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Investigations on the Effect of Antioxidant Type and Concentration and Model System Matrix on Acrylamide Formation in Model Maillard Reaction Systems

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Abstract

The formation of acrylamide in model Maillard reaction systems containing phenolic compounds was examined, with regards to phenolic type, concentration, and model system matrix. In dry glyoxal/asparagine waxy maize starch (WMS) systems, 9 out of 10 examined phenolics demonstrated an inhibiting effect, with the most significant reductions (55–60%) observed for caffeoylquinic acids. In WMS glucose/asparagine systems, examination of three different concentrations (0.1, 0.5 and 1 μmol/g WMS) suggested a 'minimum effective concentration' for epicatechin and caffeic acid, whilst addition of caffeoylquinic acids resulted in dose-dependent acrylamide reduction (25–75%). The discordant results of further studies utilising different matrices (dry and wet-to-dry) indicated that, apart from the nature and chemical reactivity, the matrix and the physical state of the reactants might be important for acrylamide formation.

Keywords: Acrylamide; glyoxal; glucose; antioxidants; phenolics; model system

1 Introduction

The formation of acrylamide, a heat-induced toxicant in certain carbohydrate rich foods has been the subject of ongoing worldwide research activities. Various aspects of acrylamide formation in foods have been studied, including the extent of human exposure and assessment of health risks, chemistry, occurrence and formation mechanisms in foods and possible mitigation strategies (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Zyzak et al., 2003). Suggested effective ways for the reduction of acrylamide in foodstuffs include modification of raw materials or product formulation (Claus, Mongili, Weisz, Schieber, & Carle, 2008), change of pH and heat-processing parameters (Bråthen & Knutsen, 2005) and process technology interventions (Rufián-Henares, Delgado-Andrade, & Morales, 2006). Research on the use of exogenous additives, such as acids, amino acids, hydrogen carbonates, proteins or antioxidants suggested that they could also be an effective means of acrylamide mitigation (Amrein, Schönbächler, Escher, & Amadò, 2004; Zhang, Ying & Zhang, 2008). The use of such additives is, however, conditional: the selected additives should not be regarded as toxic, addition levels should comply with corresponding criteria of food or chemical additives and addition should not affect the food's sensory characteristics.

Antioxidants, particularly phenolics, and antioxidant-rich extracts, are amongst the additives studied as potential acrylamide formation inhibitors. However, studies in model and various food systems indicated that the use of pure phenolic antioxidants or antioxidant extracts had an ambiguous effect on acrylamide formation; it was reported by Vattem and Shetty (Vattem & Shetty, 2003) that oregano phenolic antioxidants stimulated the formation of acrylamide whilst cranberry extracts did not cause any effect when added to fried potato slices. Antioxidants from bamboo leaves have been reported to reduce acrylamide formation in a concentration-dependent manner, when incorporated in thermally processed potato models

(Zhang, Chen, Zhang, Wu, & Zhang, 2006) and cookies (Li et al., 2012), whilst a strong correlation between the concentration of ortho-diphenolic compounds in virgin olive oil and the reduction of acrylamide in fried crisps was also reported (Napolitano, Morales, Sacchi, & Fogliano, 2008). Ismial et al. (Ismial, Ali, Askar, & Samy, 2013) reported acrylamide reduction in potato chips for ferulic, protocatechuic, caffeic and gallic acids and catechin (30–98%). Addition of 1% rosemary extract led to a significant reduction of the acrylamide content in wheat buns (Hedegaard, Granby, Frandsen, Thygesen, & Skibsted, 2008), whilst a slight decrease in acrylamide levels was observed when 0.5% green tea extract was added in a crispbread model system (Capuano et al., 2009). Ou et al. (Ou et al., 2010) reported that whilst the examined antioxidants [*tert*-butyl hydroquinone (TBHQ), butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), ferulic acid, epigallocatechin gallate (EGCG) and vitamin C] did not affect acrylamide in asparagine-glucose model systems, their corresponding oxidation products were able to directly destroy acrylamide and its precursor, asparagine.

Antioxidants have been traditionally used in the food industry in order to prevent product quality deterioration, as well as for maintaining nutritional value by controlling or retarding oxidation. They have also been studied extensively for their health-promoting properties (Crozier, Jaganath, & Clifford, 2009) which has been partly attributed to their ability to scavenge reactive oxygen species (ROS), thus inhibiting the formation of advanced glycation end-products (AGEs). For example, flavan-3-ols from green tea were found to scavenge methylglyoxal (MGO) under simulated physiological conditions (Lo et al., 2006) whereas procyanidins from cinnamon were shown to inhibit protein glycation (Peng et al., 2008). The reactivity of electron-rich phenols towards electrophilic carbonyls was also observed in food systems; condensation reactions between polyphenols and carbonyls, leading to the formation of various phenolic oligomers, polymers and adducts, have been examined in connection to

reduced astringency and colour development in wine during ageing (Pissarra, Mateus, Rivas-Gonzalo, Santos Buelga, & Freitas, 2003;).

On the basis of the reported formation of antioxidant-sugar fragment adducts in model systems, it was hypothesised that, in Maillard reaction systems, phenolic antioxidants may react with sugar fragments and/or reactive carbonyl compounds, form adducts through electrophilic aromatic substitution reactions and thus inhibit acrylamide formation. Significant reductions in Maillard reaction products in model systems with added epicatechin and hydroxycinnamic acids were attributed to similar trapping reactions between phenolics and reactive carbonyl intermediates (Jiang, Chiaro, Maddali, Prabhu, & Peterson, 2009; Lo, et al., 2006; Totlani & Peterson, 2006).

However, when examining the effect of phenolics on acrylamide, positive, negative or no effects were reported, highlighting the antioxidants' ambiguous role on acrylamide formation (Bassama, Brat, Bohuon, Boulanger, & Günata, 2010; Ou, et al., 2010; Zhang, et al., 2008; Zhu, Cai, Ke, & Corke, 2009). These studies differed greatly on many experimental parameters, in that a diverse range of antioxidants has been examined (i.e. flavan-3-ols, hydroxycinnamates), in different concentrations and purity states (chemically pure or part of antioxidant-rich plant extracts) and in different model and food systems (Maillard reaction mixtures, cereal- and potato-based systems). Therefore, it may be suggested that variability in experimental parameters may explain the conflicting observations regarding their efficacy as acrylamide inhibitors.

On the basis of previous reports suggesting carbonyl trapping as an effective means of acrylamide reduction, the objectives of this study were a) to examine the effect of different antioxidants on acrylamide in a model system with glyoxal, a key reactive dicarbonyl implicated in acrylamide formation, b) to compare asparagine-antioxidant-glyoxal

interactions with those of asparagine-glucose-antioxidant systems and c) to evaluate the impact of the matrix on the effect of antioxidants on acrylamide in different model systems.

2 Materials and Methods

2.1 Materials and Chemicals

Asparagine , D-(+)-glucose, glyoxal (40% aqueous solution), ethyl acetate, bromine, potassium bromide, hydrobromic acid (48% aqueous solution), sodium sulfate, sodium thiosulfate, (–)-epicatechin, caffeic acid, ferulic acid and epicatechin gallate were purchased from Sigma-Aldrich (Poole, Dorset, UK) and were ≥ 98% pure. 3-Caffeoylquinic acid (chlorogenic acid, 93.67%), 4-caffeoylquinic acid (cryptochlorogenic acid, 84.3%), 5-caffeoylquinic acid (neochlorogenic acid, 98.8%), 3,4-dicaffeoylquinic acid (isochlorogenic acid B, 86.3%), 3,5-dicaffeoylquinic acid (isochlorogenic acid A, 85.3%) and 4,5-dicaffeoylquinic acid (isochlorogenic acid C, 98.8%), were provided by Nestlé (Orbe, Switzerland). Waxy maize starch (WMS) was obtained from Roquette S.p.a (Cassano Spinola, Italy).

2.2 Model System Preparation and Heating

Three different model systems, waxy maize starch (WMS), wet-to-dry (WTD) and dry (freeze-dried, FD) were used in this study. The choice of model systems was based on simulating the Maillard reaction in the presence or absence of matrix (WMS) at the surface of products during the last stages of baking, while WTD systems were set up to simulate the total effect of baking, taking into consideration water evaporation but in the absence of matrix effects. WMS systems consisted of a solid matrix, resulting from freeze-drying a 5%

WMS slurry containing the reactants. In particular, WMS model systems were prepared using a suspension of WMS in deionised water (5%, 2.5 g/50 mL) with added reactants in aqueous solutions (25 µmol/g WMS for asparagine, glucose or glyoxal and 0.1, 0.5 or 1.0 µmol/g WMS for each of the phenolic compounds tested). The suspension was then gelatinised in a shaking water bath set at 90 °C for 5 min, cooled rapidly in an ice bath and aliquots (5 g) of the resulting homogenous slurry were transferred to 20-mL SPME vials (Chromacol Ltd., Welwyn Garden City, UK). The samples were frozen at −18 °C before being freeze-dried for 72 hours to a final moisture of 0.2% (Koutsidis et al., 2008). WTD and FD model systems were prepared by adding aliquots of aqueous solutions containing the reactants (2 mL total volume), in SPME vials. The FD samples were then sealed, frozen (−18 °C) and subsequently freeze-dried for 48 hours while the WTD (liquid solutions) were heated immediately after preparation. All samples were prepared in triplicate. Dry systems (WMS and FD) were sealed using metal caps with 1.5 mm silicone/PTFE septa whilst liquid model systems (WTD) remained unsealed throughout the heating process to allow for water evaporation. All samples were heated accurately at 160 °C using a Julabo heating tank (Julabo, Seelbach, Germany) containing Rhodorsil (47 V 20) heat transfer fluid (VWR, Lutterworth, UK). A Julabo ME circulator provided accurate and consistent temperature control within the heat transfer fluid (±1 °C). Following heating, model systems were rapidly cooled by submersion in an ice bath for 5 minutes.

2.3 Acrylamide Extraction and Analysis

All samples were prepared for acrylamide analysis using a direct extraction-bromination approach (Koutsidis et al., 2008). The brominating reagent (1 L) was prepared with potassium bromide (400 g), hydrobromic acid (20 mL), saturated bromine solution (320 mL) and deionised water.

For the WMS samples, the matrix was pulverised, aliquots (0.1–0.2 g) were accurately weighed, 720 ng (300 µL) of internal standard (1,2,3-^{13}C- acrylamide, 2400 ng/mL) and 5 mL of brominating reagent were added and the samples were stirred for 1 hour and then left to brominate overnight. For the FD and WTD samples, internal standard (300 µL) and 3 mL of brominating reagent were directly added in the sample vial, mixed vigorously and left overnight to brominate. Each sample was then neutralised with 3–4 drops of sodium thiosulfate (1 M) and extracted twice with 4 mL of ethyl acetate. The combined organic phase was collected and subsequently evaporated over granular anhydrous sodium sulfate in a TECHNE sample concentrator coupled to a Dri-Block DB-3D block heater (45 °C), under a gentle stream of nitrogen to a final volume of 0.5–1 mL. GC-MS analyses were performed on a Thermo Electron Co. Focus GC coupled to a Thermo DSQ mass spectrometer (Thermo Electron Co., Hemel Hempstead, UK) and an AI-3000 autosampler (Thermo Electron Co.). Aliquots of the ethyl acetate extracts (2 µL) were injected onto a DB-17MS (30 m × 0.25 mm i.d. × 0.15 µm film thickness; Agilent) column in splitless mode at 250 °C. The oven temperature was set at 110 °C for 2 min, raised at 8 °C/min to 200 °C and then at 15 °C/min to 300 °C. The carrier gas flow was 1 mL/min. The mass spectrometer was operated in the selective ion (SIM) mode (*m/z* 106, 108, 150, 152, 153, 155). The transfer line and ion source temperatures were held at 275 °C and 250 °C, respectively. The ion *m/z* 155 was used to quantify brominated $^{13}C_3$-acrylamide, and the ions *m/z* 150 and 152 were used to quantify brominated acrylamide.

2.4 Statistical Analysis

Experimental data from acrylamide analysis are shown as mean ± SD. Statistical analyses were carried out using SPSS (IBM SPSS Statistics for Windows, Version 19.0, New York, NY). Normality was determined using the Shapiro-Wilk test. One-way ANOVA with post-

hoc multiple comparisons (Tukey's test) was used to determine significant differences between means.

3 Results and Discussion

3.1 Acrylamide formation in asparagine-glyoxal model systems with added antioxidants

The hypothesis that antioxidants would react with carbonyl intermediates and prevent acrylamide generation through the formation of adducts was tested by performing screening experiments in model systems with asparagine and glyoxal. Glyoxal, a reactive α-dicarbonyl sugar fragment, was selected for this reaction because it has been shown to be an important acrylamide precursor by several authors (Amrein, et al., 2004; Zyzak, et al., 2003) and its elimination in dry waxy maize starch (WMS) systems has been closely correlated to acrylamide formation (Koutsidis, et al., 2008).

The efficacy of selected antioxidants as potential acrylamide formation inhibitors (by reacting directly with glyoxal) was investigated in low-moisture WMS systems with added asparagine, glyoxal and pure antioxidants heated at 160 °C for 15 minutes. The effect of antioxidants was investigated at 1 μmol/g WMS, while asparagine (Asn) and glyoxal (GO) were both at 25 μmol/g WMS. A dry WMS model system with added equimolar amounts of asparagine and glyoxal (25μmol/g WMS) was used as the reference (Figure 1).

The examined antioxidants were (–)-epicatechin (EC), caffeic acid (CA), ferulic acid (FA), epicatechin gallate (ECG), 3-caffeoylquinic acid (3CQA), -caffeoylquinic acid (4CQA) , 5-caffeoylquinic acid (5CQA), 3,4-di-caffeoylquinic acid (3,4DCQA), 3,5-di-caffeoylquinic

acid (3,5DCQA) and 4,5-di-caffeoylquinic acid (4,5DCQA). The selection of antioxidants was based on both structural differences and differences in antioxidant activity from caffeic acid (1.3 mM TEAC, Trolox equivalent antioxidant capacity) to epicatechin gallate (4.9 mM TEAC) (Crozier, et al., 2009). Moreover, investigations on different isomers (in the case of CQAs and DCQAs) also allowed for testing for chemical structure effects.

Addition of antioxidants (Fig. 1) had a significant effect on all examined systems ($p < 0.05$), compared to the reference sample. Epicatechin and epicatechin gallate decreased acrylamide formation by 25% and 30%, respectively, whereas caffeic acid decreased acrylamide content by 20%. A significantly more pronounced mitigation effect was observed in the case of model systems with caffeoyl- and dicaffeoyl-quinic acids (55–60% reduction), while addition of ferulic acid had a small but statistically significant stimulating effect on acrylamide (15%).

Epicatechin and epicatechin gallate are flavan-3-ols, abundant in teas, fruits, vegetables and wine (Crozier, et al., 2009), and have been studied extensively for their strong antioxidant properties. Their observed inhibitory effect on acrylamide formation could be attributed to a direct carbonyl-trapping property; it was reported that epicatechin may directly react with glyoxal and methylglyoxal to form adducts (Lo et al., 2006; Totlani & Peterson, 2006) in aqueous model systems, whereas under low moisture conditions epicatechin was found to form adducts primarily with C_6 sugar moieties, i.e., 3-deoxyglucosone (Totlani & Peterson, 2007). The acetaldehyde-induced oligomerisation of epicatechin and formation of ethyl-bridged adducts has also been shown (Doco, Es-Safi, Cheynier, & Moutounet, 1996). Moreover, catechin, epicatechin's epimer, has been shown to react with several different aldehydes in a wine model system, resulting in the formation of adducts linked by the alkyl/aryl group bridge derived from the respective aldehyde (Pissarra et al., 2003). A similar carbonyl-trapping property can be attributed to epicatechin gallate, which has also been

reported to form adducts with dicarbonyls in model systems (Lo et al., 2006; Noda & Peterson, 2007). The only structural difference between the two flavan-3-ols is located in the B ring (epicatechin gallate is epicatechin's ester with gallic acid). Since model systems with both compounds did not have significantly different acrylamide content ($p > 0.05$), it can be suggested that modification of the B ring did not significantly influence their acrylamide mitigation properties.

Hydroxycinnamic acids are a major class of phenolic compounds and are ubiquitously found in plants i.e. coffee, artichoke, pear, basil, thyme and oregano. Caffeic acid, which occurs in foods mainly as an ester with quinic acid (caffeoylquinic or chlorogenic acid) and ferulic acid are two major representatives of hydroxycinnamic acids. Both caffeic and chlorogenic acids have been reported to have anti-mutagenic and anti-carcinogenic properties *in vitro* (Crozier, et al., 2009). In this experiment, caffeic acid and all its examined derivatives with quinic acid (molar ratio of quinic: caffeic acid of 1:1 or 1:2, for CQAs and DCQAs, respectively) yielded significantly lower acrylamide levels compared to the reference system, whereas ferulic acid had a stimulating effect. These results are in agreement with a number of studies highlighting the carbonyl-trapping properties of hydroxycinnamates in glycation systems; in a study by Gugliucci et al. (Gugliucci, Bastos, Schulze, & Souza, 2009), caffeic and neochlorogenic acid (5-CQA) were reported to be effective anti-glycation agents in protein glycation model systems with methylglyoxal. DCQAs were also found to be potent anti-glycation agents in methylglyoxal-mediated protein systems; based on their findings, Cui et al. (Cui et al., 2009) suggested that the number of caffeoyl groups and their position in the quinic acid moiety might be important for the inhibition of AGE formation. In this experiment, addition of CQAs and DCQAs in the glyoxal/asparagine model system resulted in the lowest acrylamide levels for all examined antioxidants, indicating a strong glyoxal-trapping ability. However,

significant differences in acrylamide were only observed between caffeic acid and CQAs and DCQAs (CQAs and DCQAs were more effective in mitigating acrylamide).

Studies in glucose/glycine model systems with added ferulic acid showed that it can undergo decarboxylation, exposing the vinyl group, which could, in turn, undergo various pericyclic reactions with Maillard intermediates (in low moisture conditions) or form adducts with C_2 and C_3 sugar fragments, by electrophilic aromatic substitution reactions (in aqueous model systems) (Jiang, et al., 2009). In this experiment, however, ferulic acid had a stimulating effect on acrylamide formation. This was in agreement with previous studies reporting a significant increase (25%) in acrylamide in sealed aqueous model systems with asparagine, glucose and ferulic acid heated at 200 °C, (Bassama, et al., 2010) and a moderate increase in acrylamide (4.5%) (Zhu et al., 2009). Moreover, ferulic acid was previously reported as an inefficient carbonyl scavenger in glycation systems with methylglyoxal (Hu, Liu, Chyau, & Hu, 2012). Thus far, mechanistic explanations for the acrylamide-stimulating behaviour of ferulic acid have not been elucidated.

3.2 Acrylamide formation in asparagine-glucose WMS model systems with added antioxidants

The effect of phenolics on acrylamide formation in an asparagine/glucose WMS model system was investigated. Glucose is a direct precursor of glyoxal and other dicarbonyls and has been studied extensively with regards to its role in Maillard-induced acrylamide formation (Mottram, et al., 2002; Stadler et al., 2002; Zyzak, et al., 2003). Since significant reductions in acrylamide were recorded when 1 µmol/g WMS was used in systems with glyoxal, the effect of antioxidants was examined at 1 µmol/g WMS and at two lower concentrations, 0.1 and 0.5 µmol/g WMS. Asparagine (Asn) and glucose (Glu) levels were

kept at 25 µmol/g WMS while dry equimolar asparagine-glucose WMS model systems (25 µmol/g WMS) were prepared as a reference (Figure 2).

Similarly to glyoxal-containing model systems, addition of epicatechin at 1 µmol/g WMS in systems with glucose reduced acrylamide formation by 25% ($p < 0.05$). A similar decrease was observed when 0.5 µmol/g WMS was used, whereas further reduction by a factor of 10 in epicatechin concentration (0.1 µmol/g WMS) did not have a statistically significant effect on acrylamide (albeit a 13% reduction). Caffeic acid appeared to significantly decrease acrylamide only at a 1 µmol/g WMS level ($p < 0.05$) by 15%, which was also similar to the reducing effect observed in systems with glyoxal (18% reduction). However, decreasing its concentration to 0.5 and 0.1 µmol/g WMS did not have a significant effect on acrylamide formation. Based on these results it can hypothesised that the observed concentration-dependent effects, indicating a 'minimum effective concentration level' for epicatechin and caffeic acid, can be attributed to their previously studied carbonyl-trapping properties (Gugliucci, et al., 2009; Lo, et al., 2006; Totlani & Peterson, 2006).

Similarly to systems with glyoxal, addition of caffeoylquinic acids in glucose-containing systems resulted in reduced acrylamide formation for all the examined concentrations (25–75%), albeit in a non-linear manner. The highest reduction (75%) was observed in the case of 4-CQA, irrespective of the addition level, whereas samples with added 3-CQA or 5-CQA also reduced acrylamide levels significantly (25–55%). These observations indicate that, apart from antioxidant type and concentration, structural differences might also play an important role in acrylamide formation/elimination reactions. However, further studies are required to elucidate such structure–effect relationships.

Previous investigations have reported variable antioxidant-induced effects on acrylamide in systems with phenolics. In a study by Zhu et al. (Zhu, et al., 2009), similarly to the results of

this experiment, it was shown that (+)-catechin (epicatechin's epimer), caffeic and chlorogenic acid reduced acrylamide in a model aqueous system by varying degrees, whereas ferulic acid showed a slight increasing effect. A concentration-dependent inhibiting effect for epicatechin in an aqueous model system was also reported (Hedegaard, Granby, Frandsen, Thygesen, & Skibsted, 2008), whereas Kotsiou et al. (Kotsiou, Tasioula-Margari, Capuano, & Fogliano, 2011) reported an acrylamide-reducing effect of several phenolics, including caffeic and ferulic acid, in an emulsion system. In contrast, Bassama et al. (Bassama, et al., 2010) reported that addition of phenolics in an aqueous glucose/asparagine system did not have an effect on acrylamide formation apart from ferulic acid, which had a promoting effect. In the present work, ferulic acid did not have a significant effect on acrylamide at all three examined concentrations ($p > 0.05$). Cai et al. reported that chlorogenic acid added in an asparagine/glucose Maillard reaction system significantly promoted acrylamide formation mainly through increasing HMF formation, decreasing the activation energy for conversion of 3-APA to acrylamide, and inhibiting its elimination. Its quinine derivative, however, decreased acrylamide formation (Cai et al., 2014).

Addition of epicatechin gallate and DCQAs also did not have a significant effect on acrylamide levels in asparagine-glucose WMS model systems irrespective of the addition level. These compounds have not been previously studied as potential acrylamide inhibitors; however, discrepancies in their acrylamide mitigating effect depending on the carbonyl source might be explained by differences in reaction kinetics. Whilst the significant acrylamide mitigation effects observed in systems with glyoxal could be attributed to the direct carbonyl trapping properties of phenolics, in equimolar glucose-asparagine systems, dicarbonyls are not readily available; hence the kinetics of acrylamide formation would be slower and less dependent on carbonyl formation/elimination as both glucose and its fragments will be competing for the available asparagine. Moreover, it is expected that the

overall net effect of the presence of phenolic compounds on acrylamide would also depend on the reactant ratios and kinetics of their decomposition.

3.3 Influence of Model System Type/Matrix

Several model systems have been used in acrylamide formation studies under simplified conditions, simulating various cooking processes i.e. dry systems (microwave or oven conditions), semi-dry (toasting and baking conditions), oil systems (frying conditions), emulsions (multiphase systems) and aqueous systems. Much research has been conducted on dry or low moisture systems because under these conditions, acrylamide formation is accelerated (Jin, Wu, & Zhang, 2013). Sealed, freeze dried mixtures of glucose and asparagine, and low-moisture, WMS systems (simulating starchy foods) have been previously used for the study of acrylamide formation, in oven or microwave conditions (Koutsidis, et al., 2008; Koutsidis et al., 2009), whereas unsealed, semi-dry WTD models were considered as a simulation for toasting and baking conditions (Jin, et al., 2013). In order to assess the extent of matrix effects on the efficacy of phenolic compounds to mitigate acrylamide formation unsealed, 'wet-to-dry' (WTD) model systems and matrix-free dry systems (FD) were also studied. In the former, volatile dicarbonyls would readily escape while reaction kinetics would be much slower due to dilution and temperature effects. In particular and contrary to low-moisture, sealed WMS systems, where the core temperature rapidly reached the desired value of 160 °C in less than two minutes, WTD systems involved heating of an aqueous equimolar asparagine/glucose or asparagine/glyoxal (25 μmol) and antioxidant (1 μmol) mixture of reactants (2 mL) at 160 °C. During heat treatment, the temperature of the reaction mixture increased from room temperature to 100 °C, remained steady at 100 °C until complete water evaporation and then increased rapidly to 160 °C. A reaction mixture with

only asparagine/glucose or asparagine/glyoxal was chosen as the reference. Figure 3 shows the formation of acrylamide in WTD model systems with glyoxal and glucose, respectively.

In glucose-asparagine WTD systems all antioxidants exhibited significantly smaller effects compared to WMS systems, with those effects ranging from negligible increases to a 16% reduction for 3-CQA and 4-CGA. Addition of epicatechin, caffeic and ferulic acid did not have a significant effect on acrylamide formation in WTD systems ($p > 0.05$), irrespective of the carbonyl source used, whereas a small but statistically significant increase in acrylamide was recorded for epicatechin gallate in the glyoxal-containing systems (12%, $p < 0.05$). Differences in acrylamide formation observed in the case of the composite hydroxycinnamates further suggested that structural differences might play an important role in WTD systems; whilst all three examined CQAs reduced acrylamide in a slight but significant manner in systems with glucose only 4-CQA had a decreasing effect in systems with glyoxal (Fig. 3; 29%, $p < 0.05$). DCQAs, on the other hand, inhibited acrylamide formation by 15–20% ($p < 0.05$) in systems with glyoxal but did not appear to have a significant effect in systems with glucose.

In contrast to WMS reference systems, in which the acrylamide content was similar for both glucose- and glyoxal- containing systems (approx. 8 nmol/µmol Asn), the mean acrylamide value for the glyoxal-containing WTD reference model system was 0.25 nmol/µmol Asn, a seven-fold decrease from the corresponding glucose-containing model system (1.73 nmol acrylamide/µmol Asn). The formation of lower amounts of acrylamide is attributed to glyoxal's low boiling point (51 °C) and its partial loss through evaporation.

The observed differences in the ability of phenolics to reduce acrylamide between the two model systems, suggest that matrix differences might affect their potential to participate in acrylamide formation-elimination reactions: in WMS systems, when glyoxal was used as the

carbonyl source, all antioxidants apart from ferulic acid reduced acrylamide whereas in WTD systems only the DCQAs and 4-CQA had a reducing effect; a stimulating effect was also recorded for epicatechin gallate. When glucose was used as the carbonyl source, epicatechin, caffeic acid and 4-CQA had a reducing effect on acrylamide in WMS systems, whilst all three examined CQAs had a reducing effect in the corresponding WTD systems.

Acrylamide formation results in various matrices indicated that, apart from the nature and chemical reactivity, the model system employed (corresponding to differences in matrix, moisture content and time-temperature heating profiles) and the physical state of the reactants might play an important role on acrylamide formation/elimination reactions. This has been observed in studies with antioxidants: Bassama et al. (Bassama, et al., 2010) reported that addition of phenolic antioxidants (gallic, caffeic, ferulic acids) in an aqueous Maillard model system did not have an effect on acrylamide formation whereas Kotsiou et al. reported a decrease when the same antioxidants were added in an emulsion system (Kotsiou, et al., 2011).

Possible matrix effects on the efficacy of antioxidants were further examined utilising a dry model system (FD), in the absence of water. In those model systems, all reactants (25 µmol glucose and asparagine, 1 µmol antioxidant) were added to the reaction vessel in the form of aqueous solutions, frozen and lyophilised to afford a moisture-free model system, allowing for the study of the impact of antioxidants on acrylamide in the absence of a matrix. All samples were sealed and heated at 160 °C for 15 min; their acrylamide content is shown in Figure 4 together with the results from the WTD systems for comparison purposes.

The results further highlighted the effect of the system matrix on the efficacy of antioxidants as potential acrylamide inhibitors. Model systems with epicatechin, epicatechin gallate, caffeic acid, 3-CQA, 5-CQA and 4,5-DCQA had a slight but significant decreasing effect on

acrylamide content by 15–20% ($p < 0.05$), compared to the reference system, whereas the acrylamide content of systems with 4-CQA, 3,4-DCQA and 3,5-DCQA was not significantly different to the reference. Ferulic acid, on the other hand, increased acrylamide by 20%. Even though ferulic acid's stimulating effect has not yet been explained, it was proposed that certain antioxidants may promote acrylamide formation by acting as donors of carbonyl groups; Hamzalıoğlu et al. reported that curcumin formed acrylamide through a direct reaction with asparagine and suggested that antioxidants with a carbonyl group or their oxidation products having carbonyl moieties, as well as other bioactive carbonyls, such as vanillin, silymarin, ascorbic acid and dehydroascorbic acid, may react directly with asparagine to form acrylamide (Hamzalıoğlu & Gökmen, 2012; Hamzalıoğlu, Mogol, Lumaga, Fogliano, & Gökmen, 2013). Recently, Zhang et al. reported a non-linear relationship between the addition levels of flavone glycosides and inhibitory rates of acrylamide formation in the Maillard reaction, with the maximum inhibitory rate (25.3–63.5%) observed at 10^{-9} mol/L for all flavone glycosides, whereas structure–activity analyses showed that both the number and position of phenol hydroxyl functional groups play an important role in their inhibition properties (Zhang, Chen, Cheng, Jin, & Zhang, 2014).

3,4-DCQA and 3,5-DCQA yielded similar acrylamide levels to the reference system in both WTD and FD asparagine-glucose systems while erratic results were obtained for 4,5-DCQA, EC and ECG, demonstrating statistically significant reductions in acrylamide levels in FD systems but not in WTD. FA promoted acrylamide formation in FD systems while 3-CQA and 5-CQA yielded similar reductions in both WTD and FD systems and 4-CQA reduced acrylamide levels only in WTD systems. Moisture content, which was shown to have a significant effect on acrylamide formation in food systems, appeared to be an important differentiator in model systems, significantly affecting the model system's heating profile, reactant mobility and thus the chemical reactions taking place. In a study by Capuano et al.

(Capuano, Oliviero, Açar, Gökmen, & Fogliano, 2010) in oil systems with catechin, the large difference in acrylamide content between the two systems (with 4% and 16% moisture, respectively) was attributed to the different time–temperature profiles, caused by the difference in water contents, which in turn affected catechin's distribution and stability/degradation, as well as its reaction with acrylamide or intermediates. Previous studies in binary asparagine/sugar systems have shown that acrylamide formation depended on the physical state of the reaction system as well as the presence of water and its availability, which directly affected the molecular mobility and in turn, the participating reaction kinetics (Bråthen & Knutsen, 2005). Based on these studies, it could be suggested that addition of antioxidants, and subsequently their reactivity with the model system's components, i.e. potential adduct formation with glucose fragments, could also be affected by the system's matrix, ultimately redirecting the associated chemical pathways, i.e. the Maillard reaction (Jiang, et al., 2009; Totlani & Peterson, 2006; Zhu, et al., 2009). Since reaction kinetics are thought to be dependent on the model system, this could explain the different behaviour of a given antioxidant in different model systems. Another possible explanation could be that the heat treatments may have altered the state of antioxidants at varying degrees, by causing them to decompose or degrade under high temperatures, rendering them unable to take part in the occurring chemical reactions (Jin et al., 2013).

4 Conclusions

The present work examined the effects of antioxidants on acrylamide formation in model Maillard reaction systems with regards to antioxidant type and concentration and model system matrix. Reduced acrylamide levels in waxy maize starch (WMS) model systems with added glyoxal as the readily-available carbonyl source suggested a possible inhibiting effect through antioxidant-glyoxal interaction and binding; this effect was significantly more

pronounced in the case of relatively more composite antioxidants, such as caffeoyl- and dicaffeoyl- quinic acids, indicating that different types of antioxidants, with different structures, exhibit varying degrees of carbonyl-binding properties. Structural differences and concentration effects were more evident when glucose was used as the source of non-readily-available carbonyls with caffeoylquinic acids, and 4-CQA in particular, exhibiting significant mitigating activity. Model system matrix effects were also studied in dry (FD) and wet-to-dry (WTD) systems. In such systems the acrylamide mitigating effects were generally smaller. The results highlighted the complexities governing the interactions between antioxidants and acrylamide formation/elimination reactions, leading to variable results depending on the system matrix, antioxidant type and antioxidant/reactant concentration and suggest that these parameters might be partly responsible for the discordant effects reported in the literature.

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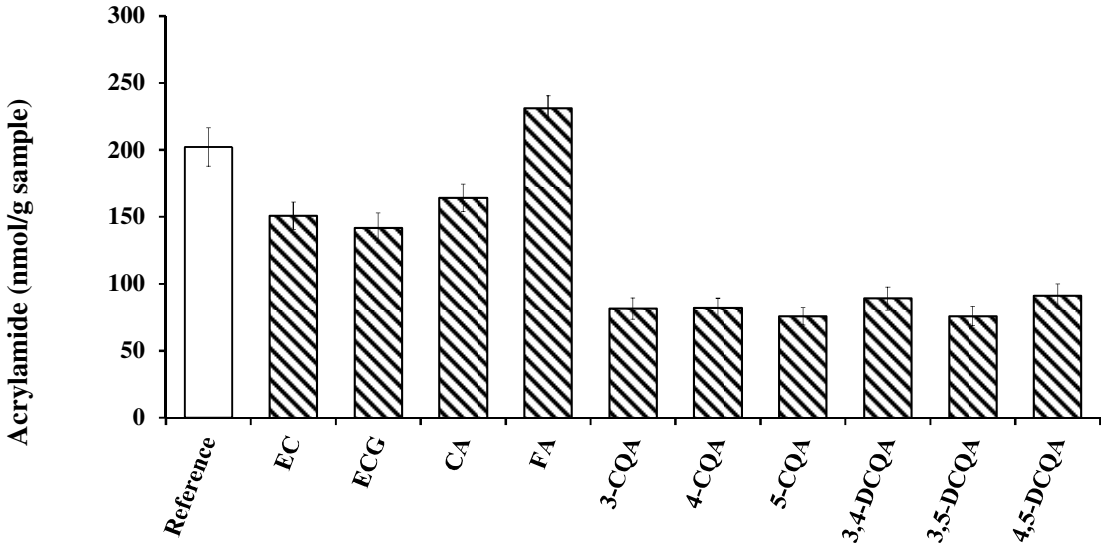
Figure Captions:

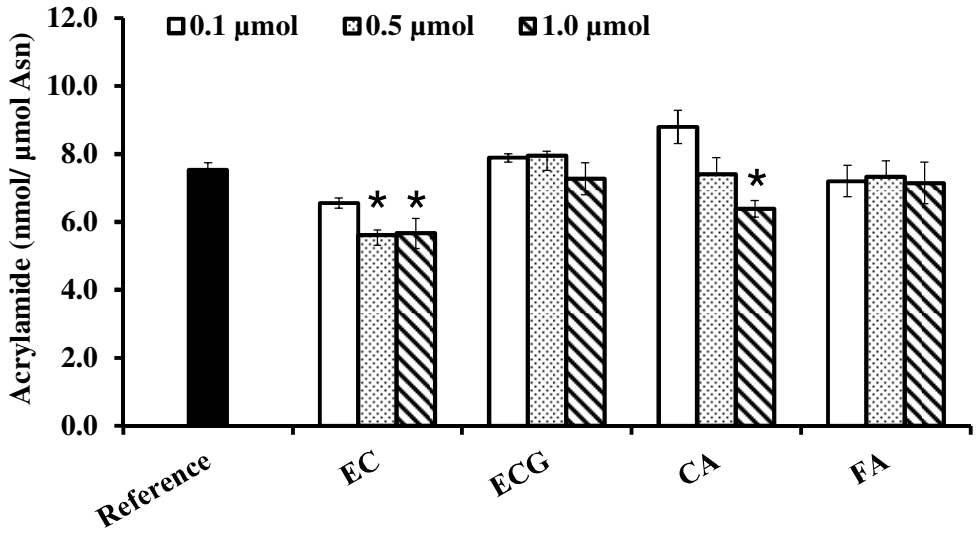
Figure 1: Acrylamide formation in freeze-dried waxy maize starch (WMS) model systems containing equimolar amounts of asparagine and glyoxal (25 µmol/g WMS) and antioxidant (1 µmol/g WMS), heated for 15 minutes at 160 °C (n = 3). All treatments resulted in statistically significant differences ($p < 0.05$) from the reference value.

Figure 2: Acrylamide formation in sealed, freeze-dried (WMS) model systems containing equimolar amounts of asparagine and glucose (25 µmol/g WMS) and antioxidant (0.1, 0.5 and 1 µmol/g WMS), heated at 160 °C for 15 minutes (n = 3) [(*) indicates statistically significant differences ($p < 0.05$) from the reference value].

Figure 3: Acrylamide formation in unsealed wet-to-dry (WTD) model systems containing (**Asn-GO**) asparagine (25 µmol), glyoxal (25 µmol) and antioxidant (1 µmol for EC, ECG, CA, FA, 3-, 4- and 5-CQAs, 3,4-, 3,5- and 4,5- DCQAs (n=3); (**Asn-Glu**) asparagine (25 µmol), glucose (25 µmol) and antioxidant (1 µmol), heated for 15 minutes at 160 °C (1 µmol for EC, ECG, CA, FA, 3-, 4- and 5-CQAs, 3,4-, 3,5- and 4,5- DCQAs) n = 3 [(*) indicates statistically significant differences ($p < 0.05$) from the reference value].

Figure 4: Acrylamide formation in sealed dry model systems (FD) and Wet-to-Dry (WTD) systems containing asparagine (25 µmol), glucose (25 µmol) and antioxidant compounds (1 µmol), heated at 160 °C for 15 minutes (n = 3) [(*) indicates statistically significant differences ($p < 0.05$) from the reference value].

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Acrylamide (nmol/ µmol Asn)
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Reference
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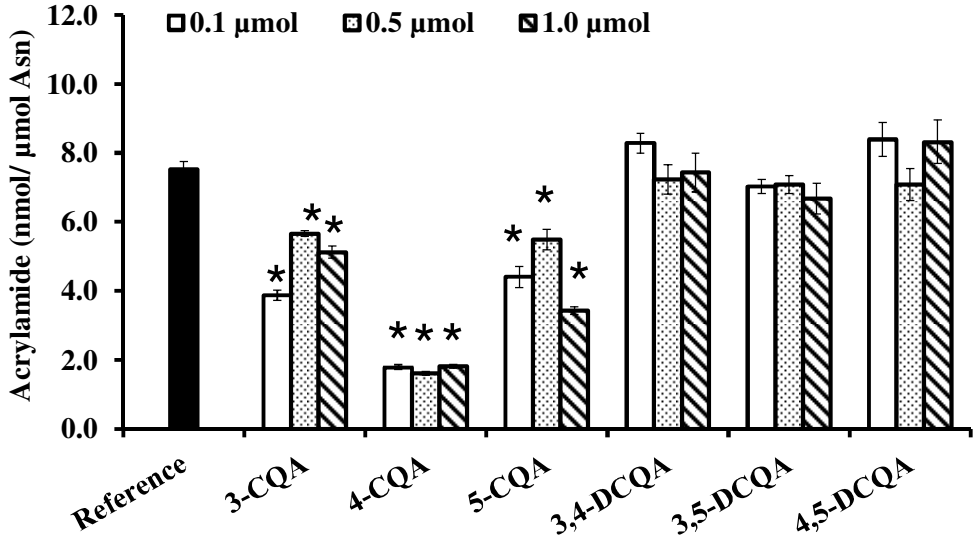
ECG
CA
FA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Acrylamide (nmol/ µmol Asn)
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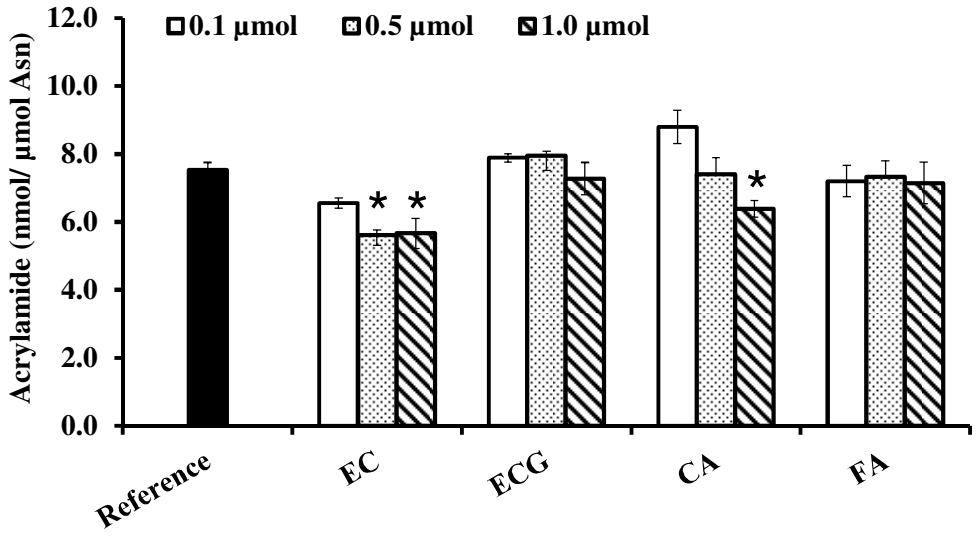
5-CQA
3,4-DCQA
3,5-DCQA
4,5-DCQA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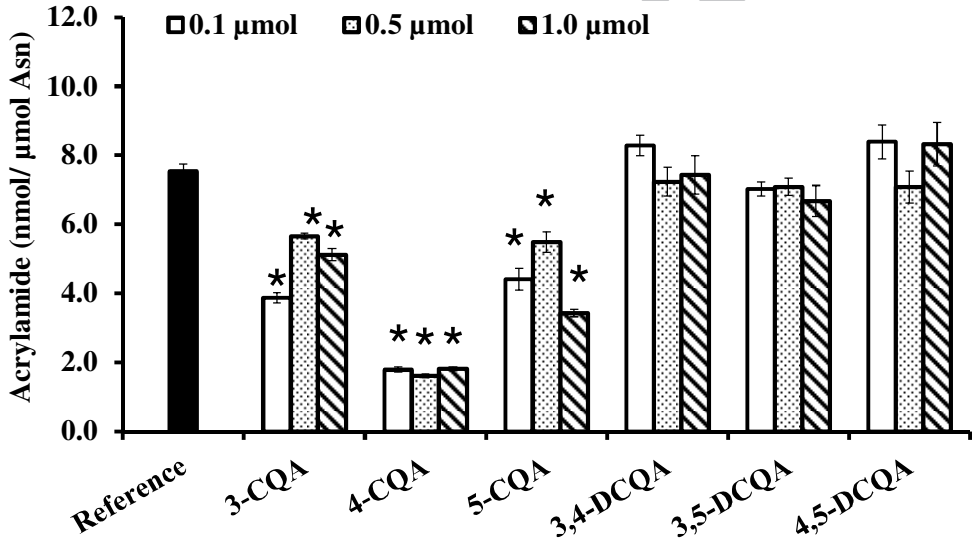
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Acrylamide (nmol/ µmol Asn)
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Reference
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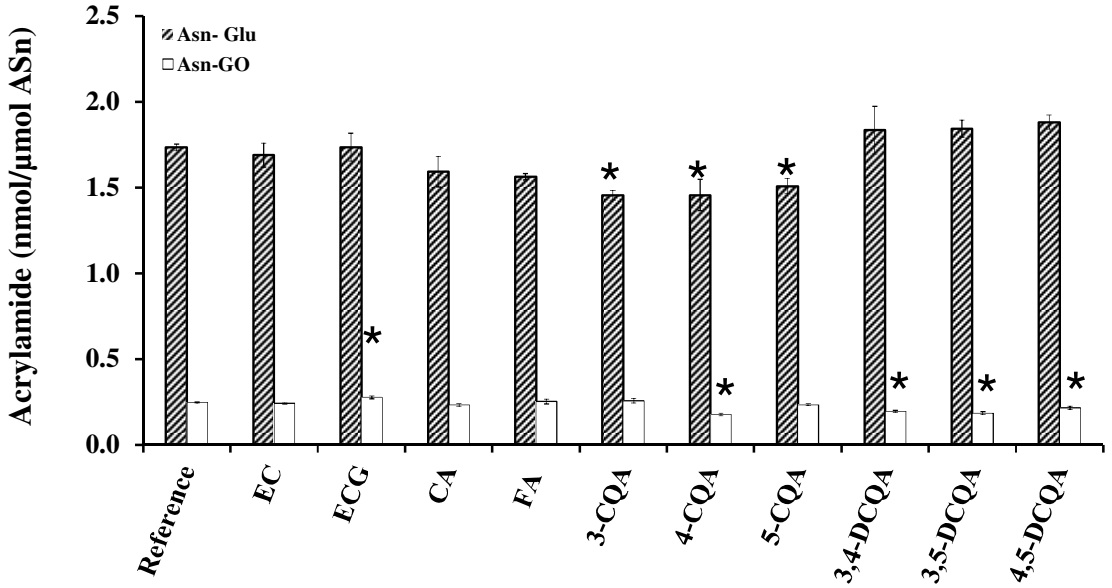

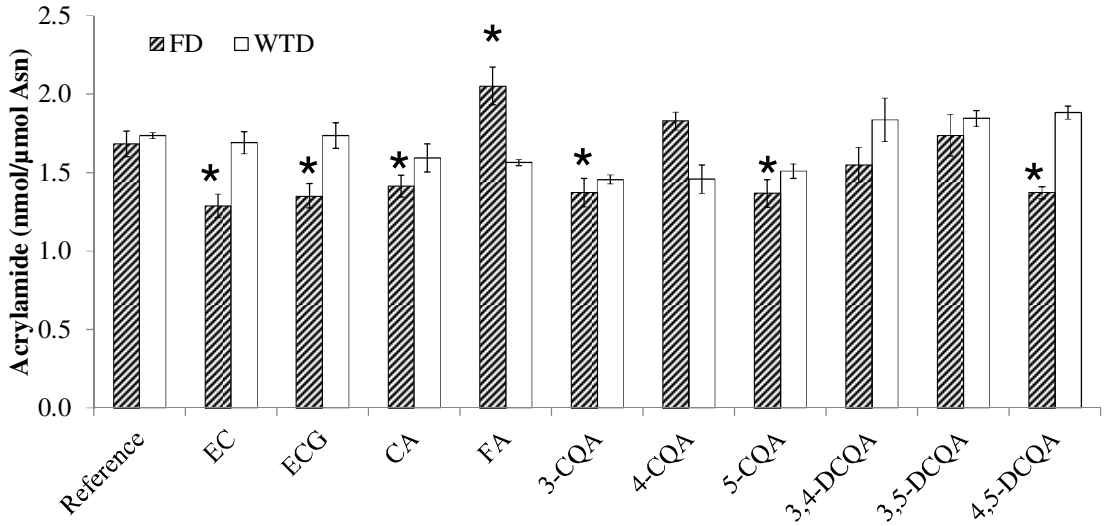

Highlights

- Caffeoylquinic and dicaffeoylquinic acids are potent scavengers of glyoxal.
- Phenolics did not as effectively inhibit acrylamide in systems containing glucose.
- Acrylamide mitigation was influenced by antioxidant type and concentration.
- System matrix and reaction conditions greatly affected the behaviour of phenolics.