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Effects of Fruit Extracts on the Formation of Acrylamide in Model Reactions and Fried Potato Crisps

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Natural products extracted from plants and fruits have attracted increasing attention for the development of effective inhibitors against the formation of acrylamide during food processing. In this study, six fruit extracts (apple, blueberry, mangosteen, longan, dragon fruit with white flesh, and dragon fruit with red flesh) were compared for their activities against acrylamide formation in chemical models containing equal molar quantities of glucose and asparagine in distilled water (160 °C for 30 min). Apple extract demonstrated potent inhibition on acrylamide formation. Blueberry, mangosteen, and longan extracts did not have significant impact, whereas dragon fruit extracts enhanced acrylamide formation. Column chromatography guided by chemical model analysis showed that the proanthocyanidin-rich subfraction played a key role in mediating the inhibitory activity. The inhibitory activity was finally corroborated in fried potato crisps. The present study identified some natural products that might have important applications in the food industry to inhibit acrylamide formation.

KEYWORDS: Acrylamide; fruit extracts; column chromatography; proanthocyanidins

INTRODUCTION

In thermally processed foods, especially starch-rich products such as fried potato, acrylamide (AM) can be formed through pathways closely associated with the Maillard reaction (*1*−*3*). Although asparagine alone could degrade to form AM (*1*), thermal degradation of asparagine in the presence of reducing sugars has been suggested to be the major route (*2*,*3*). In addition, the presence of reactive carbonyl species also strongly enhances the formation of AM from asparagine (*3*, *4*). AM has been reported to possess mutagenic and carcinogenic properties (*5*) and is classified by the International Agency for Research on Cancer as a probable human carcinogen (*6*). Reports on the presence of AM in widely consumed dietary components (*7*, *8*) have raised global concerns because these data imply that humans could be exposed to significant quantities of AM in the long term.

Extensive research efforts have been carried out aiming to develop strategies that could effectively inhibit the formation of AM during thermal food processing. Reaction time and temperature were found to play important roles in the formation of AM. At high temperatures (e.g., 160 °C), AM could be formed in much shorter times than under mild thermal conditions such as 120 °C (*9*). Therefore, AM formation might be reduced through an appropriate combination of heating time and temperature. Because fundamental food constituents including reducing sugars and amino acids, especially asparagines, are the precursors of AM, some studies showed that manipulating the content of certain food constituents, such as by the addition of lysine or glycine (*10*) or the pretreatment of potato with asparaginase to reduce asparagine content (*3*), could lead to reduced AM levels in food products. Recently, certain vitamins, especially the B vitamins, were reported to significantly inhibit the formation of AM (*9*, *11*).

Natural extracts are attractive candidates for the development of effective inhibitors of AM formation in processed foods. For example, antioxidant-rich bamboo leaf and green tea extracts were found to effectively reduce AM formation (*12*, *13*). The use of phenol-rich olive oil has also been proposed as a suitable strategy to mitigate AM formation in domestic deep-frying foods (*14*). On the basis of these previous studies, polyphenols are likely key active components in these natural extracts that inhibit AM formation. Fruit extracts are increasingly recognized as rich natural sources of phytochemicals, especially polyphenols. The present study aimed to evaluate the effects of different fruit extracts [apple, blueberry, mangosteen (*Garcinia mangostana*), longan (*Euphoria longana*), and white- and red-flesh dragon fruits (*Hylocereus undatus*)] on the formation of AM. Principal inhibitors of the most effective extract were further identified. The findings of the present study will provide useful information for the development of natural food additives that could inhibit AM formation in practical applications.

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MATERIAL AND METHODS

Reagents and Chemicals. Asparagine, acrylamide standard (≥99.8%), and glucose were purchased from Sigma-Aldrich Co. (St. Louis, MO). Activated charcoal powder was from Riedel-de Haën (Seelze, Germany). Microspin centrifuge filters (0.45 μm) were from Alltech Associates (Deerfield, IL). Blueberry, apple, mangosteen, longan, red- and white-flesh dragon fruits, potatoes, and peanut oil were purchased from a local supermarket in Hong Kong. All solvents used were of analytical grade and were obtained from BDH Laboratory Supplies (Poole, U.K.).

Preparation of Fruit Extracts. Fruit extracts were prepared according to the method of our previous study (*15*). Fresh fruits were peeled, cut into small pieces, and homogenized with a blender (Vivacio, Moulinex International, U.K.) in 95% ethanol. The homogenate was sonicated for 1 h and filtered through a filter paper (grade 1, Whatman). The extraction process was repeated three times. The ethanol extract filtrate was combined and evaporated on a rotary evaporator under vacuum to a volume of ~100 mL. The concentrated extract was mixed thoroughly with 500 g of Amberlite XAD-16 macroporous resin before loading onto a glass column (40 × 4 cm i.d.). Five bed volumes of Milli-Q water was used to remove water-soluble components (primarily simple sugars). This was followed by elution with 5 bed volumes of 95% ethanol. The ethanol eluent was evaporated to dryness with a rotary evaporator under vacuum. The dried extracts obtained were stored in a desiccator before analysis.

Chemical Model Reactions. One millimole of glucose and asparagine and two mL of deionized water (with or without a fruit extract at concentrations of 15, 25, and 35 mg/mL, respectively) were added to a glass test tube (25 mL). The openings of the tubes were tightly wrapped with aluminum foil. Reactions were performed in an oil bath at 160 °C for 30 min (*2*). At the 30 min time point, the tubes were withdrawn from the oil bath and immediately cooled in an ice−water bath to stop the reaction. Fifteen milliliters of distilled water was added to each tube, and the contents were stirred vigorously before they were subjected to centrifugation (14000 rpm, 30 min). The extraction process was repeated twice. Supernatant from the two extractions was combined and adjusted to a final volume of 30 mL with distilled water. Subsequent sample processing was performed according to a previous study by Kawata et al. (*16*). Briefly, 10 mL of the diluted supernatant was loaded onto to a syringe filled with 1 g of activated carbon that had been conditioned with 5 mL of methanol followed by 5 mL of water. Elution was performed with 9 mL of methanol, and the effluent was collected and adjusted with methanol to 10 mL. The samples were filtered through microspin nitrocellulose centrifuge filters (0.45 μm) prior to HPLC analysis.

Potato Crisp Analysis. The method was adopted from Zhang et al.'s study (*12*). Fresh potato tubers were washed, peeled, sliced (6 × 4 × 0.2 cm), and immersed for 5 min in water (control) or a solution of a fruit extract (0.01, 0.05 or 0.1%, w/w). The potato slices were removed from the immersion solution and drained for 2 min at room temperature. Frying was carried out at 170 ± 5 °C for 5 min in a peanut oil bath on an electric fryer (Princess 2611N, Fortress, Hong Kong).

The fried potato crisps were processed according to the method of Zhang et al. (*17*) with slight modifications. Briefly, 10 g of potato crisps from each treatment was homogenized in 100 mL of deionized water; 0.2% of α-amylase (6000 U/mg, Unikbio Biotech Ltd., Guangzhou, China) was added and reacted at 95 °C for 30 min. The reaction mixtures were cooled in an ice bath and centrifuged at 14000 rpm for 30 min. The supernatant was extracted three times with hexane (half the volume of the supernatant) to remove fat. The hexane phase was discarded, and the aqueous phase was filtered through a Teflon filter (0.45 μm pore size). Ten milliliters of the filtrate was loaded onto a syringe filled with 1 g of activated carbon. Subsequent steps were the same as those applied for samples from chemical model reactions.

HPLC-UV Analysis of Acrylamide. Quantification of AM was performed on a Waters 2695-2996 HPLC system (Waters Corporation, Milford, MA) equipped with a Waters 2996 photodiode array (PDA) detector. The method was adopted from our previous study with minor modifications (*11*). Chromatographic separation was carried out on a Zorbax SB-Aq column (4.6 × 250 mm, 5 μm, Agilent, Wilmington, DE). The mobile phase was Milli-Q water. The flow rate was 0.5 mL/min for the first 5 min and linearly increased to 0.8 mL/min in 3 min, and this flow rate was kept for 7 min. The total running time was 15 min, and the post running time was 15 min for equilibration of the column. AM was monitored at 205 nm. Peak identification was accomplished by comparing the retention time and UV spectral characteristics of the HPLC peaks with those obtained from standard AM solutions analyzed under the same conditions. Quantitative determination was carried out using an external calibration curve. The coefficient of determination (R^2) for AM standard curve was 0.9996. Triplicate analyses were performed for each treatment.

Fractionation of Apple Extract and Identification of Inhibitors of Acrylamide Formation in Apple. Apple extract was fractionated by using the same method reported in our previous study on the identification of heterocyclic amine formation inhibitors from natural extracts (*15*). Briefly, the dissolved apple extract was loaded onto an Amberlite XAD-16 column (40 × 4 cm), which was eluted with a stepwise gradient of ethanol−water for fractionation. HPLC analysis was carried out to compare the chemical profiles of the fractions, and those with similar profiles were combined. Fractions that effectively reduced the formation of AM in chemical models were further separated by Sephadex gel column chromatography and, eventually, LC-DAD/MS was performed to identify the principal inhibitory components (*15*).

Statistical Analysis. Statistical analyses were performed using the SPSS 13.0 statistical package (SPSS, Inc., Chicago, IL). Paired samples *t* test was applied to determine whether a particular treatment of the sample would result in significantly different content of AM as compared with the control. Treatment differences with $P < 0.05$ were considered to be significantly different.

RESULTS AND DISCUSSION

Effects of Fruit Extracts on Acrylamide Formation in Chemical Model Systems. Natural extracts were shown to have great potential as effective inhibitors of the formation of genotoxic substances in thermally processed foods (*12*, *15*). In this study, six fruit extracts were compared for their relative activities against the formation of AM in chemical model systems containing equal molar concentration of asparagine and glucose. Although it was reported that samples from AM-producing Maillard models generally have fairly simple chemical profiles that could be tackled with routine analytical techniques such as HPLC-DAD (*11*), considering the complexity of the fruit extracts tested, the reaction mixtures were cleaned by passage through mini-columns filled with activated carbon before analysis by LC-DAD. The results of the quantitative analyses are presented in **Figure 1**. Surprisingly, among the extracts, only apple extract was capable of significantly ($P < 0.05$) reducing the content of AM in the model systems. Blueberry, mangosteen, and longan extracts did not significantly affect the formation of AM even at a concentration of up to 35 mg/mL. In contrast, the addition of dragon fruit extracts strongly increased the amount of AM formed. The effect was especially prominent with respect to the extract of dragon fruit with red flesh; at a 35 mg/mL level of addition, the content of AM in the chemical model was increased by > 50% relative to the control. Another interesting phenomenon was that no dose-dependent activity was observed for most of the extracts. In particular, the three levels of addition (15, 25, and 35 mg/mL) of apple extract demonstrated comparable inhibitory effects on the formation of AM.

Apple extract has been shown to contain flavonoids, anthocyanins and proanthocyanidins, phenolic acids, lignans, and triterpenoids, which contribute to its beneficial biological activities (*18*, *19*). Apple polyphenols such as proanthocyanidins, phloridzin, and phloretin have been found to effectively trap reactive carbonyl species in simulated physiological conditions (*20*, *21*). It is likely that these polyphenols might also scavenge reactive carbonyls formed in the Maillard reaction, and this property might lead to reduced levels of AM because the presence of reactive carbonyls (especially α-dicarbonyls and

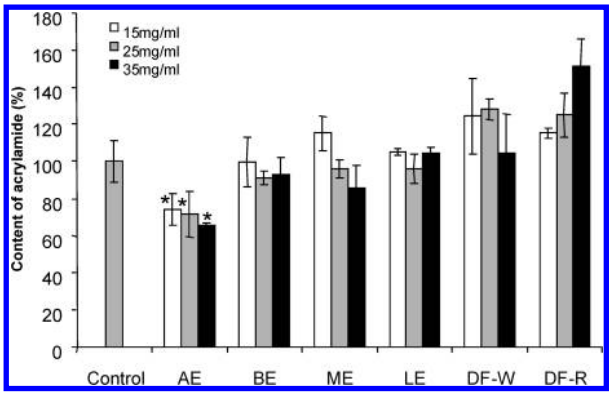

Figure 1. Effects of different concentrations of fruit extracts on the formation of acrylamide in chemical model systems. AE, apple extract; BE, blueberry extract; ME, mangosteen extract; LE, longan extract; DF-W, dragon fruit with white pulp; DF-R, dragon fruit with red pulp. Bars with an asterisk indicate significant difference ($P < 0.05$) from control.

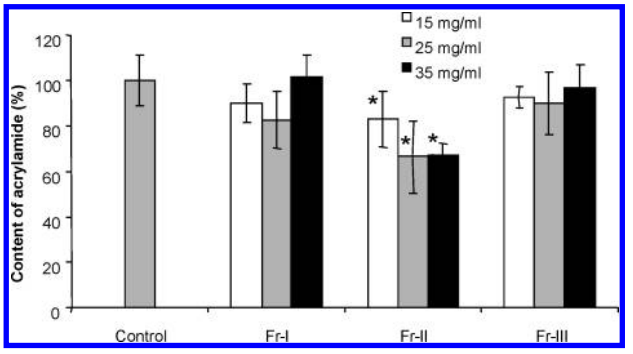

Figure 2. Effects of different fractions of apple extract on the formation of acrylamide in chemical model systems. Bars with an asterisk indicate significant difference ($P < 0.05$) from control.

α-hydroxycarbonyls) has been shown to enhance the conversion of asparagine into AM (*4*). Apart from scavenging of reactive carbonyls, quinone−amine interaction has also been proposed to modulate AM formation (*13*). In fact, apple extract was recently demonstrated by our group to have potent inhibitory activity against the formation of mutagenic heterocyclic amines, a class of potent genotoxic substances that have been central to numerous research works for the past three decades (*15*). Proanthocyanidins were eventually identified as the dominant components in apple that suppressed heterocyclic amine formation. Because apple extract exhibited the strongest inhibition of the formation of AM in the model system investigation, it was further examined following an approach similar to that used in our previous study.

Identification of Dominant Inhibitors of Acrylamide Formation from Apple Extract. Four fractions (Fr-I−IV) were obtained by Amberlite XAD-16 column chromatography of apple extract, but the amount of Fr-IV was insufficient for testing of inhibition against AM formation. Among the other three fractions, only Fr-II showed effective inhibition of the formation of AM; the other fractions did not cause significant changes in the content of AM in the chemical models (**Figure 2**). Theoretically, at the same concentration, the fraction that contains the dominant inhibitory components should demonstrate much more potent inhibition of AM formation than the crude extract. Interestingly, the inhibitory activities of the most effective fraction (Fr-II) were comparable to those of the crude apple extract for the three different concentrations (15, 25, and 35 mg/mL) examined in this study. One probable reason for this phenomenon might be that in the

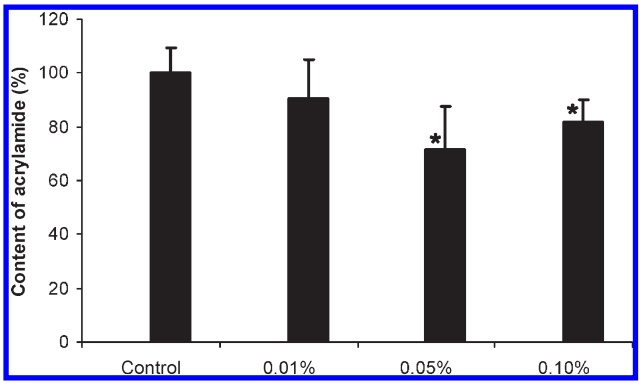

Figure 3. Effects of different concentrations of sub-fr2 from Fr-II on the formation of acrylamide in fried potato crisps. Bars with an asterisk indicate significant difference ($P < 0.05$) from control.

crude extract, some compounds that were not effective when added alone to the AM-producing models could have a synergistic effect on the inhibitory activity of the dominant inhibitor(s). Alternatively, some component(s) of Fr-II might have counteracted the activity of the dominant inhibitors.

To further characterize the roles of the different components of Fr-II in the formation of AM, Sephadex LH-20 column chromatography was performed on Fr-II, which led to two major subfractions (sub-fr1 and sub-fr2). Sub-fr1 contained a single compound (>95% purity by LC-DAD), which was identified by comparison with the data of our previous study as chlorogenic acid (*15*). Chlorogenic acid did not have a significant impact on the formation of AM in the chemical models. On the other hand, sub-fr2 strongly reduced the content of AM (data not shown). This subfraction mainly contained proanthocyanidins, including monomers, dimers, trimers, and tetramers (*15*). Among them, catechin, epicatechin, and procyanidins B1 and B2 were present in high concentrations. The above systematic chromatographic and chemical model investigation led to the identification of proanthocyanidins among the principal components in apple that inhibit AM formation.

Subsequently, the activity of sub-fr2 to inhibit AM formation was further tested in fried potato chips. Because this subfraction was highly concentrated with the postulated active inhibitors of AM formation, a lower starting concentration was used in the test. As shown in **Figure 3**, 0.01% of sub-fr2 did not significantly reduce the content of AM in fried potato crisps. Apparently, there exists a threshold level for effective inhibition of AM formation by apple proanthocyanidins. Treatment of potato slices with a solution containing 0.05 or 0.1% of sub-fr2 significantly ($P < 0.05$) reduced AM formation in the fried potato crisps. However, there was no significant difference between the effects of these two treatments. As described under Potato Crisp Analysis, instead of directly adding sub-fr2 to the potato slices, the potato slices had to take up components of sub-fr2 from the surrounding solution through passive diffusion or active absorption. Therefore, it is probable that during the treatment time allowed, the difference between the amounts of active inhibitors taken up by the potato slices from the treatment solutions might not be great enough to demonstrate significantly different AM formation inhibitory activities. There have been no previous reports on the role of proanthocyanidins in AM formation. Early studies suggested that antioxidant activities most probably contribute to the health benefits of proanthocyanidins (*22*). On the other hand, it has also been shown that certain proanthocyanidins effectively scavenged reactive carbonyls, which could be relevant to the inhibition of AM formation (*1*). Further studies should be carried out to better

understand the mechanism of the major components of this subfraction in inhibiting the formation of AM.

3.3. Conclusions. The present study compared the activities of six fruit extracts in inhibiting the formation of AM in chemical model systems. Apple extract demonstrated the strongest inhibitory effect. A series of chromatographic analyses guided by chemical model investigations revealed that the proanthocyanidin-rich subfraction contributed to the AM formation inhibitory activity of apple extract. This subfraction also effectively reduced the formation of AM in fried potato crisps. Apple has long been a popular dietary component as well as a culinary ingredient. The findings of the present study provided useful information for the development of natural food additives that could be relevant to mitigation of AM-associated health risks in practical applications.

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