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Inhibition of acrylamide formation by vitamins in model reactions and fried potato strips

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ABSTRACT

The capacities of 15 vitamins in reducing the formation of acrylamide were examined. Inhibitory activities of the water-soluble vitamins were tested in both chemical models containing acrylamide precursors (asparagines and glucose) and a food model system (fried snack products), while activities of fat-soluble vitamins were examined only in the latter model. Biotin, pyridoxine, pyridoxamine, and L-ascorbic acid exerted a potent inhibitory effect (>50%) on acrylamide formation in the chemical model system. Using the food model, it was shown that water-soluble vitamins are good inhibitors of acrylamide formation. On the other hand, only weak inhibitory effects were observed with fat-soluble vitamins. Effects of pyridoxal, nicotinic acid, and L-ascorbic acid were further examined using fried potato strips. Nicotinic acid and pyridoxal inhibited acrylamide formation in fried potato strips by 51% and 34%, respectively. Thus, certain vitamins at reasonable concentrations can inhibit the formation of acrylamide.

1. Introduction

Acrylamide has long been used in the industry to synthesize polyacrylamides for wastewater treatment, papermaking, and as monomers for the manufacture of other industrial products. Studies have shown that long-term exposure to acrylamide could cause damage to the nervous system both in humans and animals (Tilson, 1981). Early toxicological studies suggested that acrylamide in the gas phase can irritate the eyes and skin, and cause paralysis of the cerebrospinal system (Johnson et al., 1986) and act as a potential genetic and reproductive toxin (Dearfield, Abernathy, Ottley, Brantner, & Hayes, 1988). Later *in vitro* and *in vivo* studies revealed that acrylamide possesses mutagenic and carcinogenic properties (Dearfield et al., 1995).

Traditionally, vitamins and minerals have been among the most widely applied chemical agents to enhance the nutritional values of food products. Some vitamins may also help to lower the levels of toxicants formed from the Maillard reaction. Pyridoxamine, a member of the B6 complex, was demonstrated to suppress the formation of advanced glycation endproducts (AGEs) from Amadori products (Khalifah, Baynes, & Hudson, 1999; Khalifah et al., 1996). On the other hand, thiamine (vitamin B1) pyrophosphate was shown to reduce the formation of AGEs by acting as an effective post-Amadori inhibitor (Booth, Khalifah, Todd, & Hudson, 1997). Vitamins C and E were also reported as AGE-formation inhibitors in therapeutic interventions (Singh, Barden, Mori, & Beilin, 2001). However, few reports can be found on the application of vitamins to inhibit acrylamide formation during food processing. Taking into consideration that acrylamide is likely a Maillard product and that some vitamins have been reported to effectively suppress the generation of toxic Maillard products, the present study aimed to examine the effects of 15 vitamins on inhibiting acrylamide formation under different heating conditions. The tested vitamins included retinol acid (vitamin A, VA), thiamin (vitamin B1, VB1), riboflavin (vitamin B2, VB2), nicotinic acid (vitamin B3, VB3), pantothenic acid (vitamin B5, VB5), pyridoxine (vitamin B6, PN), pyridoxal (vitamin B6, PL), pyridoxamine (vitamin B6, PM), biotin (vitamin B7, VB7), folic acid (vitamin B9, VB9), vitamin B12 (VB12), L-ascorbic acid (vitamin C, VC), ergocalciferol (vitamin D, VD), α-(±)-tocopherol (vitamin E, VE), and phylloquinone (vitamin K1, VK). The B vitamins and vitamin C are water-soluble. The others are fat-soluble.

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2. Materials and methods

2.1. Reagents and chemicals

Acrylamide standard (⩾99.8%), asparagine, glucose, acetonitrile (MeCN), NaCl, $MgSO_4$, retinol acid (vitamin A, VA), thiamin hydrochloride (vitamin B1, VB1), riboflavin (vitamin B2, VB2), nicotinic acid (vitamin B3, VB3), D-pantothenic acid hemicalcium salt (vitamin B5, VB5), pyridoxine monohydrochloride (vitamin B6, PN), pyridoxal hydrochloride (vitamin B6, PL), pyridoxamine dihydrochloride (vitamin B6, PM), biotin (vitamin B7, VB7), folic acid (vitamin B9, VB9), vitamin B12 (VB12), L-ascorbic acid (vitamin C, VC), ergocalciferol (vitamin D, VD), α-(±)-tocopherol (vitamin E, VE), and phylloquinone (vitamin K, VK) were purchased from Sigma–Aldrich Company (St. Louis, MO, USA). All solvents used were of analytical grade and were obtained from BDH Laboratory Supplies (Poole, UK). Fresh potato, flour, and peanut oil were purchased from a local supermarket in Hong Kong. The Reacti-Therm III heating module model 18840 was purchased from Pierce (Rockford, IL, USA) and the stainless steel tubes were from a Shanghai Manufacturing Company (Shanghai, PR China). The food fryer (Philips, model HD6157, The Netherland) was purchased from an electric appliances store in Hong Kong. Isolute Multimode SPE columns (300 mg) were from International Sorbent Technology (Biotage, Sweden).

2.2. Effects of vitamins on acrylamide formation in chemical models

Chemical model reactions were carried out according to the method of Gokmen and Senyuva (2007) with minor modifications. Briefly, the reaction system contained 1 mmol asparagine and 1 mmol glucose with or without a testing vitamin (30 mg). The reaction medium was 2 mL deionised water. The stainless steel reaction vials were sealed and heated on the Reacti-Therm III heating module at 170 °C for 30 min. At the 30 min time point, the vials were cooled immediately in an ice bath. The reaction mixture from each sample was transferred to a 50-mL Falcon tube and the vials were then rinsed in triplicate, each with 3 mL of deionised water. The combined solvent in the Falcon tube was centrifuged at 8000*g* for 20 min. The supernatant (0.2 mL) was diluted with 1 mL deionised water and 10 μL of the resulting solution was injected into an HPLC system for quantitative and qualitative analyses. Details of the HPLC method are given in Section 2.5. The experiments were performed in triplicate.

2.3. Effects of vitamins on acrylamide formation in food model systems

The first food model (fried snack products) was developed with reference to the method of Kim, Hwang, and Lee (2006) with slight modifications. In brief, wheat flour (64.5%), glucose (1%), and asparagine (0.5%) were premixed with or without a testing vitamin (0.5%) for 5 min before adding water (34%). All ingredients were then mixed for 20 min. The dough was transformed into a round sheet with a diameter of 5 cm. The sheet was allowed to stand for 1 h at room temperature before being dried for 4 h at 70 °C. After drying, the model snack was fried at 170 ± 3 °C for 5 min on each side. The experiment was repeated three times.

The extraction of acrylamide from the snack samples was similar to the method of Mastovska and Lehotay (2006). Firstly, the fried snack of the control or the treatment sample was pulverised and transferred to a 50-mL Falcon tube. Deionised water (40 mL) was added and the tube was sealed and shaken for 1 min. This was followed by sonication for 30 min. After filtration, the filtrate (10 mL) was transferred to another 50-mL Falcon tube and MeCN (10 mL) and hexane (2 mL) were added. Anhydrous $MgSO_4$ (4 g) and NaCl (0.5 g) were then added to the mixture. After shaking for 1 min and centrifuged at high speed for 5 min, the hexane layer was discarded, and the acetonitrile extract (5 mL) was pipetted to a pear-shaped flask and dried on a rotary evaporator under vacuum. The dried sample was re-dissolved in 2 mL of deionised water and loaded onto an Isolute Multimode SPE column, which was pre-conditioned with methanol (2 mL) followed by water (2 mL). The SPE protocol was with reference to the method reported by Pedreschi, Kaack, and Granby (2004). The SPE column was eluted with deionised water (3 mL). The first 1 mL of the eluate was discarded, and the remaining eluate collected and analysed with HPLC.

2.4. Effects of vitamins on acrylamide formation in fried potato strips

The second food model was in the form of potato strips (60 mm × 10 mm × 10 mm). For each analysis, the same batch of potato strips was divided into four portions (50 g each), one for the control, and the other three for treatment with the three selected vitamins, respectively. Solution of each of the three vitamins (1%) was prepared and the potato strips were soaked in the corresponding solution for 60 min at room temperature. Water was used for soaking the control samples. The potato strips were drained for 2 min prior to frying, which was carried out in peanut oil at 170 °C for 10 min with an electric fryer (Philips). After frying and cooling, the potato strips of each treatment were dipped in 100 mL hexane to remove oil on the surface. The strips were then ground to a paste, which was then extracted with 100 mL deionised water by sonication for 30 min. Duplicate samples, each of 20 mL water extract, were taken for each treatment and transferred to a 50-mL Falcon tube. To each tube was added 20 mL MeCN, 4 mL hexane, 8 g anhydrous $MgSO_4$, and 1 g NaCl. The tubes were sealed and shaken vigorously for 1 min by hand. Then the tubes were centrifuged for 5 min at high speed. The MeCN extract (15 mL) from each sample was dried under vacuum. After re-dissolving in 2 mL water, the samples were further cleaned with SPE following the procedure described in Section 2.3. Triplicate experiments were performed.

2.5. HPLC-UV analysis

Analytical HPLC was carried out using a Shimadzu LC-20AT system (Kyoto, Japan) equipped with a diode array detector and an LC-solution software. A pre-packed Sunfire™ C18 column (250 × 4.6 mm, 5 μm, Waters Corporation, Ireland) was selected for analysis of acrylamide. The flow rate was 0.8 mL/min. The mobile phases were water (solvent A) and acetonitrile (solvent B). The elution started with 100% A for 10 min, then linear gradient to 20% B in 5 min. Then it was 20–25% B from 15 to 20 min, 25–90% B from 20 to 30 min and finally kept at 90% B till 35 min. The post running time was 15 min and the chromatograms were registered at 205 nm.

2.6. LC/MS/MS analysis

LC–MS/MS analysis was carried out on an Agilent 1100 HPLC system (Waldbronn, Germany) equipped with a binary pump, an autosampler, and a thermostatically-controlled column oven, and coupled to an Agilent 1100 MS detector. Atmospheric pressure chemical ionisation (APCI) was selected for the analysis. Chromatographic separation was on the same column as that used for LC-UV analysis. The mobile phase consisted of an isocratic mixture of 0.01 mM acetic acid in a 0.2% aqueous solution of formic acid. The flow rate was 0.8 mL/min. Data acquisition was performed either in the scan mode or selected ion monitoring (SIM) mode using the following analysis parameters: drying gas (N_2, 100 psi), flow rate of 4 L/min, nebulizer pressure of 60 psi, drying gas tem-

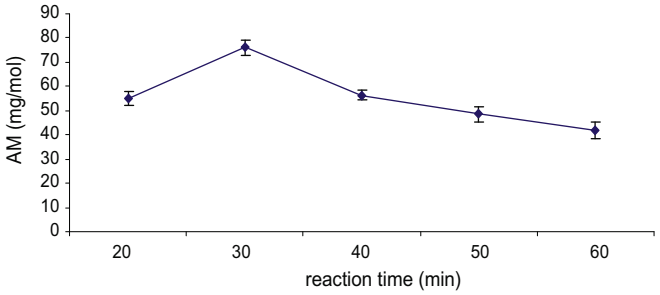

Fig. 1. Effect of reaction time on acrylamide formation in an acrylamide-producing model heated at 170 ± 3 °C. The model contains 2 mL of a solution of glucose and asparagines (molar ratio, 1:1) and was sealed and heated in an oil bath. Data values are mean ± SD (n = 3).

peratures at 325 °C, vaporiser temperature at 425 °C, capillary voltage of 4 kV, corona current of 4 μA, and fragmentor voltage of 55 eV. The ions monitored for acrylamide were m/z 72 and 55 (Gokmen & Senyuva, 2007).

2.7. Statistical analysis

Statistical analyses were performed using the SPSS statistical package (SPSS, Chicago, IL). Paired samples t-test was applied to determine whether a particular treatment of the sample would result in a significantly different content of acrylamide compared with the control. $p < 0.05$ was selected as the level decision for significant differences.

3. Results and discussion

3.1. Effects of vitamins on the formation of AM in chemical model systems containing asparagine, glucose, and water

As indicated by Stadler et al. (2002), asparagine is the main amino acid, which reacts with reducing sugars to produce acrylamide. It was reported that the highest amount of acrylamide was formed when the mole ratio of asparagines/glucose was 1:1 and when water was used as the reaction medium (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005). In this study, time-course investigation (20, 30, 40, 50, and 60 min) into the generation of acrylamide at a heating temperature of 170 ± 3 °C showed that 30 min was optimal in terms of the yield of acrylamide formed (Fig. 1). The yield of acrylamide then decreased with further increase in the duration of heat treatment. This trend is in broad agreement with that reported in a previous study which was conducted at 185 °C (Mottram, Wedzicha, & Dodson, 2002).

Because the chemical model was an aqueous system, only the water-soluble vitamins were examined for their effect on the formation of acrylamide. Cysteine was used as the positive control. As shown in Fig. 2, several vitamins, including VB1, VB3, VB7, PM, PN, and VC strongly reduced the formation of acrylamide; PN and VB3 were the two most potent inhibitors (>70% reduction relative to the control). Other water-soluble vitamins, VB2, PL, VB12, and VB5 only reduced the amount of acrylamide formed by around 20%. Interestingly, among the three commonly-consumed B6 vitamins (PN, PL, and PM), only PN and PM were effective in inhibiting acrylamide formation. This phenomenon suggested that the terminal functional group (primary amino, hydroxyl, or aldehyde) of the side chain at the four-position might play a significant role in affecting the vitamins' capability to interrupt certain steps of the pathway to the formation of acrylamide.

3.2. Effects of vitamins on the formation of AM in food model systems

Certain fat-soluble vitamins such as VA, VE, and VK are well-recognised antioxidants. Antioxidant activity has been proposed as one highly-possible mechanism to inhibit acrylamide formation. However there is still controversy over this proposition. In order to evaluate also the activity of fat-soluble vitamins (VA, VD, VE, and VK) in influencing acrylamide formation, a food model system (fried snack product) was developed using wheat flour, to which an accurately weighed amount of asparagine and glucose was added. This model served as a surrogate for a starch-rich food system. Another advantage of this model system is that it allowed the activity of both the water- and the fat-soluble vitamins to be compared in the same reaction system.

Fig. 2 presents the relative activities of the 15 vitamins in inhibiting acrylamide formation in the food model. Surprisingly, none of the four fat-soluble vitamins was effective in suppressing the formation of acrylamide. VD even enhanced acrylamide formation by >20% relative to the control. These data indicated that antioxidant activity unlikely played a significant role in interfering with acrylamide formation in such a reaction system. A comparison of the data (Fig. 2) obtained from simple chemical model investigation and those from testing in the food model showed that, with the exception of VB5 and PL, the water-soluble vitamins were, in general, much weaker in inhibiting the formation of acrylamide in the latter system. Furthermore, it appeared that the water-soluble vitamins were more effective as acrylamide formation inhibitors than the four fat-soluble vitamins.

As mentioned previously, large differences in the extent of inhibition were observed between the chemical and food model systems for several vitamins. To test whether the trace amounts of ferrous or ferric ions that might be released from the stainless steel vials during the heating process had contributed to this stronger inhibition in the former system, 0.1% PN or VB1 with 0.1% ferrous phosphate or ferric citrate was added to the food model system. However, it was found that the inhibition was not improved in the presence of ferrous or ferric ions. A probable explanation for the apparent discrepancy might be that in the food model, the matrix effect could hinder the interaction between acrylamide precursors and the added vitamins, and thus reduced their efficacy to arrest the reactions involved in acrylamide formation.

3.3. Effects of vitamins on the formation of acrylamide in fried potato strips

Fried potato products are known to contain the highest amount of acrylamide among different food items (Senyuva & Gokmen, 2005). Therefore, the effect of PN, VC, and VB3 on acrylamide formation was further examined using fried potato strips ($10 \times 10 \times 10$ mm). Taking into consideration that acrylamide was produced in a complex matrix that might contain a lot of interfering compounds; identity of the target peak in the UV-absorption spectrum was confirmed with LC–DAD–MS/MS analysis prior to actual testing of the vitamins' activity in inhibiting acrylamide formation. In addition, spiking with acrylamide standard was also done to confirm the peak identity. Representative LC-UV chromatograms are shown in Fig. 3.

PN and VB3 reduced the formation of acrylamide in fried potato strips by ~35% and ~50%, respectively (Fig. 4). The inhibitory effect of VB3 was comparable to that of the positive control cysteine. In contrast, VC did not demonstrate good activity in fried potato strips with an inhibition rate of only 11%. The effectiveness of cysteine (~60%) as a strong inhibitor of acrylamide formation was in agreement with that reported previously by Kim et al. (2006). However, cysteine treatment prior to frying was found to give the fried strips a lighter colour when compared to the control.

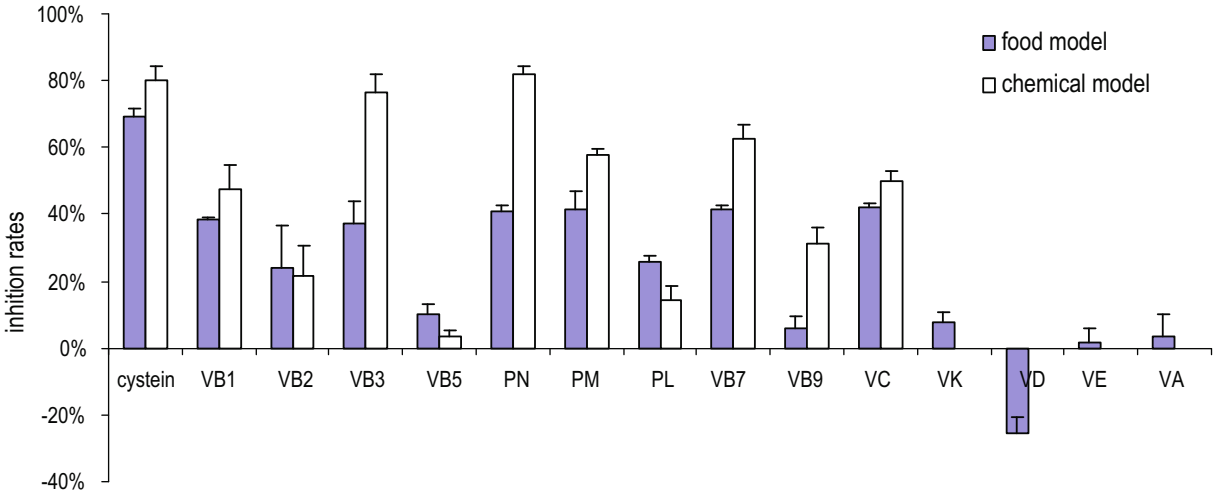

Fig. 2. Inhibition rates of vitamins to acrylamide formation in chemical and food model systems. Data values are mean ± SD (n = 3).

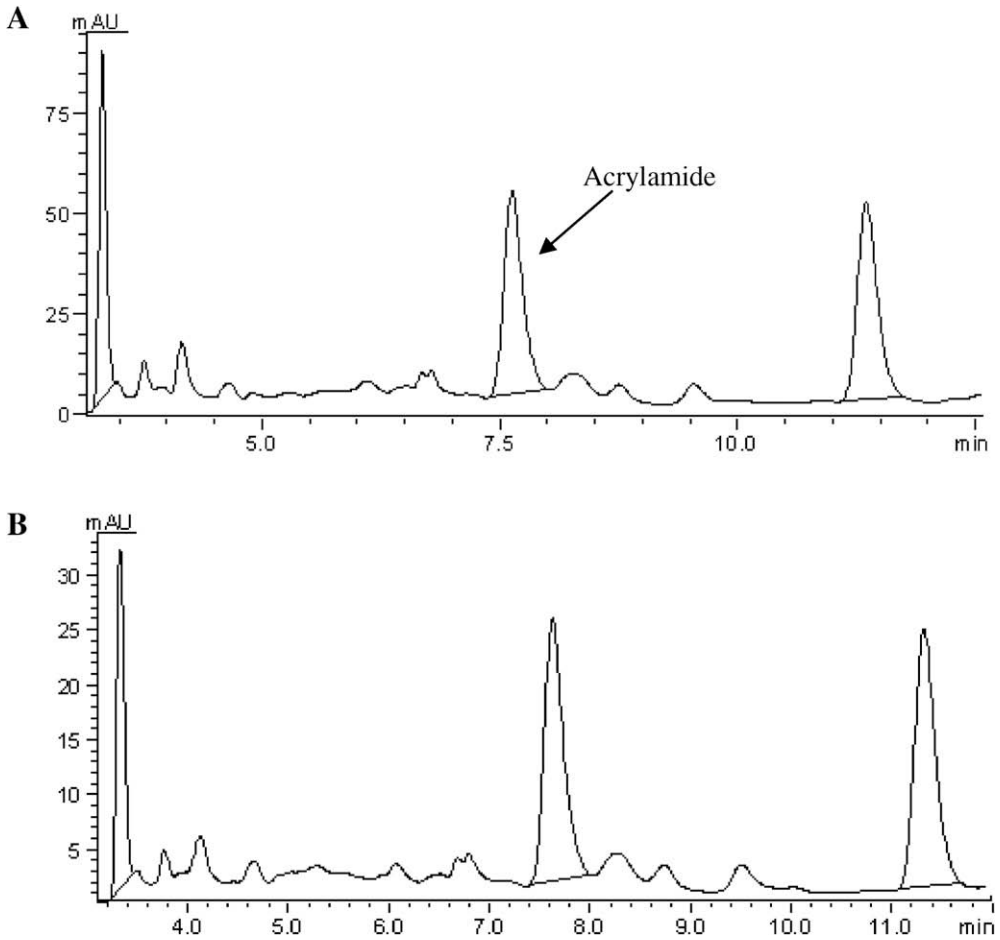

Fig. 3. HPLC chromatograms of acrylamide in water extracts from fried potato strips pretreated with water (chromatogram A) and nicotinic acid (chromatogram B).

The presence of an unpleasant odour in the cysteine-treated samples might greatly undermine the value of this amino acid as an additive in practical applications. In other words, VB3, which inhibited acrylamide formation by ~50% without a statistically significant difference (<0.05) from that achieved with cysteine, might be considered as a more promising inhibitor of acrylamide formation in food processing, because no undesirable odour generation was observed in VB3-treated samples.

Among the four fat-soluble vitamins, only VE has been previously examined for its effect on the formation of acrylamide. The reasons might be that VE is a strong antioxidant closely associated with our diet and that antioxidant activity has been proposed as

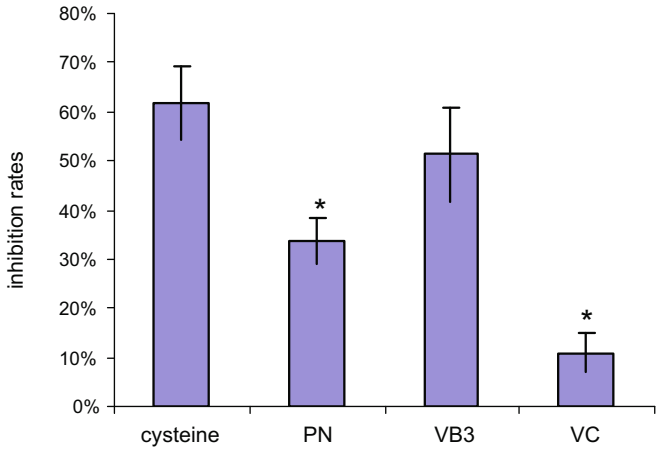

Fig. 4. Inhibition rates of vitamins on acrylamide formation in fried potato strips. Data values are mean ± SD ($n = 3$). Bars with an asterisk indicate significant difference from the control (cysteine-treated samples) ($p < 0.05$).

one of the major mechanisms to inhibit the formation of acrylamide. Nevertheless, no conclusive evidence has been found to support such proposition, and some studies even showed that the addition of certain antioxidants (butylated hydroxytoluene, sesamol, and VE) prior to cooking enhanced acrylamide formation (Tareke, 2003). The author attributed the phenomenon observed to the protective effects of the above vitamins on acrylamide from being degraded in free radical-initiated reactions. On the other hand, Taeymans et al. (2004) found that the addition of rosemary to dough in concentrations as low as 1% could significantly reduce acrylamide content by up to 60%. Addition of a flavonoid mixture to potato chips was also reported to result in a lower acrylamide content relative to the control (Ferńandez, Kurppa, & Hyvönen, 2003). The authors explained that the added flavonoids might inhibit the transformation of acrolein to acrylic acid in the pathway leading to acrylamide formation. However, this proposed inhibitory mechanism had been challenged by several research groups (Stadler et al., 2004; Vattem & Shetty, 2003; Yaylayan, Locas, Wnorowski, & O'brien, 2004).

It appeared that, among the vitamins examined in the present study, the effective inhibitors of acrylamide formation were mostly B vitamins. Antioxidant capacity has also been suggested to play important roles in mediating some of the beneficial bioactivities of some B vitamins, such as protection against oxidative damage induced by argemone oil/sanguinaring in mice (Ansari, Dhawan, Khanna, & Das, 2006). However, not all the antioxidant B vitamins have demonstrated a strong and significant inhibitory effect on the formation of acrylamide.

VC is another well-known antioxidant vitamin that has been evaluated for its activity to suppress acrylamide formation. Biedermann, Noti, Biedermann, Mozzetti, and Grob (2002) reported a slight decrease in the acrylamide content when ascorbic acid was added to a potato model at a concentration of 1%. In contrast, Rydberg et al. (2005) observed a significant reduction in acrylamide formation in a fried potato sample that was dipped in a 1.7% ascorbic acid solution prior to heat treatment. Several other studies also reported that VC acted synergistically with certain food additives such as potassium persulfate to inhibit acrylamide formation (Beileryan, Minasyan, & Chshmarityan, 2008). In the present study, VC reduced acrylamide content in the chemical model and food model systems by 50% and by 42%, respectively; but no significant inhibition was found when it was applied to potato samples prior to frying. Therefore, an integration of the data from previous studies and those from the present could not lead to a conclusive answer to the real potential of VC as an effective acrylamide formation inhibitor. Taken together the results from different categories of vitamins, including water- and fat-soluble ones, it seemed that antioxidant activity might not be the dominant mechanism responsible for the modulating effect of the tested vitamins on acrylamide formation.

Cysteine has been quite well-known as a strong inhibitor of acrylamide formation. From a mechanistic point of view, it was proposed that cysteine might act as a nucleophile that might react with acrylamide precursors and/or acrylamide itself to form stable intermediary or final products, and thus leads to a decrease in acrylamide content of the system concerned. A close examination of the structures of the vitamins tested in this study revealed that the effective inhibitors of acrylamide formation are of diverse structural characteristics. Some of them also contain nucleophilic groups, in particular, an amino group that might contribute to their inhibitory activity against acrylamide formation. Good examples are VB1 and VB7, which in our experiments, strongly reduced the formation of acrylamide in both chemical and food model systems.

4. Conclusions

In this study, the effect of vitamins on inhibiting the formation of acrylamide was systematically investigated. The results showed that some vitamins were capable of inhibiting acrylamide production. In chemical model systems, PN and VB3 were the two most effective inhibitors with an inhibition rate of >70%. Several other water-soluble vitamins, including VB7, PN, PM, and VC also reduced the formation of acrylamide by >50%. In the food model system, VB1, VB3, VB7, VC, PM, and PN caused ~40% reduction in the amounts of acrylamide formed. The effectiveness of VB3 was eventually corroborated in fried potato strips, thus suggesting its great potential for application in food processing to decrease acrylamide formation. Further studies are needed to characterise the action mechanism of the vitamins that showed strong inhibitory activity against the formation of acrylamide.

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