

ACRYLAMIDE: A POSSIBLE RISK FACTOR FOR CARDIAC HEALTH

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Received: 02 May 2018, Revised and Accepted: 15 June 2018

ABSTRACT

Acrylamide (ACR) is an important industrial chemical agent also a food contaminant formed when food rich in carbohydrates is processed at high temperatures (120°C) such as cooking, frying, roasting, toasting, and baking. It happens when amino acid asparagine reacts with sugars, especially glucose and fructose as a result of the Maillard reaction. Its potential to cause damage to humans and animals makes it a cause for concern. After its outbreak in 2002, extensive study has been going on to prevent its formation in food. Neurological effects are by far well established for ACR along with systemic toxic effects. Diet contributes to the high proportion of ACR intake on daily basis; other exposure media are occupational, environmental, and smoking. A number of studies justifying ACR cardiotoxicity, its clear mechanism and its relevance to humans are less, but some research papers suggest the possibility of cardiotoxicity or developmental cardiotoxicity. In this review, ACR cardiac toxic effects and mechanism pathway have been discussed along with mitigation strategies.

Keywords: Acrylamide, Cardiotoxicity, Maillard reaction, Mitigation strategies, Alternative mitigation strategies.© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i10.27073>**INTRODUCTION**

Acrylamide (ACR), an industrially produced α , β -unsaturated reactive molecule, colorless, non-volatile crystalline solid, soluble in water and has a molecular weight of 71.08. It is an important industrial chemical widely used to synthesize ACR. Polyacrylamide has found many applications as the soil conditioner; in water waste treatment; in the cosmetic, paper, and textile industries; and in the laboratory as solid support for electrophoresis and various analytical techniques [1]. In April 2002, ACR was discovered by the Swedish National Food Authority in carbohydrate-rich foods which are subjected to deep frying, toasting, and baking at the temperature above 120°C. ACR was classified as a Group 2A carcinogen by the International Agency for Research on Cancer [2] and category 2 mutagen by the European Union (2002) [3], its formation in food caused worldwide concern. In 2002, Tareke *et al.* analyzed the occurrence of ACR in food heated under laboratory conditions and in commercially available foodstuff and concluded that ACR levels were quite high in food subjected to heating. Carbohydrate-rich foods have higher ACR content as compared to protein-rich foods [4]. Subsequent research demonstrated ACR formation in food when heated at the higher temperature above 120°C [1].

ACR is known as a byproduct of Maillard reaction which is the key reaction for ACR formation. In another initiative, researchers from 14 countries, joined together in a research project called heat-generated food toxicants (HEATOX) to evident the potential toxic threat ACR poses to humans. A total of 50 compounds has been highlighted as potential carcinogens [5].

It is of health concern because high quantities are being consumed on daily basis, which poses the more serious problem, especially Western countries where foods such as chips and other potato products are widely consumed [6] because potato products have known to contain higher amounts (117–4215 $\mu\text{g}/\text{kg}$). Potential human dietary mean ACR intake in the general population has been estimated to range from 0.31 to 1.1 $\mu\text{g}/\text{kg}/\text{day}$ body weight (b.w.) for adults (>18 years), 0.43 and 1.4 $\mu\text{g}/\text{kg}/\text{day}$ b.w. for adolescents (11–17 years), and 0.70 and 2.05 $\mu\text{g}/\text{kg}/\text{day}$ b.w. for children's (3–10 years). Major contributors to exposure for adults were fried potatoes, coffee, and soft bread [7]. Foods contributing to dietary intake of ACR differ from country to country [8]. After

findings of ACR in food, many epidemiological studies have emerged investigating the relation of ACR and cancer and various associated toxic effects. It is reported to cause neurotoxicity [9,10], reproductive toxicity [11,12], hepatotoxicity [13], carcinogenicity [14,15], and possible developmental cardiotoxicity [16]. Neurotoxicity is the only informed toxicity observed in humans so far, other toxicological data is based on animal studies. ACR, soft electrophilic toxicant, reacting on thiol groups of proteins (cysteine, homocysteine) and glutathione (GSH) as well as protein-bound-SH groups (kinesin, dynein), whereas the metabolite glycidamide is harder electrophilic compound, reacting with nucleophilic centers of adenine and guanine in the DNA [17,18]. Hemoglobin adducts have been used as biomarkers of exposure and to estimate internal dose in occupationally exposed populations [19,20].

There are a number of studies now evident the occurrence of ACR in food and its potential risk causing capability, but this review will focus on the possible cardiotoxicity associated to ACR and summarize methods which can be utilized to reduce the formation of ACR in food. Nevertheless, few studies have been published regarding the cardiotoxic effect of ACR.

OCCURRENCE IN FOOD

People are exposed to different amounts of ACR through diet, smoking, drinking water, environmental and occupational exposure, but dietary exposure is prominent. ACR primarily found in plant-based foods; treated starchy foods such as potato, cereal, and bakery products [3,21]. A large number of data has been collected by the European Food Safety Authority, Table 1, represents levels of ACR in processed foods (values based on the European Food Safety Authority monitoring data from 2007 to 2010) [7,22,23].

The 2006 exposure assessment for ACR (AA) presented by Food and Drug Administration includes evaluation of 66 food categories (ethnic and regional foods) included. Table 2 represents the data and level of ACR in food. Mean intake was observed to be 0.4 $\mu\text{g}/\text{kg}$ b.w. per day [24].

FORMATION

The Maillard reaction is a key reaction for ACR formation: It is the reaction between the naturally present amino acid and reducing

Table 1: Distribution of ACR level in different food categories ($\mu\text{g}/\text{kg}$) by EFSA [22]

S. No.	Foodstuff	Indicative value ($\mu\text{g}/\text{kg}$)
1.	Potato-based crackers	
	French fries ready-to-eat	600
	Potato crisps from fresh potatoes and from potato dough	1000
2.	Soft bread	
	Wheat-based bread	80
3.	Soft bread other than wheat-based bread	150
	Breakfast cereals (excluding porridge)	
	Bran products and whole grain cereals, gun-puffed grain (gun puffed only relevant if labeled)	400
	Wheat- and rye-based products	300
	Maize-, oat-, spelt-, barley-, and rice-based products	200
	Biscuits and wafers	500
	Crackers with the exception of potato-based crackers	500
	Crispbread	450
	Gingerbread	1000
	Products similar to the other products in this category	500
	Roast coffee	450
4.	Instant (soluble) coffee	900
	Coffee substitutes	
	Coffee substitutes mainly based on cereals	2000
5.	Other coffee substitutes	4000
	Baby foods other than processed cereal-based foods	
	Not containing prunes	50
	Containing prunes	80
	Biscuits and rusks for infants and young children	200
	Processed cereal-based foods for infants and young children, excluding biscuits and rusks	50

ACR: Acrylamide, EFSA: European Food Safety Authority

Table 2: Mean Intake of ACR from different foods ($\mu\text{g}/\text{kg}/\text{b.w.}/\text{day}$) (FDA, 2006)

Food	Mean AA* intake ($\mu\text{g}/\text{kg b.w.}/\text{day}$)	Cumulative percentile
French fries	0.070	0.16
Potato chips	0.045	0.28
Breakfast cereal	0.040	0.38
Cookies	0.028	0.47
Brewed coffee	0.027	0.53
Toast	0.023	0.60
Pies and cakes	0.018	0.65
Crackers	0.017	0.73
Soft breads	0.014	0.77
Chile corn carne	0.014	0.80
Corn snacks	0.011	0.82
Pizza	0.007	0.87
Burrito	0.006	0.88
Peanut Butter	0.003	0.89
Breaded chicken	0.003	0.90
Bagels	0.003	0.90
Soup mix	0.003	0.91

*AA: *Assessment for ACR. ACR: Acrylamide, FDA: Food and drug administration

sugar within the food [25,26]. ACR is formed within the food during processing, when food is subjected to toasting, roasting, and frying at high temperature. The formation depends on the presence of precursors and the processing conditions such as pH value, temperature, and time. Different pathways of ACR formation are known, but formation through Maillard reaction between the amino acid (asparagine) and sugar (glucose and fructose) is the major pathway [25,27-29]. The $\alpha\text{-NH}_2$ of asparagine participates in a nucleophilic addition reaction with the aldehyde group of glucose to form a Schiff base, which undergoes Amadori rearrangement to form a derivative. The latter can undergo decarboxylation to produce ACR (Fig. 1). Research shows that the reducing sugars are the major limiting factor in potatoes [30-32], while free amino acid- asparagine is the major limiting factor in cereal products [33-35].

Asparagine and reducing sugar reaction are the major routes, other routes (from amino acids and fatty acid) also been shown to generate ACR (Fig. 2). The fatty acid oxidation product of acrolein and ammonia, acrylic acid produces ACR [36], which showed that acrolein and ammonia as an important precursor of ACR [37,38]. It is known that acrolein and acrylic acid are produced by degradation of lipids, especially from triglycerides, at the high temperature [23,26,39]. Degradation of amino acids with ammonia can give rise to ACR formation by thermal decay [40]. ACR produce from asparagine and from amino acids that generate acrylic acid directly and produce β -alanine, aspartic acid, and carnosine or indirectly formed cysteine and serine [1]. Zyzak *et al.* [29] were the first to show that 3-aminopropionamide, a biogenic amine formed during the Maillard reaction. Granvogi and Schieberle provided concrete evidence of the intermediacy of 3-APA in the reaction to ACR [41,42]. Bagdonaite *et al.* [43] reported the high content of 3-APA in potatoes, but no content was detected in roasted coffee.

As the mechanism of ACR came into light, suppression of these two precursors turned out to be a useful approach in the reduction of ACR in food. Multiple studies have been conducted up-to-date unfolding the toxicological profile of ACR. Neurotoxic properties are well documented, also non-neurotoxic effects such as genotoxic, carcinogenic, reproductive, and developmental effects have been studied on the basis of rodent studies. The review will focus on information concerning cardiotoxicity of ACR.

CARDIOTOXICITY

No epidemiological studies have been reported so far for ACR-induced cardiac abnormalities. Furthermore, the studies conducted suggesting cardiotoxicity are few. Still one cannot disregard the fact that ACR has potential to cause cardiotoxicity/cardiac ill effects. More studies are needed to enlighten the cardiac abnormalities associated with ACR.

Pre-clinical studies data

Animal experiments on rodents conducted by Field *et al.* and Friedman *et al.* suggested that no placental or lactational transfer of ACR or its active metabolite in utero or suckling offspring. Schettgen *et al.* [44] investigated ACR in mother's (mothers exposed through food) blood is able to cross human placenta and determined the internal exposure

of neonates in relation to the body burden of the mother suggesting that the transplacental exposure to ACR could affect newborn. The concentration of ACR-Hb adduct in the blood of neonates were half of the adduct level found in the blood of the mother. ACR-Hb adduct levels in mother and neonates showed a good correlation. This study suggested that transplacental exposure of fetus to acrylamide might raise concerns. Hence, this study proves the hypothesis by field and Friedman wrong. ACR can be hypothesized to cause developmental cardiotoxicity, but no concrete evidence was generated for ACR toxicity to neonates.

In 2008, Mogda *et al.* [45] reported ACR (2.8 mM for 6 weeks)-induced oxidative stress and myocardial damage in rats. Histopathological findings of the heart tissue showed hemorrhage of the myocardial muscle fibers accompanied by myocardial degeneration and loss of

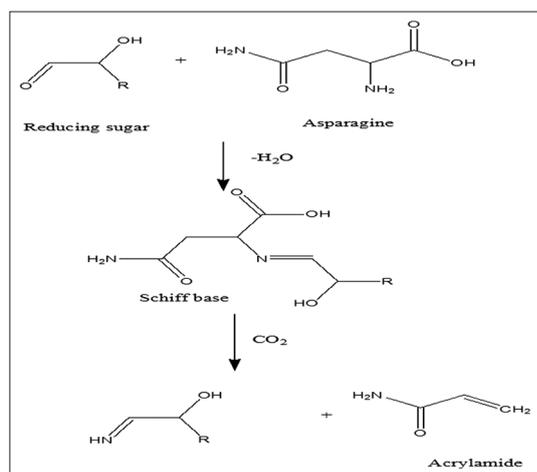


Fig. 1: Acrylamide formation from asparagine [32]

striations suggesting signs of cardiac toxicity. In another rodent study, ACR administered 0.34g/kg diet for 11 days showed reduced organ weight, reduced levels of antioxidant in heart tissue. An absolute and relative weight of liver, testes, and heart was recorded. Absolute decrease in heart weight was recorded 0.64 ± 0.034 as compared to negative control 0.70 ± 0.10 and level of tissue antioxidant levels showed a significant decrease compared to control group indicating oxidative damage by ACR [46].

Swamy *et al.* [47] studied the effect of different doses 0.2, 0.4, and 0.6 mg of ACR in developing chick embryo heart. Damage to the heart tissue was proportional to the dose: 0.2 mg - mild degeneration, 0.4 mg - tissue degeneration with necrotic and degenerative changes, and 0.6 mg - collapsed myocardial fibers. Impaired antioxidant defense, analyzed by measuring superoxide dismutase (SOD), GSH peroxidase, catalase, and GSH S-transferase activity.

Rat cardiomyocyte properties with prolonged exposure to ACR concentrations corresponding to dietary levels appeared with altered morphology and increase in the number of immunoreactive signals for connexin 43 at cell junction related to cardiac pathologies. Alteration in the cardiomyocyte morphology, contractions properties, and gap junction distribution was reported in response to ACR toxicity [48].

Jayadev *et al.* [49] evaluated dietary ACR exposure in male F344 rats. Hematological data, serum enzyme levels, and body and organ weights were analyzed, a significant increase in these parameters was observed in response to toxic insult. A marked increase in lipase levels, decrease in total cholesterol to high-density lipoprotein (HDL) ratio was observed. Lower HDL is an important marker of an increased risk of cardiovascular disease and the ACR related decrease in HDL may be an indicator of disruption in cardiovascular physiology.

Concurrent exposure of aluminum (50 mg/kg b.w.) and ACR (20 mg/kg b.w.) toxicant to rats produced additive/synergistic

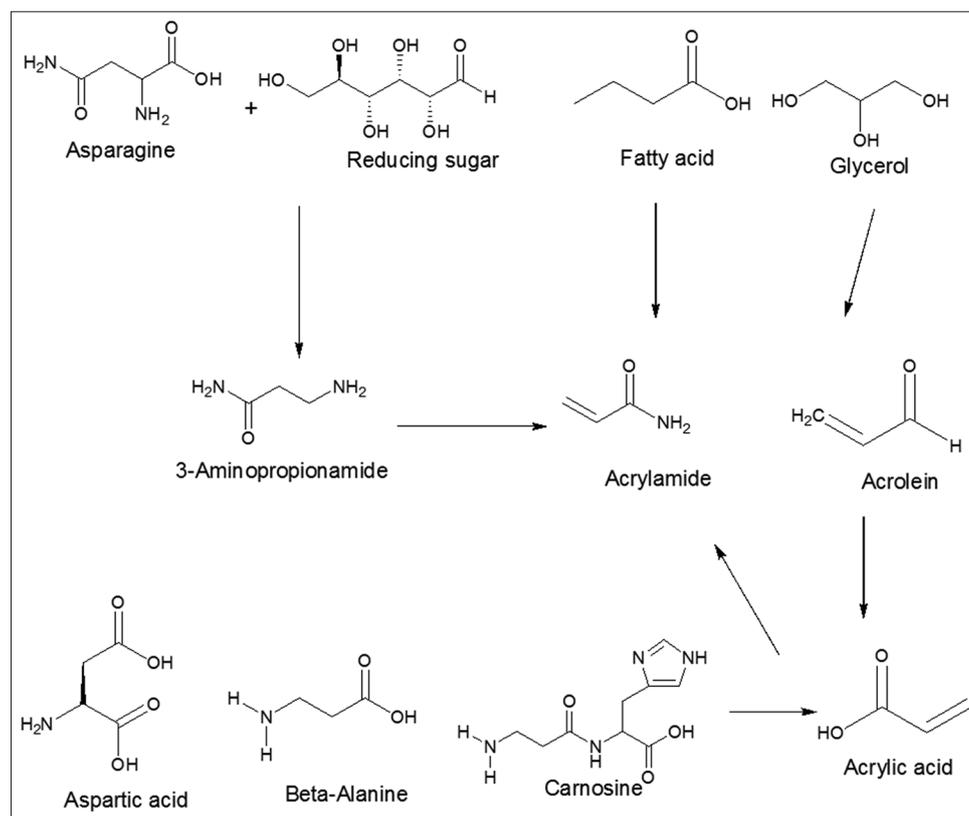


Fig. 2: Different routes of acrylamide formation

interactions in hearts of rats. Morphological and physiological changes in cells were observed resulting from coexposure. Significant reduction in b.w. and heart weight were reported. Increased free radical production, leading to the membrane damage evidenced by lipid peroxidation in the form of malondialdehyde (MDA) and increased level of advanced oxidation products in cardiac tissue. GSH levels depleted in heart, decreased the permeability of membrane leading to leakage of enzymes creatine kinase and lactate dehydrogenase, their activities reflect the alterations in the heart. Histological examination of heart showed inflammation and marked vascular congestion [50].

Recently, a pioneer acute toxicity study showed cardiac developmental toxicity of ACR in zebrafish during cardiogenesis. Cardiomyocyte proliferation was reduced significantly and reduced myocardial and endothelial cell number indicated subsided development of ventricle and atrium. Cardiomyocyte proliferation was reduced significantly and reduced myocardial and endothelial cell number and down regulated expression of cardiac progenitor genes indicated subsided development of ventricle and atrium. The study findings conclude that prenatal period may be more vulnerable to ACR developmental cardiotoxicity in humans; transplacental exposure of ACR is the cause of concern to neonate's health and heart could be the target for the developmental cardiotoxicity and potential risk factor for congestive heart failure [16]. Table 3 represents the studies evaluating the effect of phytochemicals on ACR toxicity. Table 4 represents the studies of ACR toxicity evaluation.

Underlying mechanism

ACR toxicity is likely related to its binding to sulphhydryl groups on protein by forming covalent bonds with electron-rich residues on biological macromolecules and inactivating the enzymes involved in

DNA repair [17,18,51]. ACR alkylates proteins such as hemoglobin, enzymes, and DNA [1], through a Michael addition-type reaction, which has implications for its genotoxicity and carcinogenic potential. Hemoglobin ACR adduct formation emerged as a useful biomarker of human exposure [52,53]. Similar was observations were reported in other studies [20,44,54].

Oxidative stress

A heart is composed primarily of long-lived, post-mitotic cells, which prefer fatty acids as the substrate for energy production, so it becomes more susceptible to oxidative damage than other tissues [55].

Oxidative stress, a consequence of increased production of free radical and reactive oxygen species and/or decrease in antioxidant defense, leads to damage and disruption of biological membrane and macromolecules [56]. Availability of more evidence shows that cardiovascular mechanisms are strongly linked to the production of free radicals or imbalance between antioxidants-oxidants [57,58]. It is well known that there is an indirect and direct relation between oxidative damage that shares a mutual pathway of molecular and cellular damage. Numerous studies represent the relationship between neurotoxicity and oxidative stress [9,17,59-62]; the review emphasizes the relation between ACR oxidative stress and cardiotoxicity.

In an *in vitro* study, ACR-induced oxidative stress in human erythrocytes; increases Methb formation, SOD activity, MDA formation, and hemolysis due to the destruction of erythrocytes cell membrane [64]. Reactive oxygen species are continuously generated by the slow autooxidation of hemoglobin produces methemoglobin; unable to carry oxygen [63]. Chronic administration of acrylamide at a dose

Table 3: Effect of phytochemicals on ACR toxicity

Treatment	Model	Effect of treatment on ACR toxicity	Proposed mechanism of action	References
Catechin and neem leaves extract	Rat	Restore GSH, MDA, and CAT in liver and modulate injuries in liver, kidney, lung, heart, spleen, and stomach	Detoxification with GSH, antioxidant, and anti-inflammatory activities	[45]
<i>Curcuma longa</i> powder	Rat	Restore body and organ weight, GSH, MDA, SOD in liver, heart, and kidney. AST, ALT, ALP in liver	Detoxification with GSH, antioxidant and anti-inflammatory, hepatoprotective	[46]
Extra virgin olive oil	Rat	Restore GSH, CAT, Gpx, SOD, in heart, decrease CK-MB, LDH in heart, decrease in lipid profile, and modulate injuries in cardiac muscle	Antioxidant and Anti-inflammatory, Lipid level lowering, cardioprotective	[50]

GSH: Glutathione reduced, MDA: Malondialdehyde, CAT: Catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, CK-MB: Creatine kinase-muscle/brain

Table 4: ACR cardiotoxicity evaluation

Model	Significant effect of ACR toxicity	Proposed mechanism of action	References
Human erythrocyte	Reduced GSH, SOD, CAT levels, Increased MDA, hemolysis, and Methb formation	Oxidative stress and inhibited antioxidant defense	[63]
Chick embryo	Reduced CAT, SOD, GPx, and GSH in the heart of embryo. Loss of heart weight and b.w. Myocardial fibers degeneration	Oxidative stress and inhibited antioxidant defense	[47]
Rat	Primary cardiomyocyte altered cell morphology, irregular contraction patterns, and increase in immunoreactive signal for connexin 43 at cell junctions	Diminished cell-cell adhesion and impaired ion ingredients	[48]
F344 rat	Lowered total HDL, total testosterone, blood hematocrit level, lymphocyte count, hemoglobin level, MCV, MCH, increased serum lipase		[49]
Zebrafish	Reduced myocardial cell number and endocardial cells, collapsed developmental of ventricle and altered cardiac genes expression	Downregulation of cardiac genes	[16]

HDL: High-density lipoprotein, MCV: Mean corpuscular volume, Mean corpuscular hemoglobin, MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

which does not induce neurotoxicity alters blood viscosity. Increased blood viscosity means decreased fluidity of blood which might increase chances of cardiovascular disease [65]. A subsequent study evaluated the toxicity of ACR exposure in male F344 rats. Hematological findings show decreased levels of hemoglobin, mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentrations, and lowered hematocrit value [49]. These systemic effects can be a possible cause of anemia, and anemia is strongly known to link with cardiovascular disease [66]. A Korean cohort study, enrolled 476,529 Korean adults to investigate the relationship between hemoglobin concentration and the incidence of cardiovascular disease. To minimize bias, subjects with previous history of cardiovascular disease or cancer were excluded. Those with extreme or low level (above 10 g/dl or above 20 g/dl) Hb levels were also excluded. Finally, 407,858 subjects were included, both men and women. The subjects were subdivided into different quintiles due to a gender difference in hemoglobin levels. The findings of the study demonstrated a positive correlation between hemoglobin levels and cardiovascular disease risk factors. Low or high hemoglobin levels showed a high risk for CVD both for men and women [67]. Some of the parameters were left out such as red blood cell distribution width (RDW), mean corpuscular volume, and mean corpuscular hemoglobin which is linked to CVD, especially RDW, it is known to be related to CVD mortality [68]. Furthermore, anemia or low hemoglobin levels contribute to myocardial ischemia and left ventricular hypertrophy [69]. The link between anemia and hypoxia; anemia can cause hypoxia due to decreased hemoglobin level and through several other hemodynamic and no hemodynamic compensatory mechanisms [70]. Another cohort study conducted by Choncol and Neilson [71], the study states that hemoglobin levels are independently associated with increased risk for new cardiac events. As ACR-induced oxidative stress has been observed to alter the hemoglobin levels, it may be an indicator of perturbation in cardiovascular physiology.

Depletion of antioxidants due to ACR toxicity has been indicated in various studies. Decreased level of GSH and SOD results in increased oxidative damage to proteins and DNA. Mogda *et al.* and Shawakia *et al.* [45,46] reported a significant decrease in antioxidant levels and lipid peroxidation in heart tissues. ACR forms covalent bonds with sulfhydryl groups of proteins, however, the reactivity of free thiols is greater than that of other soft nucleophile centers, and consequently, the preferential *in vivo* target is SH groups on protein cytosine residues and GSH [19] which explains depletion of GSH stores due to the oxidative stress. ACR generates reactive oxygen species which enhances lipid peroxidation. Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides [72]. Lipid peroxyl radicals propagate into MDA. MDA as the biomarker of lipid peroxidation is used to investigate oxidative damage of proteins induced by ACR [45,46].

Increase levels of cholesterol, triglycerides, and HDL levels are related to cardiac abnormalities. Cardiovascular disease related to increasing lipids levels are atherosclerosis, endothelial dysfunction, vascular inflammation, myocardial infarction, coronary heart disease, and peripheral artery disease [73]. ACR-induced reduction in HDL level may increase the risk of cardiovascular disease [49]. Lower HDL is an important marker of an increased risk of cardiovascular disease [74].

Gene expression

Downregulated expression of cardiac genes is reported in a recent study by Huang *et al.* [16] during cardiogenesis. Altered cardiac gene expression strongly linked to disrupted cardiogenesis which resulted in the formation of the cardiovascular system deficient embryo of zebrafish. This is the only study up-to-date representing the alteration in gene expression due to ACR in the heart.

Myocardial apoptosis

Loss of cardiac myocytes is a fundamental part of the myocardial injury that initiates or aggravates cardiomyopathy and leads to

premature death [75,76]. An important mode of myocardial cell loss is apoptosis [77-79] which has been demonstrated in the myocardium of heart failure patients [80,81]. Apoptosis may be one of the pathways for ACR to induce cardiac injury but no apoptosis have been reported in the above-cited studies.

MITIGATION STRATEGIES

No treatment is currently devised or known for ACR cardiotoxicity; antioxidants might emerge as potential therapeutics in the near future. Modifying our eating habits or modifying the food itself turned might be the treatment we need as "Prevention is better than cure."

Establishment of a reliable, rapid, and simple test method still remains a challenge although a number of the potential strategies and analytical methods have been developed. This review summarizes the mitigation strategies for preventing the formation of ACR in food.

Falling ACR in food at domestic and industrial level can help out the community not only form food hazards but also make awareness about the food safety. Food Drink Europe a confederation of Food industry provides ACR toolbox (Fig. 3). The "toolbox" reflects the results of more than 10 years of cooperation between the food industry and national authorities of the European Union to investigate pathways of formation of ACR and potential intervention steps to reduce exposure.

Toolbox defines food products based on potato-based products, cereal/grain-based products, coffee, roasted grain and substitutes, infant foods. The objective of the toolbox is to provide practical tools that food manufacturers can evaluate to reduce ACR according to a particular situation. The Agronomical approach involves minimizing reducing sugar, selection of crop varieties free of asparagine. Recipe changes including the addition of calcium salts and amino acids, change in pH, and fermentation process in potato-based products. In case of cereal-based products, focus on mitigation of ACR in whole grain and bran products. Pretreatment with commercially available enzyme asparaginase accounts for the majority of more recent measures in the toolbox. Some effective mitigation methods have been used only for few specific products because they are in direct with conflict with health target [82].

Food business operators follow ALARA: "As low as reasonably achievable," means that food business operators should take every reasonable measure to reduce the presence of a given contaminant in a final product taking into account other legitimate considerations, change of parts of a process, or even a whole process if technologically feasible by operator can be done. The tools identified contained in the Food Drink Europe Toolbox are possible trial planned to limit ACR levels in finishing product through interventions at various stages of manufacturing [83].

In respect to these, this review will summarize the effective and potentially exploitable technological interventions of ACR minimization. The strategies up-to-date can be summarized into two prominent approaches.

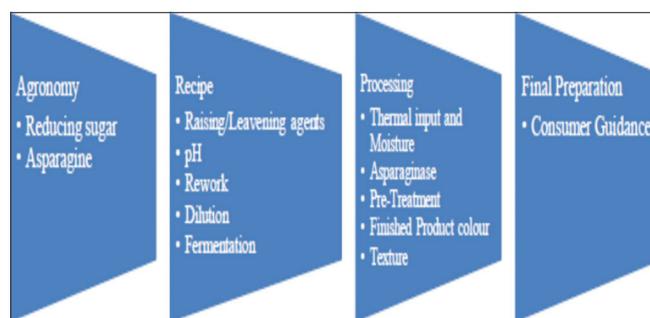


Fig. 3: Toolbox parameters

1. Removal of precursor - agronomical selection.
2. Interference with the Maillard reaction - Processing and ultimately prevent the formation of undesirable Maillard products.

Raw materials

Asparagine content is higher in potatoes products, which represents the major factor for the high amount of ACR formation [84-87]. The correlation between sugar content and ACR in 16 different varieties was reported by De Wilde [88]. Potato varieties with low reducing sugar can be effective in reduction of ACR level [31,89,90]. Variety, harvest year, fertilization, storage, soil properties, condition influence formation of ACR in potatoes products [87,91,92], and cereal products [93].

Soil properties highly influence the crop yield due to fertility, water holding capacity, temperature, and drainage. A study investigated the effect of tuber mineral composition, on the expression of asparagine and reducing sugar in tubers [94]. Fertilization is a major factor in crop production; nitrogen fertilization level greatly affects the formation of ACR. Decreased nitrogen fertilization enhances ACR formation due to increased reducing sugar level [91], whereas moderate level in combination with potassium results in decreased ACR level by reducing asparagine and reducing sugar level [95]. The effect of nitrogen and sulfur fertilization on free amino acids, sugars, and ACR formation potential; the effect is type and variety dependent increase in sugars and amino acids on deprivation of nitrogen fertilization, also increased ACR in response to increased nitrogen [96]. Legal fertilization limit should be taken into account to obtain ACR free crop. Climate condition near to harvest period may affect the susceptibility of ACR formation. De Meulenaer *et al.* showed the significant impact of variable climatological conditions on reducing sugar and total free amino acid of tubers [97].

Harvested tubers are stored up for months, storage duration influence sugar accumulation in tubers. Senescent sweetening results from an enzymatic process which occurs more rapidly at higher storage temperature (>8°C) followed by sprout growth [33]. Cold temperature and senescent sweating are the causes of sugar accumulation in potatoes during storage. Storing at low temperature (>8°C) [98] turned out to be a useful approach with major effect on reducing sugar accumulation [99-103]. Storing at 8°C proved to ideal temperature [104] and reducing sugar is not influenced at 8°C [87,105].

Process changes

Initial strategies involved the control of processing conditions; cutting, blanching, lowering pH, frying, drying, use of additives such as enzymes, amino acids/protein rich substances, antioxidants, divalent, and monovalent cations have been extensively studied and utilized to mitigate ACR formation.

The peripheral region of tubers have high reduced sugar content, slicing the region may contribute to ACR reduction [106]. Blanching is an important process in the production of French fries, during this process enzymes are inactivated a gelatinized layer of starch is developed which limits oil absorption and improve texture [107]. Blanching assisted reduction in ACR formation in the final product [5,108-111]. Hence, these conditions can be manipulated to optimize reducing sugar extraction and ACR free final product. However, potential loss of mineral and vitamin occurs on excessive blanching.

Time and baking temperature have the strong correlation information of ACR, level of ACR increases with increment in temperature [112,113], but a slow linear decrease occurs with time [114]. The similar correlation of baking temperature and time was observed [115,116]. ACR could also be formed at the temperature below 100°C (Biedermann and Grob). Higher temperature combined with prolonged heating times produces the reduced level of ACR due to elimination/degradation reactions [117]. It can be a tool of interest that can be exploited industrially but such intense heat treatment is responsible for changes in sensory attributes, such as color, flavor, and texture and these changes may lead to the formation of the inconsumable

product [118]. A water content of food may have some role in the formation of ACR during heating. Lower moisture promotes ACR formation [114,119,120]; keeping humidity high proven to be effective in ACR mitigation in bakery products [112]. The approach is utilized for foods with relatively high residual moisture, whereas for most cereal derivatives lower moisture contents are required. In respect to the above problem, the use of deck ovens appeared to be more convenient and more advantageous in reducing ACR than convection ovens, which are based on forced air circulation.

Use of additives

Use of additives such as organic acids, amino acids, and mono- and divalent cations, as a mitigating strategy to control ACR content in foods, has been extensively discussed.

Mono- and divalent cations reduce ACR formation. They could interact with asparagine and prevent the formation of Schiff base, ultimately reduction in ACR [90,121-123]. Antioxidants can play important role in the reduction of ACR, an addition of rosemary herb, bamboo leaves extract to the oil used for frying potatoes slices resulted in decreased ACR formation [124,125]. Biedermann *et al.* [126] reported a weak decrease in ACR level by the addition of ascorbic acid to the potato model. However, controversy surrounds the antioxidants potential to reduce ACR, positive and negative relationships have been observed [28,125,127-130]. The correlation between antioxidant phytochemicals and ACR has been reviewed [61]. The variation in result might be due to the presence of amino acids in extracts, decrease in pH, or the variability might be due to different food models. Effect of additives such as amino acids or protein-rich substances reduced the ACR content in foods [115,131,132]. Formation of ACR decreased 50% when cysteine and methionine were added to the cracker and potato dough [133,134]. On the contrary, cysteine does not contribute the formation of ACR in crisps bread was investigated [135] due to acidic pH, adduct formation could not occur. High pH favors ACR formation, acidic pH and enzymatic extraction had no significant effect on concentrations [136]. Reduced pH drastically reduces ACR levels during frying and baking [115,137] but at the expense of high levels of process contaminants such as 3-monochloropropane-1, 2-diol [138]. Instability of Schiff base at acidic pH might be the reason for ACR formation reduction.

Enzymatic approach emerged as a potential approach by means of which ACR reduction could be done. Fermentation can effectively reduce the ACR level before food processing step [138].

Asparaginase is an enzyme distributed in animals, plants, and living organism [139]. It has received "generally recognized as safe" status from the US government. It has also been given a favorable evaluation as a food additive by the Joint FAO/WHO expert committee. It has been known to possess excellent therapeutic properties and clinically acceptable for its antitumor activity. Microbial production was initially reported in *Escherichia coli* and other bacterial strains such as *Corynebacterium glutamicum*, *Erwinia carotovora*, and *Staphylococcus aureus* [140]. It accounts for the 40% of the total worldwide sale of antileukemic and antilymphoma agents [141]. Numerous studies have been emerged monitoring the mitigation of ACR formation by means of asparaginase treatment. Prevent as from DSM and Acrylaway from Novozymes A/S are the two commercially available products used in industry for ACR mitigation [142]. Asparagine hydrolyzes to aspartic acid and ammonia can reduce ACR formation in foods by removal of the precursor asparagines [29,33,143-148]. Blanching can enhance the performance of enzyme [149].

ALTERNATIVE STRATEGIES

High temperature leads to the formation of thermal process contaminants such as ACR, chloropropanols, and furan [150]. Lowering the temperature is an appropriate approach as summarized in above-mentioned studies; also the thermal process is used for drying purposes. Attaining decrease in temperature cannot be considered an effective approach because effective drying cannot be attained. Vacuum

technology provides the accelerated drying process at the lower temperature. Vacuum lowers the boiling point of water and it leads to evaporation at lower temperatures. This way the drying can take place at a lower temperature (below 100°C) without compromising the final product properties [151].

A combined conventional and vacuum baking process effectively reduces ACR formation in biscuits by decreasing the thermal load. The dough was partially baked at 220°C for 2–4 min under usual situation was post-baked under vacuum drying at 180°C and 500 mbar for 4–6 min until desired moisture content was obtained [152]. Frying condition significantly alters the formation of ACR level, since it is the final stage where ACR is formed. Vacuum frying of potatoes slices at reduced oil bath temperature has been demonstrated to reduce ACR level 98% preserving nutritional value [153]. Browning and flavor development caused by the Maillard reaction occur at this step. Frying at high thermal load generates higher ACR [154,155]. As frying occurs at a temperature of 160–195°C, lowering the temperature is the way to reduce ACR, which is more suitable if done under vacuum conditions. 51% ACR content reduction as the frying temperature decreased from 180°C to 165°C and 63% reduction during vacuum frying as the temperature drop was 140–125°C in potato chips of different potato cultivars [156]. Vacuum frying operates below atmospheric pressure, preferably at, <50 Torr [157]. The boiling point of both oils and the moisture in food are lowered due to low pressure [158]. Furthermore, lack of air prevents oxidation which provides food with better nutrition and quality [159-161]. 50% reduction of ACR formation was achieved by vacuum roasting in coffee beans as compared to conventional roasting [162]. Radiofrequency (RF) a promising approach has been introduced as the final drying step to decrease thermal load, doing so desired moisture content is attained in the final product. Earlier, RF has been utilized in food processing such as thawing of frozen foods, tempering, cooking, and roasting successfully [163]. Few studies have been carried out evaluating the effect of RF on ACR formation. The effect of RF heating on ACR formation was investigated in leavened cakes and short dough biscuits. The alternative baking process provided 35% decrease in ACR in short dough biscuits then in baked by conventional heating [164]. Another study examined the effect of RF post-drying of partially baked cookies on the ACR content, texture, and color of the final product. ACR content reduction was reported to be 52.3% and 30% reduction when post-dried in RF oven and subjected to conventional baking in the oven. RF in combination with conventional baking resulted in less surface browning of cookies [165]. Kocadagli *et al.* stated that MRP (Maillard Reaction Products) might be considered as useful recipe modifiers to have less ACR in cookies to be obtained by means of a combined conventional baking and RF post-baking drying process. It delivers acceptable color and texture properties to cookies containing less ACR [166]. Fiore *et al.* reported that the ACR concentration was 250% higher in the conventional cooked samples than in those cooked in a combined RF oven. They concluded that RF cooking could be proposed as an effective mitigation strategy for ACR formation in potato samples [167].

The main motive of the process should be preserving the attributes of the final product which are directly linked to commercial success. For satisfying the market and industrial demand, a need to create new approach is demanded which offers high variability with efficiency.

Infrared radiation processing has become quite popular in recent years and is being utilized for the different thermal process in the industry such as drying, frying, and pasteurization [168]. Infrared drying has been extensively studied in recent years. It offers numerous advantages, such as intermittent energy source, easy control of the process parameters, uniform temperature distribution, and clean operational environment along with space saving [169-171]. IR can be integrated easily with convective, conductive, vibration, freeze, vacuum, and microwave technologies which increase its applicability to a wider extent [166,172-174]. Par-cooking far-infrared heating and dry steam treatments used to make low-fat crisps may also reduce ACR. Specific studies utilizing solely infrared radiation heating in the reduction of ACR are fewer, however, from the perspective of the decrease in thermal load; reduction in degradation and

preserving food quality with less energy requirement, IR heating is an efficient method. A recent study by Omotosho *et al.* evaluated the effect of conventional frying and infrared frying on the ACR formation in *Musa paradisiaca*. IR frying showed a significant reduction in ACR formation in the ripe and unripe sample [175].

CONCLUSION

Cardiotoxicity of ACR is not fully elucidated as only few research papers have demonstrated this toxicity. Evidence suggests that oxidative stress mechanism and altered myocardial gene expression plays a vital role in ACR-induced cardiotoxicity. Accordingly, dietary components which possess antioxidant properties are likely to be protective against ACR-induced cardiotoxicity. The prenatal period may be a window of vulnerability to ACR in humans and efforts should be made to reduce the transplacental exposure of AA to the fetus and pregnant mother. A firm conclusion cannot be drawn based on few studies; more research is needed to establish the cardiotoxicity of ACR.

AUTHORS CONTRIBUTION

Taranbir Singh has majorly performed role in concept, design, literature survey, data and scientific writing.

Ajay Singh Kushwah has provided the help in developed concept and designs the intellectual content, innovations, and also provides scientific advice, guidance, and mentorship in scientific writing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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