 and 1.5-4.2 µg kg^{-1} b.wt./day for adults and children, respectively[44]. Foods contributing the most to dietary intake would differ from country to country, according to different dietary patterns and the way how foodstuffs are processed and prepared. The mean daily acrylamide consumption found was 0.534 µg kg^{-1} b.wt./day, which is similar to that found for other European countries such as Germany, Norway, France, Sweden and Belgium in studies carried out in different population segments[40]. The adolescents from a Canadian urban Center that median total daily intake of acrylamide was estimated at 0.29 µg kg^{-1} b.wt./day and upper 97.5th percentile value was found to be 2.85 µg kg^{-1} b.wt./day, which is approximately 5-fold greater than median intake[45]. Average contribution of main food groups to total daily acrylamide intake as estimated with the 2-day food dairy were: deep-fried French fries had the highest contribution representing 50% of total daily acrylamide intake. The mean exposures as well as the 95th percentile were calculated for general population[46]. The dietary acrylamide exposure in the Chinese population from each food group in the 12 provinces in 4 total diet study (TDS) regions. The acrylamide total intakes of 12 provinces ranged from 0.056-0.645 µg kg^{-1} b.wt./day with overall average of 0.286 µg kg^{-1} b.wt./day. Another study showed that the daily intakes corresponding to a 1×10^{-5} upper-bound risk level are estimated to be 1 and 9 µg/person, respectively for high and average consumers (assuming a default body weight of 75 kg)[47].

EFFECT OF ACRYLAMIDE ON HEALTH

Neurotoxicity: Acrylamide is a well-known neurotoxic compound that produces central and peripheral distal axonopathy. Peripheral neuropathy and hemoglobin adduct formation have been seen in occupationally exposed humans and experimental animals to acrylamide[48-50]. Neurotoxicity of acrylamide results from high levels of exposure and concerns workers occupationally exposed to acrylamide through inhalation or dermal absorption[51]. Longer exposure resulted in more severe symptoms, including cerebellar dysfunction followed by neuropathy[36]. Long-term consumption of low levels of acrylamide contributed to the causes of Alzheimer's or other degenerative diseases of the human brain. In rat and mice studies, No observable Effect Level (NOEL) for neurotoxic effect has been estimated ranging from 0.2-10 mg kg^{-1} b.wt./day and is far above dietary exposure[51]. However, it has been postulated that neurotoxicity of acrylamide might be cumulative and thus that dietary exposure might not be negligible[52]. *In vivo* proteomic and in chemico studies demonstrated that acrylamide forms covalent adducts with highly nucleophilic cysteine thiolate groups located within active sites of presynaptic proteins and the resulting protein inactivation disrupts nerve terminal processes and impairs neurotransmission[53]. In addition, since acrylamide can bind to sulfhydryl groups on cysteine residues of proteins and causes nerve terminal degeneration and inhibition of axonal transport, it is possible that acrylamide is associated with some neurodegenerative diseases[54].

Carcinogenicity: Several studies in rodents supported the evidence that acrylamide is a multi-organ carcinogen being able to cause tumors to many organs such as; lung, uterus, skin, mammalian gland and brain, etc[55]. Although acrylamide is clearly carcinogenic in experimental animals, epidemiological studies on individuals exposed occupationally to acrylamide have not indicated any consistent dose-related increase in cancer at any organ site, with the possible exception of the pancreas ovaries and endometrium[55,56]. It is widely accepted that the carcinogenicity of acrylamide would stem from its conversion in mammalians to glycidamide which has been shown to be mutagenic and genotoxic *in vitro* and *in vivo* test systems[57,58]. The acrylamide-induced DNA adduction and consequent mutagenesis has been postulated as the key process in acrylamide carcinogenicity although the chain of events starting from glycidamide DNA adducts formation and leading to mutation has not been directly investigated (The first evidence of acrylamide and glycidamide inhibition of a mitotic/meiotic motor protein and speculated) that this could be an alternative mechanism to DNA adduction in the production of cell division defects and potential carcinogenicity[59,60]. Also, a hormonal effect in rat endocrine (thyroid) and mammary glands has also been postulated for carcinogenicity of acrylamide. A prospective epidemiological study has found that increased dietary intake of acrylamide is associated with increased risks of post-menopausal endometrial and ovarian cancer particularly among neversmokers[61]. This was further supported by a large prospective cohort study among women in the U.S.[56]. They observed that risk for endometrial cancer and possibly ovarian cancer was greater among high acrylamide consumers. In addition, a positive association between dietary acrylamide intake and renal cell cancer risk was observed in a prospective cohort study[62]. Moreover, one case-control study reported that consumption of French fries during preschool years was associated with a slightly increased risk of breast cancer later in life[63]. There was a positive association between acrylamide-hemoglobin levels and estrogen receptor positive breast cancer[64]. On the other hand epidemiological studies indicated that dietary acrylamide intake is not associated with other cancer risks, prostate cancer, gastrointestinal cancer, bladder cancer, thyroid cancer, brain cancer and lung cancer. Generally, epidemiological studies have provided valuable information regarding the associations between dietary acrylamide intake and cancer in human. It is suggested that more epidemiological studies are needed to substantiate the evidence[65-68].

Reproductive and developmental toxicity: Acrylamide has also been reprotoxic in rodents and mutagenic in somatic cells *in vitro* and *in vivo* as well as in germ cells *in vivo*[69-72].

The reproductive and developmental toxicity of acrylamide has been reported in laboratory animals. When mice or rats were treated with exogenous acrylamide, reproductive and developmental toxicity manifests as disrupted mating, reduced fertility rates, increased reabsorption of fetuses, reduced litter size in pregnant females, abnormal sperm and decreased sperm count in males[73]. The molecular mechanisms of reproductive toxicity are associated with chromosomal damage, alkylation of SH groups, depletion of GSH and/or DNA damage[59]. Nevertheless, there is no information as to the potential for reproductive and developmental effects of acrylamide in humans[73].

FACTORS AFFECTING THE ACRYLAMIDE FORMATION IN FOODS DURING PREPARATION

There are numerous factors affecting acrylamide formation whether by reducing or increasing its content in foods during preparation. It has been documented that most if not all the acrylamide found in foods is formed during preparation. Its intake is harmful to the body due to its carcinogenic effect. Therefore; it is crucial to avoid or reduce its content during preparation. Many effective methods regarding the reduction of acrylamide were found in the actual food matrixes. A number of mitigation strategies to reduce the acrylamide content in foods have been proposed and tested. They have been mainly focused on potato products and cereal-based products that are two of the major contributors to dietary exposure in most of the populations. Conversely, only very limited process options for coffee products are available for reducing acrylamide levels without affecting the final quality[74].

The mitigation approach referred to certain steps which include changing in recipes and formulations. This included for example selection of foods such as potato varieties and cereal species or varieties low in acrylamide precursors. Also, it includes addition of some nutrients or compounds such as proteins, amino acids like glycine, cysteine, organic acids and acidulants, calcium ions, cyclodextrin, natural antioxidants or antioxidant extracts etc, replacement of reducing sugars with sucrose and of ammonium bicarbonate with sodium bicarbonate. In addition variations in preparation can be followed like changing in process conditions and/or cooking methods including changing of time, temperature of frying or baking, changing the type of oven, prolonged fermentation of bakery products etc. Also, the removal of acrylamide after formation by means of vacuum has also been proposed, but its impact on manufacturing practices and food quality has not yet been clearly established[75]. One of the most promising tools to control acrylamide content in heat treated foods is the addition of the enzyme asparaginase. The enzyme asparaginase (L-asparagine amidohydrolase) is an enzyme able to catalyze the hydrolysis of asparagine to aspartic acid and ammonia thus lowering the content of asparagines as a precursor. Asparaginase has been successfully applied at lab scale both to potato products and cereal-based products with percentage of reduction up to 85-90% with no effect on products taste and appearance[11]. This has been used for some products at industrial scale. Some preliminary results achieved at lab scale highlighted that asparaginase pretreatment of green beans may represent a viable way to reduce acrylamide concentration in roasted coffee as well. Nevertheless, the high cost of the enzyme may represent a serious constraint on its application on a large scale[76-78].

It has been also emphasized that some mitigation strategies are associated with an increase in other risks or a loss in benefits. For example, replacement of ammonium bicarbonate with sodium bicarbonate as rising agent for fine bakery products results in an increase of sodium intake[79]. Some of the mitigation strategies that have been proposed and bring about changes in organoleptic properties of foods like excessive browning as result of glycine addition, generation of off-flavors, insufficient browning as a result of changing in time temperature profile etc. This can dramatically affect the final quality and consumer's acceptance[78,80].

Generally, all potential strategies to prevent acrylamide formation may be resumed in two major approaches, removal of the acrylamide precursors or interference with the Maillard reaction, which is essential for the desired and characteristic flavor and color formation of food products like potatoes[81]. Therefore, this constitutes the first challenge for food scientists on how to reduce acrylamide formation without affecting the final product specifications and its quality. Numerous researches have been performed which demonstrated the necessity of a farm-to-fork approach in order to reduce acrylamide like in fried potato products. Several relevant aspects regarding acrylamide formation should be considered at different stages of producing food products including methods of treatment and type of additives[81].

Influence of food size: Acrylamide is formed in the surface layer of the food like potato product and therefore, the size and the cut shape of the product (surface-to-volume ratio) will influence the final acrylamide contents. Accordingly, thinner and smaller cut sizes result in increased acrylamide formation upon final frying[82]. Larger French fries ("pont-neuf") 20$\times$20 mm contained somewhat less acrylamide, which is probably related to the lower proportion of the crust on the total mass[83]. These differences are compared to 7$\times$7 mm French fries which are small, but larger pieces require longer frying (prolonged pre- frying). However, thinner pieces (such as 6$\times$6 mm) are more difficult to fry to the adequate point, since few seconds of overtime are sufficient to initiate general browning and shooting acrylamide formation. Fine pieces from the outer part of the potato are a problem since they reach optimum frying earlier than the bulk rapidly getting brown and contain acrylamide in excess[83] of 1000 μg kg^{-1}.

Influence of washing and soaking in water: Washing and soaking the food may decrease acrylamide formation during heat treatment. There are quite significant changes that water soluble components, such as glucose and fructose are washed out from the external layer of the potato. Matthaus *et al.*[84] reported a reduction in acrylamide formation of 69% following soaking at 50°C for 15 min. The glucose content was decreased by 32% in potato slices soaked for 90 min and 25% for 40 min in distilled water. Other reducing sugars such as fructose and sucrose followed a similar trend. Asparagine content tended to remain unchanged even after a 90 min soak[85]. Acrylamide formation was reduction by 50% in French fries following soaking in distilled water[86] for 1 h and frying at 190°C.

Influence of temperature: Temperature has an effect on acrylamide formation in foods as requires temperatures[3] $\geq$120°C. In addition, acrylamide synthesis is mainly limited to the surface of the heated material[87]. When beef patties (commercially available as frozen hamburgers) were fried (without addition of oil) at temperatures of 180, 200, 220 and 240°C for 3 min on each side, a significant dependence of acrylamide formation on temperature was demonstrated[8]. In raw hamburgers the acrylamide content was below the detection level (5 μg kg^{-1}). Acrylamide was absent (below the detection limit of 10 μg kg^{-1}) in fresh potatoes. Acrylamide content in potatoes that were oven-dried for 72 h at 50°C was only 50 μg kg^{-1} of dry weight. In contrast, at all applied frying temperatures (120-230°C) acrylamide concentrations above 1000 μg kg^{-1} were produced, with considerably higher levels in the grated potatoes (max. 18000 μg kg^{-1}).

Accordance with studies (model reaction conditions)[88]. Acrylamide is formed in the last moments of a frying, roasting or baking process. With an initial temperature of 180°C, optimum French fries were obtained after 4-5 min. After 6 min, the local browning at the ends spread over the whole pieces accompanied by a steep increase in the acrylamide content from about 80-320 µg kg^{-1}. At 170°C the comparable optimum product was obtained after 6 min. The acrylamide content was as low as 40 µg kg^{-1}. The general browning and the rapid increase in acrylamide content started only 2 min later[83]. The large variations in mean concentrations of acrylamide in deep-fried French fries purchased in restaurants or home-prepared. Acrylamide contents were found to be higher with a 10 min cooking at around 190°C compared to a 2.5-3 min cooking[45] at 170-177°C.

Influence of time: The length of time affects acrylamide formation. The highest levels of acrylamide were present after heating for 150 sec (mean 4400 µg kg^{-1} of heated product) in microwave. A heating time of 100 sec resulted in ~100 time's lower levels[8]. Another study investigated the effect of time (20, 30, 40, 50 and 60 min) on the generation of acrylamide at a heating temperature of 170$\pm$3°C and found that 30 min were optimal in terms of formed acrylamide yield. The yield of acrylamide decreased with further increase in the duration of heat treatment[89].

Influence of cooking method: The level of acrylamide was below the detection level (5 µg kg^{-1}) of boiled beef-potato, boiled fish as well as in broth from the boiling tests. When heating carbohydrate rich foods relatively higher contents of acrylamide 150-1000 µg kg^{-1} were measured. After microwave-heating of fish and potato no detectable levels of acrylamide were found in the fish. In French fried potatoes and potato crisps exhibited relatively high levels of acrylamide (median values of 424 and 1739 µg kg^{-1}, respectively)[8]. Toasting of soft bread produced a moderate formation of acrylamide (increasing from 13-53 µg kg^{-1}). Analyses of fried potato revealed acrylamide contents at 10-100 times higher levels than in the protein-rich foods. Frying as a method of cooking dramatically affect acrylamide levels of the products as they are eaten, since acrylamide is actually formed during this last step. Simultaneously to acrylamide formation, browning, texture and flavor development caused by the Maillard reaction equally occur during frying. Therefore, acrylamide formation is correlated to color development given that both are linked to the Maillard reaction in addition to applied frying conditions (time and temperature) that have the same effect in similar manner. Intense frying conditions (time and temperature) lead to darker fries and higher acrylamide contents[90,91]. On the other hand, frying at lower temperatures (below 140°C) results in increased frying time and enhances fat uptake[82]. Thus, frying time and oil temperature should be controlled in order to avoid high acrylamide levels meaning that they should not exceed 170-175°C. Lower temperatures towards the end of the process may reduce acrylamide formation. So, the product/oil ratio may influence a drop of initial frying temperature and therefore longer frying periods would be needed but these results in higher acrylamide contents. The real oil temperature towards the end of frying depends on the amount of potato added to a given amount of oil. Most acrylamide is formed in the last moments of the frying process, when the surfaces are sufficiently dried and heated. The cool potato and water evaporation extract a large amount of heat from the oil. On adding 100 g potato to 1 L of oil (10%), the temperature typically drops by 20-35° depending on the fryer[83]. The heating immediately starts but is unable to substitute the consumed energy i.e., the regulation of the oil temperature fails in the moment that is most relevant for acrylamide formation. With 200 g L^{-1} (20%) temperature usually drops from 170 to below 130°C with the effect that French fries tend to be soft and oily. The relevant temperature for acrylamide formation depends on the initial oil temperature, thermal mass of the fryer, its heating capacity, heat transfer characteristics of the oil and the amount of potato related to the amount of oil. The type of oil used for frying was investigated on acrylamide formation in a potato model system and French fries. Some studies indicated that palm oil relatively to rapeseed and sunflower oils generated higher acrylamide contents[92]. Other authors reported that the oil type did not influence acrylamide in the final product[84,93]. Moreover, consideration to oil oxidation and hydrolysis products which have been proposed as possible acrylamide precursors, but they are negligible in acrylamide formation in fried potato products[33]. Temperature and time of frying process have been shown to be significant factors affecting the amount of acrylamide formed in potato foods as well as the development of desirable aroma components. The use of low temperature of frying (under 180°C) reduces the concentration of acrylamide produced, but quality characteristics of the product like texture, color and oil contents are negatively affected and consequently the acceptability of the product by the consumer is deeply reduced[14].

Influence of protein source: In a previous study, samples of fish fillet (cod), lean beef, lean pork, chicken fillet and soy flour (soaked in water) were heated in a fry pan for 2.5 min on each side at 220°C without addition of oil[8]. The results showed that different protein-rich foods exhibited different acrylamide concentrations ranging between 5 and 50 μg kg^{-1} with lower levels in fish. The adding of different relative amounts (0-100%) of cod fish meat mixed with grated potato and then heated in a frying pan or oven revealed a decrease in acrylamide content by 70% at equal amounts of potato and fish. This reflected a protective action of protein by elimination of forming acrylamide via reaction with nucleophilic groups (-SH,-NH_2) on the amino acid side-chains[94].

Effect of pH: At pH of ~8, the α-amino group of asparagines is protonated to a lower degree than is the case for the other amino acids (glutamine, glycine, lysine and alanine). At relatively high pH values, asparagines are more prone to react with electrophiles and aldehyde groups in carbohydrates resulting in Schiff base formation. Which is the initial step in the formation of acrylamide. This explained the comparatively high levels of acrylamide obtained upon addition of asparagines or carbohydrates. When citric acid was added to homogenized potato (Cv. Rosor) prior to heating in the oven at 180°C for 25 min, the acrylamide content was reduced[87].

ADDITION OF NATURAL SOURCES OF ANTIOXIDANTS

Antioxidants are presented in this section in some details as in this study they are related to the section concerned with trials to reduce the acrylamide content in foods during preparation and also in the section concerned with the biological experiment.

Eggplant peels: Eggplant (*Solanum melongena* L.) a common vegetable grown in the subtropics and tropics is consumed throughout the world not only as a kind of tasty food but also with underlying medical value. The whole eggplant fruit possessed antioxidant activities and is ranked amongst the top 10 vegetables in terms of antioxidant capacity due to its high content of anthocyanin, which is a major phenolic in eggplant[95,96]. This compound is the most important antioxidants with a variety of physiological functions such as anti-mutagenesis, anticancer and vision improvement[97,98].

Antioxidant activities and phenolic compounds are found also in both the pulp and skin of eggplant[99].

Green tea: Green tea extract has been used in various food applications such as bread, dehydrated apple products and biscuits as well as various meats products[100-103]. Green tea extract effectively reduced the formation of acrylamide and achieve a maximum reduction rate of (74.3%) due to its content of flavonoids which have superior antioxidant properties largely due to the phenolic hydroxyl groups in their chemical structures[104,105]. The lowest concentration of acrylamide was obtained by submerging the potatoes in green tea extract before frying, achieving a 62% reduction of the toxin. The fried potatoes treated with the green tea extract presented the lowest concentration of acrylamide (118 mg kg^{-1}) indicating a reduction of 62%, which was significantly different from that in the control potatoes. Also the concentration obtained with the potatoes treated with ascorbic acid which showed a reduction of 61%. These results are encouraging since there are currently no reports on the use of green tea extract to decrease acrylamide in fried potatoes. However, some publications have already mentioned the effect of green tea on the formation of the toxin[106]. For example, the acrylamide level was a reduction in bread by adding the aqueous extract of green tea at different concentrations achieving a reduction of 72.5% with a concentration of 0.1 g kg^{-1} of the tea. Using green tea in the soaking water of potato chips reduced 14, 14 and 31% of the acrylamide formation after 20, 40 and 60 min, respectively[107,108].

Onion peels: Onion skins contained significantly higher content of flavonoids than the edible portion by around 2-10 g kg^{-1}. Onion peel contained flavonoid levels that are 48 fold on that in its flesh and cell-based investigations found that the peel has a greater capacity for controlling lipid peroxidation than the flesh does[109].

Further, dry onion skin has a different configuration of quercetin derivatives compared to fleshy scales were as much as 53% of total quercetin is present in the free form[110]. The major flavonoids found in dry peel of onion that has usually been considered as waste contain large amounts of quercetin, quercetin glycoside and their oxidative product which are effective antioxidants against the lethal effect of oxidative stress[111,112]. Also, they reported that they have a liver protective effect, immune enhancement potential, anti-infection, anti-stress, anti-cancer and other pharmacological properties[113].

Rosemary: Rosemary, a spice with a known antioxidant property had been proposed as one effective inhibitor of acrylamide. It was shown that acrylamide contents were lowered in fried potato slices when rosemary herb was added to either the corn or olive oil[10]. It was found that the added of aqueous rosemary extract, rosemary oil and dried rosemary leaves to a bread model and found that the content of acrylamide was reduced by 62, 67 and 57%, respectively compared to bread without rosemary[114]. Also, the added of rosemary extract to the frying oil significantly reduced the acrylamide content by up to 38%[115]. That rosemary extract has exceptional carry-over protective effects for deep-fried food, because of its high heat stability. The effect of rosemary extract and the other antioxidants on the reduced formation of acrylamide during frying might be ascribed to different mechanisms although mainly to the radical scavenging activity that prevents hydroperoxide formation and thus secondary oxidation products formation by stabilizing radicals formed during the induction and propagation steps of lipid oxidation. Using rosemary in the soaking water of potato chips reduced 11, 48 and 24% of the acrylamide after 20, 40 and 60 min, respectively[108].

Sage: The main antioxidant effect of Sage has been reported to relate to the presence of phenolic diterpenes (such as carnosic acid and carnosol) and especially rosmarinic acid[116]. The addition of sage retarded lipid oxidation in Chinese-style sausage[117,118]. Sage leaves can be considered a source of powerful antioxidants which can be used as a substitute for synthetic additives in food production process in order to prevent or delay oxidative deterioration of food products[119]. Sage has been reported to have excellent activities in scavenging radicals, reducing metal ions and inhibiting lipid peroxidation[120]. The phenolic compounds such as carnosol, carnosic acid and rosmarinic acid in the plant may account for the antioxidant activity of sage. Some researchers have reported that sage or sage extracts can effectively retard lipid oxidation of muscle foods[121]. Adding sage to the soaking water of potato chips reduced 7, 14 and 36% of acrylamide after 20, 40 and 60 min soaking, respectively[108].

Black berry: Black berry (*Morus nigra*) contains large amounts of flavonoid pigments (anthocyanins) that give blackberries their characteristic red to blue color[122]. Many studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables[123,124]. Berry flavonoids are best known not only for their anti-oxidant and anti-inflammatory action, but the research also has shown that their action extends to impacting on cell signaling mechanisms[125]. Polyphenolics included several classes of phenolic compounds that are secondary plant metabolites and an integral part of both human and animal diets. Interest in food phenolics has increased greatly because of their antioxidant capacity (free radical scavenging and metal chelating activities) and possible beneficial roles in human health such as; reducing the risk of cancer, cardiovascular disease and other pathologies[126]. Acrylamide increased thiobarbituric acid reactive substances (TBARS) while decrease the activities of GSH and antioxidant enzyme (Catalase, SOD, GST and GPX) in liver, kidney, brain, lung and testes in rats while the oral intake of black berry decreased the risk of acrylamide[108,127].

Bioactive milk peptides and lactic acid bacteria: Peptides generated from the digestion of various proteins are reported to have antioxidant activities. Studies with peptides contained histidine have demonstrated that these peptides can act as metal-ion chelators, active-oxygen quencher and hydroxyl radical scavenger. The ability of protein hydrolysates to inhibit deleterious changes caused by lipid oxidation appears to be related to the nature and composition of the different peptide fractions produced, depending on the protease specificity[128]. Antioxidant peptides contained of 5-18 amino acid residues. Antioxidative peptides from foods are considered safe and healthy compounds with low molecular weight, low cost, high activity and easy absorption. They have some advantages in comparison to enzymatic antioxidants, that is with simpler structure and having more stability in different situation and no hazardous immunoreactions[129]. The peptides generated from the digestion of milk proteins are reported to have antioxidant activity. These peptides are composed of 5-11 amino acids including hydrophobic amino acids, proline, histidine, tyrosine or tryptophan[130]. Treatment with milk bioactive peptides improved activities of antioxidant enzymes (catalase, superoxide dismutase, reduced glutathione, glutathione-S-transferase and glutathione peroxidase) in healthy and diabetic rats[131]. Casein hydrolysate exerted (Tyr-Phe-Tyr-Pro-Glu-Leu) scavenging activity towards the superoxide anion, hydroxyl radical and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. It is suggested that the casein-derived Glu-Leu sequence is important for this radical scavenging action thus the primary structure of the protein plays a role in determining the antioxidant activity[132]. Also, hydrolysates of WPI have been shown to possess AO activity. About 5 h digestion with alcalase produced a hydrolysate with strong reducing power (Ferric reducing antioxidant power FRAP). When fractionated on the basis of molecular mass, the low molecular weight fraction (0.1-2.8 kDa) was most potent[133]. A corolase PP digest of β-Lg produced the most potent peptide (f19-29, Trp-Tyr-Ser-Leu-Ala-Met-Ala- Ala-Ser-Asp-Ile). Synthetic β -Lg f19-29 had a higher radical scavenging ability than BHA (2.62 μmol Trolox/μmol peptide vs. 2.43 μmol Trolox/μmol BHA). The Antioxidant (AO) activity was attributed to the presence of tryptophan (Trp), Tyr and Met residues in the peptide. The radical scavenging ability of another β -Lg peptide (f42-46, Tyr-Val- Glu-Glu-Leu) was compared to an equimolar mixture (Tyr + Val + 2(Glu) + Leu) of amino acids and the peptide was more potent (0.8μmol Trolox/μmol peptide vs. 0.4 μmol Trolox/μmol amino acid mixture). This suggested that in some instances the peptide bond or structural conformation of the peptide can enhance AO activity[134]. Lactic acid bacteria convert reducing sugars in vegetables to lactic acid thus lowering pH. Previous studies have shown that lactic acid fermentation of potato reduces sugar levels amounts of Maillard products including acrylamide, consequently burnt taste and color of deep fried potato products. Several lactic acid bacteria (LAB) (e.g., Lactococcus lactis, Lactobacillus helveticus) have been reported to release bioactive peptides by the process of fermentation. Despite of the important potential of this process strategy in acrylamide reduction, its application is not very occurred. This system consisted of a number of distinct intracellular peptidases including endo-peptidases, amino-peptidases, di-peptidases and tripeptidases. From these studies it can be concluded that, bioactive peptides from dairy product could be reduce the harmful effect of acrylamide in human body[135].

EFFECT OF NATURAL ANTIOXIDANTS OF ACRYLAMIDE FORMATION

Antioxidants could inhibit acrylamide formation in high-temperature processed foods through three ways. The first one is to destruct the formed acrylamide by their oxidized products. The second one is forming the quinones or carbonyl compounds such as vitamin C which then reacts with the main precursor of acrylamide and asparagine. The inhibition effect depends on that they are easily oxidized and the rate of oxidation and their oxidized products react with asparagine. The third one is inhibiting production of carbonyl compounds produced from frying oil[136].

Previous study, examined the capacities of 15 vitamins in reducing the formation of acrylamide. Inhibitory activities of the water-soluble vitamins were tested in both chemical models containing acrylamide precursors (asparagines and glucose) and a good model system (fried snack products) while the activities of fat soluble vitamins were examined only in the latter model. 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. On the other hand, only weak inhibitory effects were observed with fat-soluble vitamins. 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. Thus, certain vitamins at reasonable concentrations can inhibit the formation of acrylamide[88].

Also, a similar phenomenon occurs in ascorbic acid cases at low concentrations (0.2, 0.5, 0.8 and 1.2%). Ascorbic acid inhibited acrylamide formation with a highest inhibitory rate of 58%, but the increase of ascorbic acid concentration to 1.5% promoted acrylamide formation[137] by more than 90%. Such findings suggested that the effect of an antioxidant towards acrylamide is not only determined by the type of the antioxidants but also its concentration, which makes the case more complicated. But, this may partly explain the different behaviors of the same antioxidant among studies, since the applied concentrations were mostly different. Acrylamide level was significant reduction following the addition of caffeic and ferulic acid[138]. On the same side, several antioxidant compounds, including tert-butylhydroquinone (TBHQ), butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), ferulic acid and vitamin C showed a reduction or elimination of acrylamide[136]. Also using of eggplant peel in the soaking water of potato chips before frying reduced 24, 46 and 28% of the acrylamide after soaking for 20, 40 and 60 min, respectively[108].

CONCLUSION

Acrylamide was found in many types of foods arrowed the world. Acrylamide formation in foods depended on the type of product, ingredients and cooking method. Natural antioxidants are useful in reduction the formation of acrylamide during foods processing. My studies investigated the vital effect of antioxidant from natural sources in reduction of acrylamide formation during manufacturing or cooking of foods. The natural antioxidant also plays an important role in eliminate the harmful effect of acrylamide in the body by increasing the activity of antioxidant enzymes and reduction of free radicals level. Antioxidant could be reduced the acrylamide risks by three ways, (1) Destruct the formed acrylamide, (2) Formed the quinones or carbonyl compounds such as vitamin C, which are precursor of acrylamide and asparagine and (3) Inhibiting production of carbonyl compounds produced from frying oil.

SIGNIFICANCE STATEMENT

This study discovered the important role of antioxidant from natural sources in reduction of acrylamide formation in foods that can be beneficial for the production of more healthy foods. This study will help the researchers to uncover the critical areas of production of foods with a very low dose of acrylamide that many researchers were not able to explore. Thus a new theory on reduction the acrylamide level in fried or other foods with low cost natural antioxidant may be arrived at.

REFERENCES

1. Skog, K. and J. Alexander, 2006. Acrylamide and Other Hazardous Compounds in Heat-Treated Foods. Woodhead Publishing Limited and CRC Press LLC., USA.
2. Rosen, J.D., 2002. Acrylamide in food. Is it a real threat to public health? American Council on Science and Health, USA.
3. EC., 2002. Opinion of the scientific committee on food on new findings regarding the presence of acrylamide in food. July 3, 2002. European Commission, Belgium.
4. Kapp, C., 2002. WHO urges more research into acrylamide in food. Lancet, Vol. 360. 10.1016/S0140-6736(02)09378-9.
5. LoPachin, R.M., J.F. Ross, M.L. Reid, S. Das, S. Mansukhani and E.J. Lehning, 2002. Neurological evaluation of toxic axonopathies in rats: Acrylamide and 2,5-hexanedione. Neurotoxicology, 23: 95-110.
6. WHO., 2002. FAO/WHO consultation on the health implications of acrylamide in food. Summary Report, Geneva, June 25-27, 2002. World Health Organization, Geneva, Switzerland.
7. SNFA., 2002. Swedish National Food Administration: Analysis of acrylamide in food. Swedish National Food Administration, Sweden.
8. Tareke, E., P. Rydberg, P. Karlsson, S. Eriksson and M. Tornqvist, 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem., 50: 4998-5006.
9. FAO. and WHO., 2002. Health implication of acrylamide in food. The final report of a joint FAO/WHO consulation. FAO. and WHO., Geneva, Switzerland, June 25-27, 2002.
10. Becalski, A., B.P.Y. Lau, D. Lewis and S.W. Seaman, 2003. Acrylamide in foods: Occurrence, sources and modeling. J. Agric. Food Chem., 51: 802-808.
11. Zyzak, D.V., R.A. Sanders, M. Stojanovic, D.H. Tallmadge and B.L. Eberhart *et al*., 2003. Acrylamide formation mechanism in heated foods. J. Agric. Food Chem., 51: 4782-4787.
12. Friedman, M. and C.E. Levin, 2008. Review of methods for the reduction of dietary content and toxicity of acrylamide. J. Agric. Food Chem., 56: 6113-6140.
13. Gokmen, V., T.K. Palazoglu and H.Z. Senyuva, 2006. Relation between the acrylamide formation and time-temperature history of surface and core regions of French fries. J. Food Eng., 77: 972-976.
14. Pedreschi, F., K. Kaack and K. Granby, 2006. Acrylamide content and color development in fried potato strips. Food Res. Int., 39: 40-46.
15. EFSA., 2009. Scientific report of EFSA prepared by data collection and exposure unit (DATEX) on "Monitoring of acrylamide levels in food". The EFSA Scientific Report No. 285, pp: 1-26.
16. Amrein, T.M., H. Lukac, L. Andres, R. Perren, F. Escher and R. Amado, 2005. Acrylamide in roasted almonds and hazelnuts. J. Agric. Food Chem., 53: 7819-7825.
17. Amrein, T.M., L. Andres, F. Escher and R. Amado, 2007. Occurrence of acrylamide in selected foods and mitigation options. Food Addit. Contaminants, 24: 13-25.
18. Fredriksson, H., J. Tallving, J. Rosen and P. Aman, 2004. Fermentation reduces free asparagine in dough and acrylamide content in bread. Cereal Chem., 81: 650-653.
19. Fink, M., R. Andersson, J. Rosen and P. Aman, 2006. Effect of added asparagine and glycine on acrylamide content in yeast leavened bread. Cereal Chem., 83: 218-222.
20. Tateo, F., M. Bononi and G. Andreoli, 2007. Acrylamide levels in cooked rice, tomato sauces and some fast food on the Italian market. J. Food Compos. Anal., 20: 232-235.
21. Jin, C., X. Wu and Y. Zhang, 2013. Relationship between antioxidants and acrylamide formation: A review. Food Res. Int., 51: 611-620.
22. Yasuhara, A., Y. Tanaka, M. Hengel and T. Shibamoto, 2003. Gas chromatographic investigation of acrylamide formation in browning model systems. J. Agric. Food Chem., 51: 3999-4003.
23. Granvogl, M. and P. Schieberle, 2006. Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. J. Agric. Food Chem., 54: 5933-5938.
24. Yaylayan, V.A., A. Wnorowski and C.P. Locas, 2003. Why asparagine needs carbohydrates to generate acrylamide. J. Agric. Food Chem., 51: 1753-1757.
25. Granvogl, M., M. Jezussek, P. Koehler and P. Schieberle, 2004. Quantitation of 3-aminopropionamide in potatoes a minor but potent precursor in acrylamide formation. J. Agric. Food Chem., 52: 4751-4757.
26. Eriksson, S., 2005. Acrylamide in food products: Identification, formation and analytical methodology. Ph.D. Thesis, Department of Environmental Chemistry, Faculty of Science, Stockholm University, Stockholm.
27. Stadler, R.H., I. Blank, N. Varga, F. Robert and J. Hau *et al*., 2002. Food chemistry: Acrylamide from Maillard reaction products. Nature, 419: 449-450.
28. Pollien, P., C. Lindinger, C. Yeretzian and I. Blank, 2003. Proton transfer reaction mass spectrometry, a tool for on-line monitoring of acrylamide formation in the headspace of Maillard reaction systems and processed food. Anal. Chem., 75: 5488-5494.

29. Kalita, D., D.G. Holm and S.S. Jayanty, 2013. Role of polyphenols in acrylamide formation in the fried products of potato tubers with colored flesh. Food Res. Int., 54: 753-759.
30. Riha, 3rd, W.E., H.V. Izzo, J. Zhang and C.T. Ho, 1996. Nonenzymatic deamidation of food proteins. Crit. Rev. Food Sci. Nutr., 36: 225-255.
31. Eppendorfer, W.H. and S.W. Bille, 1996. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilisation and phosphorus and potassium deficiency. J. Sci. Food Agric., 71: 449-458.
32. Yaylayan, V.A., C.P. Locas, A. Wnorowski and J. O'Brien, 2005. Mechanistic Pathways of Formation of Acrylamide from Different Amino Acids. In: Chemistry and Safety of Acrylamide in Food, Friedman, M. and D. Mottram (Eds.)., Springer, New York, USA., pp: 191-203.
33. Mestdagh, F., B. De Meulenaer and C. Van Peteghem, 2007. Influence of oil degradation on the amounts of acrylamide generated in a model system and in French fries. Food Chem., 100: 1153-1159.
34. Claus, A., G.M. Weisz, A. Schieber and R. Carle, 2006. Pyrolytic acrylamide formation from purified wheat gluten and gluten-supplemented wheat bread rolls. Mol. Nutr. Food Res., 50: 87-93.
35. Manson, J., M.J. Brabec, J. Buelke-Sam, G.P. Carlson and R.E. Chapin *et al*., 2005. NTP-CERHR expert panel report on the reproductive and developmental toxicity of acrylamide. Birth Defects Res. Part B: Dev. Reprod. Toxicol., 74: 17-113.
36. Pennisi, M., G. Malaguarnera, V. Puglisi, L. Vinciguerra, M. Vacante and M. Malaguarnera, 2013. Neurotoxicity of acrylamide in exposed workers. Int. J. Environ. Res. Public Health, 10: 3843-3854.
37. Friedman, M., 2003. Chemistry, biochemistry and safety of acrylamide. A review. J. Agric. Food Chem., 51: 4504-4526.
38. Freisling, H., A. Moskal, P. Ferrari, G. Nicolas and V. Knaze *et al*., 2013. Dietary acrylamide intake of adults in the European prospective investigation into cancer and nutrition differs greatly according to geographical region. Eur. J. Nutr., 52: 1369-1380.
39. FDA., 2006. The 2006 exposure assessment for acrylamide. FDA/Center for Food Safety and Applied Nutrition, USA.
40. Delgado-Andrade, C., M. Mesias, F.J. Morales, I. Seiquer and M.P. Navarro, 2012. Assessment of acrylamide intake of Spanish boys aged 11-14 years consuming a traditional and balanced diet. LWT-Food Sci. Technol., 461: 16-22.
41. Cummins, E., F. Butler, R. Gormley and N. Brunton, 2008. A methodology for evaluating the formation and human exposure to acrylamide through fried potato crisps. LWT-Food Sci. Technol., 41: 854-867.
42. Cummins, E., 2006. Risk assessment of acrylamide formation in fried potato products. Master's Thesis, National University of Ireland.
43. FAO. and WHO., 2011. Joint FAO/WHO expert committee on food additives: Evaluation of certain contaminants in food. Seventy-Second [72nd] report of the joint FAO/WHO expert committee on food additives. FAO. and WHO., Rome, Italy.
44. EFSA., 2011. Results on acrylamide levels in food from monitoring years 2007-2009 and exposure assessment. EFSA J., Vol. 9. 10.2903/j.efsa.2011.2133.
45. Normandin, L., M. Bouchard, P. Ayotte, C. Blanchet and A. Becalski *et al*., 2013. Dietary exposure to acrylamide in adolescents from a Canadian urban center. Food Chem. Toxicol., 57: 75-83.
46. Zhou, P.P., Y.F. Zhao, H.L. Liu, Y. Jian, X.W. Li, Y.A.N.G. Xin and Y.N. Wu, 2013. Dietary exposure of the Chinese population to acrylamide. Biomed. Environ. Sci., 26: 421-429.
47. Tardiff, R.G., M.L. Gargas, C.R. Kirman, M.L. Carson and L.M. Sweeney, 2010. Estimation of safe dietary intake levels of acrylamide for humans. Food Chem. Toxicol., 48: 658-667.
48. Konings, E.J.M., A.J. Baars, J.D. van Klaveren, M.C. Spanjer and P.M. Rensen *et al*., 2003. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food Chem. Toxicol., 41: 1569-1579.
49. Yousef, M.I. and F.M. El-Demerdash, 2006. Acrylamide-induced oxidative stress and biochemical perturbations in rats. Toxicology, 219: 133-141.
50. Ibrahim, R.M., 2005. Effect of different doses of acrylamide on some hematological and biochemical parameters on rats. M.Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.
51. WHO., 2005. World Health Organization. Summary report of the sixty-fourth meeting of the joint expert committee on food additive (JECFA). FAO and WHO, Rome, Italy, International Life Sciences Institute, Washington, DC., pp: 1-47.
52. LoPachin, R.M., 2004. The changing view of acrylamide neurotoxicity. NeuroToxicology, 25: 617-630.
53. LoPachin, R.M. and T. Gavin, 2012. Molecular mechanism of acrylamide neurotoxicity: Lessons learned from organic chemistry. Environ. Health Perspect., 120: 1650-1657.
54. Erkekoglu, P. and T. Baydar, 2014. Acrylamide neurotoxicity. Nutr. Neurosci., 17: 49-57.
55. Rice, J.M., 2005. The carcinogenicity of acrylamide. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 580: 3-20.
56. Wilson, K.M., L.A. Mucci, B.A. Rosner and W.C. Willett, 2010. A prospective study on dietary acrylamide intake and the risk for breast, endometrial and ovarian cancers. Cancer Epidemiol. Prevent. Biomarkers, 19: 2503-2515.
57. Martins, C., N.G. Oliveira, M. Pingarilho, G. Gamboa da Costa and V. Martins *et al*., 2006. Cytogenetic damage induced by acrylamide and glycidamide in mammalian cells: Correlation with specific glycidamide-DNA adducts. Toxicol. Sci., 95: 383-390.

58. Mei, N., J.X. Hu, M.I. Churchwell, L. Guo, M.M. Moore, D.R. Doerge and T. Chen, 2008. Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. Food Chem. Toxicol., 46: 628-636.
59. Shipp, A., G. Lawrence, R. Gentry, T. McDonald and H. Bartow *et al*., 2006. Acrylamide: Review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit. Rev. Toxicol., 36: 481-608.
60. Chatzizacharias, N.A., G.P. Kouraklis and S.E. Theocharis, 2008. Disruption of FAK signaling: A side mechanism in cytotoxicity. Toxicology, 245: 1-10.
61. Hogervorst, J.G., L.J. Schouten, E.J. Konings, R.A. Goldbohm and P.A. van den Brandt, 2007. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian and breast cancer. Cancer Epidemiol. Prevent. Biomarkers, 16: 2304-2313.
62. Hogervorst, J.G., L.J. Schouten, E.J. Konings, R.A. Goldbohm and P.A. van den Brandt, 2008. Dietary acrylamide intake and the risk of renal cell, bladder and prostate cancer. Am. J. Clin. Nutr., 87: 1428-1438.
63. Michels, K.B., B.A. Rosner, W.C. Chumlea, G.A. Colditz and W.C. Willett, 2006. Preschool diet and adult risk of breast cancer. Int. J. Cancer, 118: 749-754.
64. Olesen, P.T., A. Olsen, H. Frandsen, K. Frederiksen, K. Overvad and A. Tjonneland, 2008. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish diet, cancer and health study. Int. J. Cancer, 122: 2094-2100.
65. Wilson, K.M., E. Giovannucci, M.J. Stampfer and L.A. Mucci, 2012. Dietary acrylamide and risk of prostate cancer. Int. J. Cancer, 131: 479-487.
66. Mucci, L.A., P.W. Dickman, G. Steineck, H.O. Adami and K. Augustsson, 2003. Dietary acrylamide and cancer of the large bowel, kidney and bladder: Absence of an association in a population-based study in Sweden. Br. J. Cancer, 88: 84-89.
67. Hogervorst, J.G.F., L.J. Schouten, E.J.M. Konings, R.A. Goldbohm and P.A. van den Brandt, 2009. Lung cancer risk in relation to dietary acrylamide intake. J. Natl. Cancer Inst., 101: 651-662.
68. Hogervorst, J.G., L.J. Schouten, E.J. Konings, R.A. Goldbohm and P.A. van den Brandt, 2009. Dietary acrylamide intake and brain cancer risk. Cancer Epidemiol. Prevent. Biomarkers, 18: 1663-1666.
69. Tyl, R.W. and M.A. Friedman, 2003. Effects of acrylamide on rodent reproductive performance. Reproduct. Toxicol., 17: 1-13.
70. Besaratinia, A. and G.P. Pfeifer, 2004. Genotoxicity of acrylamide and glycidamide. J. National Cancer Inst., 96: 1023-1029.
71. Manjanatha, M.G., A. Aidoo, S.D. Shelton, M.E. Bishop, L.P. McDaniel, L.E. Lyn Cook and D.R. Doerge, 2006. Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female big blue mice. Environ. Mol. Mutag., 47: 6-17.
72. Ghanayem, B.I., K.L. Witt, L. El-Hadri, U. Hoffler, G.E. Kissling, M.D. Shelby and J.B. Bishop, 2005. Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: Evidence supporting a glycidamide-mediated effect. Biol. Reproduct., 72: 157-163.
73. Lineback, D.R. and J.M. Jones, 2011. Acrylamide in foods: Data and more questions. Nutr. Today, 46: 216-223.
74. Guenther, H., E. Anklam, T. Wenzl and R.H. Stadler, 2007. Acrylamide in coffee: Review of progress in analysis, formation and level reduction. Food Addit. Contamin., 24: 60-70.
75. Anese, M., M. Suman and M.C. Nicoli, 2010. Acrylamide removal from heated foods. Food Chem., 119: 791-794.
76. Vass, M., T.M. Amrein, B. Schonbachler, F. Escher and R. Amado, 2004. Ways to reduce the acrylamide formation in cracker products. Czech J. Food Sci., 22: 19-21.
77. Capuano, E., A. Ferrigno, I. Acampa, L. Ait-Ameur and V. Fogliano, 2008. Characterization of the Maillard reaction in bread crisps. Eur. Food Res. Technol., 228: 311-319.
78. Capuano, E., A. Ferrigno, I. Acampa, A. Serpen, O.C. Acar, V. Gokmen and V. Fogliano, 2009. Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies. Food Res. Int., 42: 1295-1302.
79. Cook, N.R., J.A. Cutler, E. Obarzanek, J.E. Buring and K.M. Rexrode *et al*., 2007. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: Observational follow-up of the Trials Of Hypertension Prevention (TOHP). Br. Med. J., Vol. 334. 10.1136/bmj.39147.604896.55.
80. Hamlet, C.G., D.E. Baxter, P.A. Sadd, I. Slaiding, L. Liang and R. Muller, 2005. Exploiting process factors to reduce acrylamide in cereal-based foods. Report C014. RHM Technology Ltd., Food Standards Agency, UK.
81. Vinci, R.M., F. Mestdagh and B. De Meulenaer, 2012. Acrylamide formation in fried potato products-present and future, a critical review on mitigation strategies. Food Chem., 133: 1138-1154.
82. Foot, R.J., N.U. Haase, K. Grob and P. Gonde, 2007. Acrylamide in fried and roasted potato products: A review on progress in mitigation. Food Addit. Contaminants, 24: 37-46.
83. Grob, K., M. Biedermann, S. Biedermann-Brem, A. Noti and D. Imhof *et al*., 2003. French fries with less than 100 μg kg^{-1} acrylamide: A collaboration between cooks and analysts. Eur. Food Res. Technol., 217: 185-194.
84. Matthaus, B., N.U. Haase and K. Vosmann, 2004. Factors affecting the concentration of acrylamide during deep-fat frying of potatoes. Eur. J. Lipid Sci. Technol., 106: 793-801.

85. Pedreschi, F., K. Kaack and K. Granby, 2004. Reduction of acrylamide formation in potato slices during frying. Food Sci. Technol., 37: 679-685.
86. Jung, M.Y., D.S. Choi and J.W. Ju, 2003. A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. J. Food Sci., 68: 1287-1290.
87. Mottram, D.S., B.L. Wedzicha and A.T. Dodson, 2002. Food chemistry: Acrylamide is formed in the Maillard reaction. Nature, 419: 448-449.
88. Taubert, D., S. Harlfinger, L. Henkes, R. Berkels and E. Schomig, 2004. Influence of processing parameters on acrylamide formation during frying of potatoes. J. Agric. Food Chem., 52: 2735-2739.
89. Zeng, X., K.W. Cheng, Y. Jiang, Z.X. Lin and J.J. Shi *et al*., 2009. Inhibition of acrylamide formation by vitamins in model reactions and fried potato strips. Food Chem., 116: 34-39.
90. Mestdagh, F., J. Maertens, T. Cucu, K. Delporte, C. Van Peteghem and B. De Meulenaer, 2008. Impact of additives to lower the formation of acrylamide in a potato model system through pH reduction and other mechanisms. Food Chem., 107: 26-31.
91. Gokmen, V. and B.A. Mogol, 2010. Computer vision based image analysis for rapid detection of acrylamide in heated foods. Qual. Assur. Safety Crops Foods, 2: 203-207.
92. Gertz, C. and S. Klostermann, 2002. Analysis of acrylamide and mechanisms of its formation in deep fried products. Eur. J. Lipid Sci. Technol., 104: 762-771.
93. Mestdagh, F.J., B. De Meulenaer, C. Van Poucke, C.L. Detavernier, C. Cromphout and C. Van Peteghem, 2005. Influence of oil type on the amounts of acrylamide generated in a model system and in French fries. J. Agric. Food Chem., 53: 6170-6174.
94. Rydberg, P., S. Eriksson, E. Tareke, P. Karlsson, L. Ehrenberg and M. Tornqvist, 2003. Investigations of factors that influence the acrylamide content of heated foodstuffs. J. Agric. Food Chem., 51: 7012-7018.
95. Wu, X., G.R. Beecher, J.M. Holden, D.B. Haytowitz, S.E. Gebhardt and R.L. Prior, 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Agric. Food Chem., 52: 4026-4037.
96. Hanson, P.M., R.Y. Yanga, S.C.S. Tsoua, D. Ledesmaa, L. Englea and T.C. Leeb, 2006. Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics and ascorbic acid. J. Food Compos. Anal., 19: 594-600.
97. Philpott, M., C.C. Lim and L.R. Ferguson, 2009. Dietary protection against free radicals: A case for multiple testing to establish structure-activity relationships for antioxidant potential of anthocyanic plant species. Int. J. Mol. Sci., 10: 1081-1103.
98. Todaro, A., F. Cimino, P. Rapisarda, A.E. Catalano, R.N. Barbagallo and G. Spagna, 2009. Recovery of anthocyanins from eggplant peel. Food Chem., 214: 434-439.
99. Huang, H.Y., C.K. Chang, T.K. Tso, J.J. Huang, W.W. Chang and Y.C. Tsai, 2004. Antioxidant activities of various fruits and vegetables produced in Taiwan. Int. J. Food Sci. Nutr., 55: 423-429.
100. Wang, R. and W. Zhou, 2004. Stability of tea catechins in the breadmaking process. J. Agric. Food Chem., 52: 8224-8229.
101. Lavelli, V., C. Vantaggi, M. Corey and W. Kerr, 2010. Formulation of a dry green tea apple product: Study on antioxidant and color stability. J. Food Sci., 75: C184-C190.
102. Mildner Szkudlarz, S., R. Zawirska Wojtasiak, W. Obuchowski and M. Goslinski, 2009. Evaluation of antioxidant activity of green tea extract and its effect on the biscuits lipid fraction oxidative stability. J. Food Sci., 74: S362-S370.
103. Mitsumoto, M., M.N. O'Grady, J.P. Kerry and D.J. Buckley, 2005. Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. Meat Sci., 69: 773-779.
104. Zhang, W., C. Hong, B. Pan, Z. Xu, Q. Zhang and Q. Zhang, 2008. A comparative study of the adsorption properties of 1-naphthylamine by XAD-4 and NDA-150 polymer resins. Colloids Surfaces A: Physicochem. Eng. Aspects, 331: 257-262.
105. Zhang, Y. and Y. Zhang, 2008. Effect of natural antioxidants on kinetic behavior of acrylamide formation and elimination in low-moisture asparagines-glucose model system. J. Food Eng., 85: 105-115.
106. Morales, G., M. Jimenez, O. Garcia, M.R. Mendoza and C.I. Beristain, 2014. Effect of natural extracts on the formation of acrylamide in fried potatoes. LWT-Food Sci. Technol., 58: 587-593.
107. Zhang, Y. and Y. Zhang, 2007. Formation and reduction of acrylamide in Maillard reaction: A review based on the current state of knowledge. Crit. Rev. Food Sci. Nutr., 47: 521-542.
108. Ibrahim, R.M., 2014. Acrylamide content in foods preferred by university students and reducing its level in fried foods and harmful effects on rats. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, Egypt.
109. Jang, J.R. and S.Y. Lim, 2009. Effects of onion flesh and peel on chemical components, antioxidant and anticancer activities. J. Life Sci., 19: 1598-1604.
110. Wiczkowski, W., K. Nemeth, A. Bucinski and M.K. Piskula, 2003. Bioavailability of quercetin from flesh scales and dry skin of onion in rats. Polish J. Food Nutr. Sci., 12: 95-99.
111. Gulsen, A., D.P. Makris and P. Kefalas, 2007. Biomimetic oxidation of quercetin: Isolation of a naturally occurring quercetin heterodimer and evaluation of its *in vitro* antioxidant properties. Food Res. Int., 40: 7-14.
112. Prakash, D., B.N. Singh and G. Upadhyay, 2007. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chem., 102: 1389-1393.

113. Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.
114. Hedegaard, R.V., K. Granby, H. Frandsen, J. Thygesen and L.H. Skibsted, 2008. Acrylamide in bread. Effect of prooxidants and antioxidants. Eur. Food Res. Technol., 227: 519-525.
115. Urbancic, S., M.H. Kolar, D. Dimitrijevic, L. Demsar and R. Vidrih, 2014. Stabilisation of sunflower oil and reduction of acrylamide formation of potato with rosemary extract during deep-fat frying. LWT-Food Sci. Technol., 57: 671-678.
116. Durling, N.E., O.J. Catchpole, J.B. Grey, R.F. Webby, K.A. Mitchell, L.Y. Foo and N.B. Perry, 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. Food Chem., 101: 1417-1424.
117. Lu, Y. and L.Y. Foo, 2002. Polyphenolics of Salvia-A review. Phytochemistry, 59: 117-140.
118. Zhang, L., Y.H. Lin, X.J. Leng, M. Huang and G.H. Zhou, 2013. Effect of sage (*Salvia officinalis*) on the oxidative stability of Chinese-style sausage during refrigerated storage. Meat Sci., 95: 145-150.
119. Generalic, I., D. Skroza, I. Ljubenkov, A. Katalinic, F. Burcul and V. Katalinic, 2011. Influence of the phenophase on the phenolic profile and antioxidant properties of Dalmatian sage. Food Chem., 127: 427-433.
120. Grzegorczyk, I., A. Matkowski and H. Wysokinska, 2007. Antioxidant activity of extracts from *in vitro* cultures of *Salvia officinalis* L. Food Chem., 104: 536-541.
121. Fasseas, M.K., K.C. Mountzouris, P.A. Tarantilis, M. Polissiou and G. Zervas, 2008. Antioxidant activity in meat treated with oregano and sage essential oils. Food Chem., 106: 1188-1194.
122. Felgines, C., O. Texier, C. Besson, D. Fraisse, J.L. Lamaison and C. Remesy, 2002. Blackberry anthocyanins are slightly bioavailable in rats. J. Nutr., 132: 1249-1253.
123. Stintzing, F.C., A.S. Stintzing, R. Carle, B. Frei and R.E. Wrolstad, 2002. Color and Antioxidant properties of cyanidin-based anthocyanin pigments. J. Agric. Food Chem., 50: 6172-6181.
124. Jiao, Z., J. Liu and S. Wang, 2005. Antioxidant activities of total pigment extract from blackberries. Food Technol. Biotechnol., 43: 97-102.
125. Seeram, N.P., 2008. Berry fruits: Compositional elements, biochemical activities and the impact of their intake on human health, performance and disease. J. Agric. Food Chem., 56: 627-629.
126. Bravo, L., 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev., 56: 317-333.
127. Ibrahim, R.M., M. Yousef, I. Nawar, A. Hassanein and D. Hikal, 2019. Utilization of black berry juice to reduce the oxidative stress in rats treated with acrylamide. Asian J. Biol. Sci., 12: 9-16.
128. Pihlanto, A., 2006. Antioxidative peptides derived from milk proteins. Int. Dairy J., 16: 1306-1314.
129. Sarmadi, B.H. and A. Ismail, 2010. Antioxidative peptides from food proteins: A review. Peptides, 31: 1949-1956.
130. Kamau, S.M., R.R. Lu, W. Chen, X.M. Liu, F.W. Tian, Y. Shen and T. Gao, 2010. Functional significance of bioactive peptides derived from milk proteins. Food Rev. Int., 26: 386-401.
131. Awad, S., M.I. El-Sayed, A. Wahba, A. El Attar, M.I. Yousef and M. Zedan, 2016. Antioxidant activity of milk protein hydrolysate in alloxan-induced diabetic rats. J. Dairy Sci., 99: 8499-8510.
132. Suetsuna, K., H. Ukeda and H. Ochi, 2000. Isolation and characterization of free radical scavenging activities peptides derived from casein. J. Nutr. Biochem., 11: 128-131.
133. Peng, X., Y.L. Xiong and B. Kong, 2009. Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. Food Chem., 113: 196-201.
134. Hernandez-Ledesma, B., A. Davalos, B. Bartolome and L. Amigo, 2005. Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/MS. J. Agric. Food Chem., 53: 588-593.
135. Baardseth, P., H. Blom, G. Skrede, L.T. Mydland, A. Skrede and E. Slinde, 2006. Lactic acid fermentation reduces acrylamide formation and other Maillard reactions in French fries. J. Food Sci., 71: C28-C33.
136. Ou, S., J. Shi, C. Huang, G. Zhang, J. Teng, Y. Jiang and B. Yang, 2010. Effect of antioxidants on elimination and formation of acrylamide in model reaction systems. J. Hazard. Mater., 182: 863-868.
137. Yuan, Y., C. Shu, B. Zhou, X. Qi and J. Xiang, 2011. Impact of selected additives on acrylamide formation in asparagine/sugar Maillard model systems. Food Res. Int., 44: 449-455.
138. Kotsiou, K., M. Tasioula-Margari, E. Capuano and V. Fogliano, 2011. Effect of standard phenolic compounds and olive oil phenolic extracts on acrylamide formation in an emulsion system. Food Chem., 124: 242-247.