REDUCING ACRYLAMIDE EXPOSURE: A REVIEW OF THE APPLICATION OF SULFUR-CONTAINING COMPOUNDS - A CARIBBEAN OUTLOOK

ABSTRACT

Acrylamide, a known neurotoxin, reproductive toxin, genotoxin, probable carcinogen, hepatotoxin, and immunotoxin, has sparked intense curiosity due to its prominent presence in thermally processed, carbohydrate-rich foods. Acrylamide formation occurs via the Maillard reaction at temperatures of ≥ 100ºC. Thorough investigation on acrylamide mitigation through the application of sulfur-containing compounds: to raw materials, and during food processing have been conducted. Although prominent results in acrylamide reduction has been observed, limitations are considered. These limitations involve the social and economic challenges of a population, such as the Caribbean. This study seeks to answer just how effective the application of sulfur-containing compounds are in reducing acrylamide exposure, especially when this applies to a developing region.

Keywords: [Acrylamide, Potato, Wheat, Asparagine, Cysteine, Methionine]

1. INTRODUCTION

Common to all carbohydrate-rich foods thermally processed at high temperatures, is the occurrence of the Maillard reaction (1-3). Resulting from this reaction, are the formation of alluring flavors, as well as the produ various compounds. A particular product of the Maillard reaction which has acquired keen scientific interest, as a r its toxic nature and threat to human safety, is called acrylamide (ACR) (2-propenamide). During food proc acrylamide is primarily formed between the amino acid, asparagine, and reducing sugars: glucose or fructose (1, 2, 4). Additional formation of ACR has occurred: by the heating of asparagine alone (5); by the reaction between asp and other carbonyl sources (6, 7); and through the oxidation of acrolein in the presence of asparagine and ammo The formation of ACR has been documented at temperatures of ≥120ºC, and in some cases, below 100ºC (9). A be physically identified as a white crystalline solid, void of odor and color, with a melting point of 84.5°C and a boiling point of 136°C. It is formed in the lab by the hydration of acrylonitrile, and is soluble in water, methanol, ethanol, and acetone (10, 11). In addition, ACR is identified as a monomer of polyacrylamide, which possesses several uses (3, 10, 12).

Comment [RW1]: Please depict this reaction, and its mechanism in a figure. Include the formation of asparegine.

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ACR has maintained its popularity since the 2002 discovery of its presence at high concentrations in carbohydrate-rich foods (13). Tareke's 2002 publication on the analysis of acrylamide during the heating of different foodstuffs resulted in a series of investigations on the analysis, sources, metabolism, toxicity, and mitigation of ACR (3). Several food samples were analyzed for ACR occurrence. The analysis of raw materials for food preparation, has led to the determination of ACR-forming potential. The analysis of a wide range of processed foods has led to the detection of significant levels of ACR. In raw materials, ACR occurrence is influenced by: soil composition, farming regimes, crop cultivars, and harvest season. In prepared foods, ACR concentrations are influenced by: treatment methods, processing conditions, and product formation (14). Raw materials that have been heavily assessed for the occurrence of ACR, are: wheat grains and potatoes (15-17). Large quantities of ACR can be found in processed foods such as: potato fries, crisp bread, potato chips, cereals, biscuits/crackers, baked goods, coffee, nuts and nut butter (10, 18-22). A wide range of

Caribbean based foods, including: banana chips, fried and roasted breadfruit (A*rtocarpus* altilis), banana fritters, and fried dumplings (fried bake), showed ACR concentrations ranging from 65-3,640 µg/kg (23).

With the use of food consumption data from the Netherlands and USA, short term daily ACR intake was estimated ranging from 0.8-3.0 µg/kg bw per day in the 95th percentile, and extends to 6.0 µg/kg bw per day in the 98th percentile. Long term uncertainty estimates calculated on the basis of food consumption within developed countries indicate a range of 0.3-0.8 µg/kg bw per day (24). An assessment of the consumption of foods containing high amounts of ACR is lacking for many developing regions such as the Caribbean. A comprehensive compilation of the food supply for the Caribbean region illustrates that refined carbohydrates, sugars, and fats are more prevalent than foods such as fruits and vegetables. Although not listed specifically, the categories of foods that are most prevalent are known to contain, and generate high levels of ACR. Food consumption assessment in the Caribbean could aid in providing information on the: types of foods consumed by the population, the safety of the foods consumed, and the correlation between socio-economic and demographic factors (25).

Comment [RW4]: Can the authors show some of these data in the form of a table? Also, include some relevant data about the Caribbean, i.e. amount of land cultivated per year in the region, tons of harvested crops (potatoes, wheat, etc). Other information about preferences in the consumption per capita of the population of the region.

Due to its distinct chemical structure, ACR is able to undergo a number of chemical reactions when it is absorbed by the body. Its reactivity with amino acids, thiols, hydroxyl groups, and DNA centers depends on the infamous Michael-type addition. Eighty-five percent of ingested ACR reacts with key cellular thiols forming mercapturic acid conjugates. These non-toxic metabolites are excreted from the body in the form of urine. ACR undergoes alkylation reactions with thiols of proteins, and adduct formation with hemoglobin (HB) pigments. About 15% of ingested ACR is made active by the cytochrome p-450 (CYP2E1) enzyme. The resulting metabolite formed is called Glycidamide (GA). GA may undergo: hydrolysis, conjugation with light, conjugation with HB forming GA-HB adducts, and may interact with the DNA resulting in genetic mutations (26, 27).

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Former studies have correlated ACR's dietary intake or exposure, and its toxic metabolite glycidamide to the following: neurotoxic effects on humans and rodents (28-30); carcinogenic effects on rodents (3, 26, 31, 32); reproductive effects on rodents (33, 34); and genotoxic effects on rodents and cells of humans (35-37). Current studies have also indicated ACR to be immunotoxic (38, 39), and hepatotoxic (40, 41). Amidst the current progress made on the study of ACR toxicity (42-44), it is still classified as a group 2A probable carcinogen by the International Agency for Research on Cancer (IARC), due to insufficient epidemiological studies relating the induced carcinogenicity of ACR to human populations. However, research is presently ongoing to clarify the potential genotoxicity of ACR, and the mechanisms by which ACR may contribute to induction of carcinogenicity in rodents, and humans (45-49).

Given the span of ACR's toxicity and presence in relatively high quantities of various home-cooked, as well as commercial products, its existence is alarming. Over recent years, literature has presented thorough investigations on the utilization of amino acids and antioxidants on the mitigation of ACR levels and toxicity. Among these groups of additives, are compounds which contain a sulfur atom. A broad spectrum of sulfur-containing compounds involved in the mitigation of ACR include: thiols, thiolsulfinates, thioethers, allyl sulfides and sulfates. In some instances, elemental sulfur can be classified as an additive. Sulfur-containing compounds are characterized as: strong reducing agents, strong fatty acid hydroperoxide detoxifiers, excellent anti-browning agents, cellular detoxification activators, and radical oxygen scavengers. Although such characteristics render these compounds effective agents in the mitigation of ACR levels and toxicity, there are limitations to their utilization.

This review examines the outcomes of studies published between 2007-2018, and in certain instances, comparisons are made to outcomes published in previous literatures. Emphasis is placed on the application of sulfur-containing compounds on the mitigation of ACR levels in: raw materials and food processing, in conjunction with associated limitations. These limitations include the socio-economic challenges faced by the Caribbean region. Hopefully, from this review, light can be shed on avenues for further research in this area of study.

2. THE EFFECT OF SULFUR-CONTAINING COMPOUNDS ON ACRYLAMIDE-FORMING POTENTIAL IN RAW MATERIALS

In a report released in June 2015, The European Food Safety Authority explained the risk assessment of ACR in food. It was mentioned that: fried potato products, coffee, biscuits, crackers, crisp bread, and soft bread contributed the most significantly to ACR exposure among adults, children, and babies. Apart from the storage and processing conditions of these foods, the raw materials from which they are made greatly impacts the formation of ACR (50). Common raw ingredients of these foods include: wheat grains, and potato tubers. Research has been conducted on the occurrence of ACR precursors (mainly glucose and asparagine) in these raw foods, and how their levels can be reduced.

2.1 Wheat

In cereal grains, asparagine makes up about 5% of the total fraction of free amino acids (51). Recent studies have indicated asparagine levels as the limiting factor for ACR production in wheat grains (52). In wheat, asparagine functions as: a transport for nitrogen, a compound for storage, and serves as building blocks for the formation of various wheat proteins (52). A specific mode of action involved in the endeavors of mitigating asparagine concentrations in wheat, is by the comprehension of the correlation between: sulfur content, nitrogen content, asparagine concentrations, and ACR formation (53).

Elmore et al (54) showed that sulfur-deficient wheat-flour contained greater levels of asparagine, producing ACR concentrations six times in excess of the amounts detected in sulfur-sufficient wheat-flour. Headspace analysis of sulfur-deprived, heated flour showed Strecker aldehydes, products of aldol condensations, and alkylpyrazines. On the other hand, sulfur-sufficient flour demonstrated products of sugar degradations: thiophenes, and pyrroles. The products of the sulfur-deprived flour obtained by headspace analysis are reactive intermediates of the Maillard reaction. In the final stage, these intermediates are converted into various colored pigments and flavor compounds, and simultaneously, acrylamide is produced. In contrast, headspace analysis of the sulfur-sufficient flour suggests a lack therein of the Maillard reaction by the degradation of sugars, and the absence of reactive carbonyl intermediates. The author further stated that apart from a correlation established between sulfur concentrations of the heated flour, and the products indicated in the headspace, genetic factors also play an influential role.

Curtis et al (17) showed the assessment of different strains of wheat grains, having differing concentrations of free asparagine; these were analyzed under sulfur-sufficient, and sulfur-deficient conditions. All strains displayed asparagine accumulation under sulfur-deprived conditions; some to a greater extent than others. All strains showed asparagine mitigation under sulfur-sufficient conditions. Additionally, after milling, baking and drying, fractions of the grain samples were analyzed for ACR. A quadratic correlation of R^2=0.9945 was obtained between the two variables, asparagine concentrations and ACR levels. ACR levels, in relation to sulfur content in the wheat grains showed a negative correlation. To further illustrate, 800 μg/g sulfur corresponded to 6.8 μg/kg ACR, while 1.7-3.1 mg/kg sulfur corresponded to 40-76 mg/kg ACR (5). These results suggested that an inverse relationship exists between the presence of sulfur and ACR levels in wheat grains. It was also observed that genetic factors do not affect the ACR mitigating potential presented by sulfur-sufficient conditions, but rather may implicate the potential of the plant to accumulate ACR precursors, namely asparagine.

The success of sulfur-sufficient conditions in mitigating asparagine can be explained by the dependence of asparagine concentrations in plants, on the S:N ratio as opposed to the N level only. Sulfur-deficient conditions resulted in a reduction of prolamins from 51.0% to 27.0% in plants and consequently, an increase in aspartate and asparagine from 5.7% and 5.3% to 19.2% and 18.5%, respectively (55). Prolamins are a group of storage proteins in plants that are made up of cysteine and methionine, and can be found mainly in the seeds of cereal grains. It has been suggested that the accumulation of asparagine results from an attempt to provide alternative nitrogen storage when prolamin production is marred (55). Sulfur fertilizers can be added to the soil or surface of plants, including wheat, in the form of elemental sulfur, ferrous sulfate, or aluminum sulfate to aid in counteracting the accumulation of asparagine (56).

Comment [RW6]: This ratio should be defined as "sulfur to nitrogen ratio (S:N)"

A recent study (2016), was conducted on rye grains; a grain closely related to Triticum (Wheat genus). The results showed that free asparagine increased by 70% in the presence of sulfur-deficient conditions. However, nitrogen application resulted in a three-fold increase in free asparagine levels. Additionally, there was a statistically significant association observed between nitrogen and sulfur, especially under deprived conditions of both elements (57). These results are quite similar to outcomes obtained from former investigations conducted on barley (55), and wheat grains (58). These studies confirm that the effect of sulfur application on asparagine reduction is transparent among various types of grains, and is consequently a better application towards ACR mitigation than nitrogen fertilizers.

2.2 Potatoes

The application of nitrogen-based fertilizers to potatoes, in comparison to sulfur fertilizer application has been investigated in recent years. The application of sulfur to potatoes was deemed unnecessary, due to lack of established benefits. Nitrogen nutrition on the other hand, was observed beneficial for its promotion of vegetative growth, its increase to size and yield, and its canopy senesce. Despite its benefits, previous studies have shown its threat to safety through its contribution to asparagine accumulation (59, 60). In potatoes, asparagine levels account for 33-59% of the total free amino acid (61). Despite such high levels of asparagine, previous studies have denoted reducing sugars as the limiting factor when it comes to ACR formation (16, 59). As of 2017, the Acrylamide Toolbox by Food and Drink Europe (62), illustrated the significance of the ratio of asparagine to total free amino acids in evaluating the formation of ACR in potatoes than was assessed previously. It was further stated that the direct relationship between reducing sugar levels and ACR levels is not consistent. The relative ACR exposure in potato varieties can be indicated more precisely by consideration of the asparagine to free amino acid ratio.

Muttucumaru (63) assessed 13 varieties of potatoes in a field trial; varieties were categorized based on suitability toward methods of production: French fried, chipped, and boiled. Varieties were treated with differing combinations of sulfur and nitrogen fertilizers, and immediate analysis followed harvesting. Results showed a rather conflicting illustration of nitrogen's effect on glucose concentrations, as it differed diffusely within production types and variety types. Sulfur's application demonstrated a clear inverse relationship to glucose concentrations. To further illustrate: a reduction from 0.46-0.34 g/kg glucose was observed with sulfur application ranging from 0-40 kg/ha. This was the first study assessing sulfur's outcome on potatoes in a field trial (63). Other studies have shown that absence of nitrogen increases glucose levels (64), and sulfur results in a decrease in glucose levels in potatoes grown in pots beneath glass (65). All types showed an increase in total free amino acids, including asparagine, due to nitrogen application. These results are synonymous with previous studies (59, 60). On the contrary, asparagine levels in French fried type potatoes remained unaffected by sulfur application, but chipped and boiled type potatoes showed conflicting results. Previously, sulfur demonstrated a decrease in free asparagine levels in pot samples (65). It was suggested by the author that possibly pot based trials did not provide conditions conducive for the effect of sulfur deficiency in asparagine (63). Muttucumaru (63) concluded that nitrogen application on potatoes can influence ACR formation to an extent that depends on types and variety. However, sulfur-based application can mitigate the ACR forming potential induced by high nitrogen application, particularly in French fried varieties, possibly due to its effective reduction of glucose levels. Although beneficial towards the mitigation of ACR, the reduction in glucose levels may affect the palatability of the potatoes, as reducing sugars contribute significantly to the flavor attributes of potatoes (66).

Comment [RW7]: Chronologically, references 59, 60, 64, and 65 were published before 63. I believe that the results should be presented in that order.

According to the Acrylamide Toolbox (2017), current leads being explored in the area of potato and potato tubers include: the development of potato varieties with lower asparagine content, and exploration of factors such as storage, and farming methods (fertilizer regimes) on asparagine levels. The application of both sulfur and nitrogen fertilization can impose conflicting effects on this ratio based on potato variety, and so, no optimum ratio has been established (62). Further investigations on the mitigating potential of sulfur-based fertilizers on asparagine levels in potatoes is needed, in aiding with the precise assessment of its effectiveness toward ACR formation. Additionally, the investigation of sulfur and nitrogen fertilizers and their effects towards asparagine levels, should be extended towards other crops besides wheat or cereal grains, and potatoes. Over the years, demands for gluten-free (lacking prolamin) products has elevated. Various products and baked goods have been processed from gluten-free alternatives (67-69). Competitive gluten-free flour on the market include: cassava, coconut, and most recently, breadfruit. From such an investigation, further insight can be obtained on effective utilization of sulfur compounds to produce raw materials with reduced ACR-forming potential.

2.3 Limitations

2.3.1 Environmental conditions, and genetic factors

The quest of sulfur-containing compounds to mitigate ACR levels through reduction in asparagine concentration, is limited by the natural accumulation of asparagine by plants when they are subjected to stressful conditions. These conditions include: drought and salt stress, the effect of soil contaminated with heavy or toxic metals, and the attack of plants by pathogens (70-73). Not all stresses can be avoided, or identified by farmers until it may be too late. Also, a farmer may induce plant stress indirectly by attempting to prevent it. Muttucumaru (74) showed that irrigation may promote ACR formation in potatoes; it was suggested that farmers irrigate only if necessary as water availability can influence the amino acid, and glucose concentrations in potato tubers.

The implications of environmental factors on asparagine accumulation can be further understood by considering a plant's genetic composition. Asparagine synthetase was investigated thoroughly in wheat, and four asparagine synthetase genes were observed: TaASN1, TaASN2, TaASN3, and TaASN4 (75-77). The most influential gene in wheat is TaASN2. Nevertheless, it is the TaASN1 gene that is expressed in response to saline, drought, and sulfur and nitrogen deficiencies (57, 78). In potato tubers, two genes were identified: StASN1 (found in high concentrations in the tuber), and StASN2 (found throughout the entire plant) (79). So far, a detailed model network has been constructed on asparagine metabolism, illustrating established relationships between stress response, and many genes vital for asparagine metabolism in wheat plants (80). However, some relationships have not yet been established, or understood. While this model network aids in confirming the accuracy of existing relationships, there is room for the exploration of novel relationships. Further applications may involve its use in the genetic engineering of cultivars of wheat grains; asparagine synthesizing genes can perhaps be modified to resist stimulation from certain environmental conditions**.**

2.3.2 Crop yield

The use of sulfur fertilizers is common in the cultivation of cereal grains, particularly wheat, as it promotes grain and protein yield (81). On the other hand, sulfur-based applications are limited in potato tubers. Although they are able to mitigate ACR forming potential, they add no established benefit to crop yield (63, 82). Farmers may not view the purchase of sulfur fertilizers as the reception of good value. Creating educational programs that will enable farmers to understand the correlation between acrylamide-forming potential, and the use of certain fertilizers, or introducing incentives for the purchase of sulfur fertilizers may aid in generating a positive reception towards the use of sulfur fertilizers. Moreover, the investigation of an appropriate S:N ratio fertilizer application that can result in a considerable reduction of asparagine concentrations, and efficient crop yield, may help in creating a win-win outcome for both the public, and the farmers. However, a clear and precise relationship between sulfur and asparagine levels in potato tubers first needs to be established.

2.3.3 Potato palatability

In a chapter written by Jansky (66), it was indicated that sucrose and reducing sugars were key determinants in the attribution of potato flavor. Additionally, flavors obtained from baked potato and potato chips were considered to be caused by the occurrence of pyrazines; these are products of the Maillard reaction (1) (83). Studies have shown that the application of sulfur to potato cultivation is advantageous in reducing ACR formation, by lowering the glucose (63) and asparagine levels (65). Sulfur application could negatively affect the palatability of processed potatoes as a result of a reduction in glucose levels. A reduction in glucose levels means a reduction in the occurrence of the Maillard reaction. This leads to a reduction in the generation of flavor compounds and hence, the palatability of potato and potato products.

2.3.4 Agricultural implications in the Caribbean region.

Wheat grains cannot be cultivated in the Caribbean region. This is due to the lack of an environment conducive to the biology of the crop; a biology which does not favor heavy rainfall and tropical conditions (84). Nevertheless, wheat makes its way to Caribbean mostly in the form of cereal. The diet of developing regions consists of 27% cereal calories, and 3 % protein requirements. Additionally, many people prefer wheat flour as opposed to the traditional flour from staple crop such as cassava, simply because of the certain status attached to it (85). Wheat grain based products such as: bread and wheat flour which are the most prevalent in the Caribbean, are also the products which contribute the most significantly to ACR exposure (18, 22, 50). Although research concerning the application of sulfur in the cultivation of wheat grains have been increasing, there is no guarantee that the wheat products imported into the Caribbean region were produced from wheat grains cultivated under sulfur-sufficient conditions.

Besides the high influx of processed wheat into the Caribbean, Traditional products of cassava such as cassava flour and farine (dried, unprocessed cassava) are still widespread due to affordability. Many islanders have known these products to provide sustenance during times of economic crises (86). Other popular gluten free products emerging on the market include: breadfruit flour, banana flour, and coconut flour (67, 87, 88). There is no existing research on the levels of ACR precursors in the raw materials from which these products are made. The effect of sulfur or nitrogen application on the levels of ACR precursors in the respective raw materials have not been investigated. It is imperative that the variety of raw materials cultivated in the Caribbean be assessed for the levels of ACR precursors. This will encourage more stringent measures on farming regimes and the type of food products generated within the Caribbean. Even if a suitable farming regime was obtained to reduce levels of ACR precursors in raw materials, it would be challenging to implement such a policy across the Caribbean region. In the Caribbean, there is a lack of agricultural policies governing agricultural practices or production for a sustainable development. Instead, a grave dependence of the economy on agriculture has resulted in a short-term vision of economic growth rather than a long term plan for sustainability.

Potato is a staple crop grown widely across the Caribbean region, in contrast to wheat grain. Although it grows best at around 20ºC, it can be cultivated under a wide range of climate conditions. To combat the effects of periodic droughts and the attack of pest and diseases, fertilizers are employed in the cultivation of potatoes. Caribbean farmers mainly employ the use of nitrogen fertilizers (89). According to World Fertilizer Trend and Outlook to 2018, the Caribbean and Latin America were listed as the third highest consumer of Nitrogen fertilizer in the world. The high usage of nitrogen fertilizers in the Caribbean may be due to the ease of accessibility, and production. In the region, nitrogen application is the most prevalent in the form of ammonia fertilizer (89). This type of fertilizer is produced within the region, in Trinidad and Tobago (90). Trinidad and Tobago has an abundant source of natural gas, which is essential for two thirds of ammonia production (89). Sulfur fertilizers on the other hand, are produced in the U.S and may not be readily available to Caribbean farmers. Sourcing from within the Caribbean, as opposed to sourcing from the U.S saves cost (91).

Moreover, agriculture is observed as the primary means of employment in the Caribbean; 16% of the overall employment is linked to agriculture. More specifically, agriculture accounts for employment of 30% in Guyana, 25% in Dominica, 20% in St. Lucia, and 18% in Jamaica. Any decline in agriculture affects the economic and social stability of rural areas. Most of the agriculture within the region occurs in rural areas. If crop yield were to decline, the poverty rate of various Caribbean islands would increase (92, 93). For this reason, nitrogen fertilizers are heavily relied upon for the associated benefit towards tuber size and yield. The use of sulfur fertilizers becomes even less appealing, having no established benefit to crop yield. ACR precursors can be more prevalent in potato tubers cultivated in the Caribbean region.

3.0 THE EFFECTS OF SULFUR-CONTAINING COMPOUNDS ON ACRYLAMIDE MITIGATION IN FOOD PROCESSING

The analysis of the application of sulfur-containing compounds on the mitigation of ACR levels in food processing is conducted under food preparation conditions, and/or in various food matrices. Ismial et al (94) assessed the effects of different soaking treatments on the ACR levels in potato slices, in comparison to the maximum permissible level set by the World Health Organization (WHO). The slices were soaked in: tap water for 15 minutes; distilled water for 60 minutes; acidic solutions, salt solutions, amino acid solutions and phenolic solutions for 60 minutes at room temperature. Frying at 190±5ºC was followed by immediate analysis of ACR levels. L-cysteine, and L-glycine (0.05) were among the most effective in reducing ACR, showing significant reductions of 84.74% and 84.94%.

Similar solutions were used in an assessment of the blanching treatment on potato slices. The treatment was carried out at 65 ºC, for 5 minutes and illustrated that $MgCl_2$ (0.1 M) and L-cysteine (0.05 M) were the most effective in ameliorating ACR levels at 97.97% and 97.17%, respectively (94). Blanching and soaking treatments were effective in decreasing ACR levels in potato chips by 60% due to the leaching of the glucose content (95, 96). Cysteine was effective in the enhancement of ACR reduction by preventing the Maillard reaction possibly through the replacement of asparagine in the interaction with the carbonyl compounds (97). Also, any ACR formed, may have participated in a Michael-type addition with the nucleophilic S atom, and the amino group of cysteine (98). On the contrary, other additives like $MgCl_2$ dries out glucose, preventing its participation in the Maillard reaction (99). The discrepancies in the mitigating ability of cysteine between the soaking treatment, and the blanching treatment may be attributed to the higher temperature conditions administered during blanching. Blanching involves the scalding of the raw material through boiling, whereas, soaking occurs at room temperature. Additionally, Ismial et al (94) illustrated the effects of soaking and blanching on the quality of taste, texture, appearance, odor and color of the potato slices. In both types of treatment, (0.1 M) cysteine showed a relatively poor rating, whereas, (0.05 M) showed a relatively fair rating. The other additives as listed above, were rated as relatively good.

Casado et al (100) analyzed the ACR mitigating potential of various additives in ripened olives, using an alkali-treated olive juice heated at 120ºC for 30 minutes. Among the various salts, amino acids, and antioxidants used, L-cysteine, L-arginine, and sodium bisulfite showed the strongest mitigating ability. The taste and ACR reduction of black, ripened olives were assessed by additional compounds of N-Acetyl-L-cysteine, reduced glutathione, methionine, tea, oregano, rosemary, and garlic. The thiols: cysteine, N-acetyl-L-cysteine, and glutathione were effective, but affected the savor. On the other hand, sodium bisulfite was effective and the savor was unaffected. Arginine, along with garlic showed results that were promising. Sodium bisulfite could be an excellent additive to food as it shows effective acrylamide mitigating potential, without any effect to food quality. However, sulfites' presence in food is questionable, as it has been linked to certain health issues including: dermatitis, diarrhea, asthma, and abdominal pain (101, 102). Its presence as an ingredient, is not permissible in the preparation of certain foods (103-107). Further research is needed in the recognition of suitable additives that are effective in reducing ACR levels, without impeding the health of consumers or affecting the food quality. Other investigations illustrated the effect of garlic's absence and presence, in a low moisture system containing 1.2 mmol of both glucose and asparagine. A 0.05g (mass fraction) of garlic was added to the system, and heating occurred at 200ºC for six minutes. A generation of 674.0 nmol of ACR occurred in the absence of garlic. However, this amount was quickly reduced by 43% in the presence of garlic (108). Garlic is known to contain biologically active sulfur-containing compounds, such as allicin (109).

The mitigation effects of 10 amino acids, including cysteine, and methionine were investigated on 10 μmol ACR in a reaction model system. Investigations occurred after heating at 160ºC, for 15 minutes. At natural pH and an adjusted pH of 7, cysteine displayed mitigating effects of 94.4% and 94.8%, respectively. Asparagine showed the lowest mitigating effect at natural pH, and glutamine, at pH 7. Methionine, although not as successful as cysteine, showed mitigating effects from a value of: 10.28±0.23 µmol to 7.92±0.35 µmol ACR (natural pH), and from 10.11±0.21 µmol to 7.24±0.06 µmol ACR (pH 7) (110). In another study observing the ACR mitigation during a heat treatment of canned coffee, cysteine and dithiothreitol showed positive results. Cysteine showed efficient mitigation of 95% for 6 minutes. However, cysteine in combination with dithiothreitol was unsuccessful (111). The combination of both reactants may have resulted in the oxidation of the sulfhydryl groups of cysteine and dithiothreitol, forming disulfide bonds thus lowering the availability of sulfur atoms to participate in Michael-type additions with ACR.

3.1 Limitations

3.1.1 Unpleasant food taste.

A great limitation of the employment of cysteine in the mitigation of ACR levels is that when added to foods in high levels, the foods become unsavory and in some cases, produces undesirable odors (94, 100, 112, 113). It also hinders bread leavening (112). Due to such limitations, cysteine has not gained popularity in food preparation. Methionine, however, has been explored in terms of its significance as a flavorant in foods (114). Although studies conducted on methionine's impact towards food quality have been inadequate, there was a study on the effect of amino acids on the quality of proteins in Arabian bread, and methionine demonstrated no effect towards protein quality (115). Further exploration into the possibility of adding methionine as an ingredient in food preparation, may result in new approaches toward ACR mitigation.

3.1.2 Reactivity

Studies have shown that the ACR mitigating potential of some sulfur compounds are greater than others. A reason for this outcome may be due to their differences in chemical reactivity. Variation in chemical reactivity have been correlated to size and/or intramolecular forces of attraction. In a study conducted by Bent et al (116), the chemical reactivity of thiols was compared. Measurements were conducted by investigation of the loss of the sulfhydryl group, thus forming the thiolate anion in the presence of ACR over time. Using a tris/HCl buffer system, cysteine displayed a greater chemical reactivity than glutathione (GSH), and captopril, respectively, over the pH range of 7.10-9.10. The results suggested that cysteine's performance was due to its small size and lower thiol dissociation constant. However, GSH is larger than captopril and displayed faster chemical reactivity. Therefore, size could not explain the chemical reactivity between the two. It was observed that significant intramolecular hydrogen bonding was the determinant of the stabilization of the transition state species, ACR-SR$^{\neq}$, of the two thiols. The magnitude of intramolecular hydrogen bonding in GSH was greater than was observed in captopril which increased the nucleophilicity of the S^- of GSH and hence its reaction with ACR.

Also, methionine's lower ACR mitigating potential in comparison to cysteine, can be correlated to its tendency to behave as a precursor of ACR, producing substantial amounts of acrolein in the presence of appropriate reagents (8, 114). Further studies are needed to investigate the chemical reactivity of cysteine, in comparison to methionine, with ACR. This may eventually shed light on the prospect of using methionine as an ingredient during food processing.

3.1.3 Cultural methods of food preparation and food importation in the Caribbean

In the Caribbean, the most prevalent means by which sulfur application can mitigate ACR exposure may be in the preparation of foods. Multiple Caribbean cuisines are heavily prepared with seasonings of vegetables and spices. These culinary influences originated from the Caribbean's history of ethnic and cultural diversity passed on from: Africa, France, Spain, India, the Netherlands, and from the indigenous Amerindians. The most common seasonings used which contains sulfur compounds include: garlic, onions, chives, curry, black pepper and tomatoes. These are added to both home-cooked, and commercial products before processing occurs.

However, there are several challenges to consider. The Caribbean's cultural and ethnic diversity not only influences the ingredients in food preparation, but the method by which foods are prepared. Many common dishes are prepared by baking, frying or roasting; these dishes include: roast and fried bake, roti, banana fritters, roast corn, roast dasheen, roast and fried breadfruit, cassava bread, cornmeal patties, roast and fried plantain, roast and baked cassava and baked potato (117, 118). These methods of cooking occur at higher temperatures than boiling. Acrylamide formation is increased in response to an increase in temperature within the range of 120ºC-180ºC (119). This was highlighted in Bent's work (120) where it was seen that breadfruit roasted and then fried, and breadfruit chipped then fried, produced more acrylamide as the temperature rose from 150ºC- 200ºC.

Additionally, there is the issue of food importation. The Caribbean's market has shifted from a local production of fruits, vegetables, root crops and tubers, to a massive importation of processed foods, wheat and maize products, meat and dairy. Food importation in several Caribbean countries has surmounted a total of US $4 billion, and this is expected to increase by the year 2020 to values of $US8-10 billion (121). This may result in an increase of ACR exposure in the region. The use of seasonings which contain a wide spectrum of sulfur compounds that may be effective in mitigating ACR levels during food processing, encounters two counteracting effects: a) popular or cultural methods of cooking which subjects the food to high temperatures, a condition favorable for ACR formation; b) poor food security, which results in a high influx of imported processed foods, maize and wheat products which adds to ACR exposure in the Caribbean. Considering the lack of awareness in the region on ACR exposure, a threat to safety may subsist, that may be too severe to fathom.

4. CONCLUSION

This review examined the application, and limitations of sulfur-containing compounds on the mitigation of ACR exposure, in raw material and food processing. It is clear from the research reviewed that a wide range of sulfur-containing compounds are successful in the reduction of ACR levels in the various situations mentioned, but the success is accompanied by few challenges worthy of consideration. In raw materials, the success of sulfur-containing compounds in mitigating free asparagine is abased by: the plants' genetic makeup in response to stressful conditions, the palatability of the final product, and addition of the 'useless' sulfur fertilizers to potato tubers, as far as crop yield is concerned. In food processing, the success of sulfur-containing compounds is restricted by: the negative impacts of cysteine concentrations towards food quality, and factors that affect the reactivity of sulfur-containing compounds with ACR. In addition, a Caribbean outlook was included, highlighting the social and economic factors of a developing region in the context of sulfur-containing compounds' application toward the mitigation of ACR exposure. Hopefully, this review contributes towards the global improvement of reducing ACR exposure, and improving food safety and security.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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