Original Article

Study on reduction of acrylamide in fried bread sticks by addition of antioxidant of bamboo leaves and extract of green tea

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This paper investigated the efficiency of antioxidant of bamboo leaves (AOB) and extract of green tea (EGT) on the reduction of acrylamide in fried bread sticks and summarized the optimal levels of two additives. Seven experimental groups including a control group were organized for both of additives. Fried bread sticks were made via traditional processing technology. The flour was mixed with different levels (0.002–4.9 g/kg flour) of AOB and EGT, respectively. The acrylamide level in fried bread sticks was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). The sensory evaluation was performed in double blind manner. Results showed that nearly 82.9% and 72.5% of acrylamide were reduced when the AOB and EGT addition levels were 1 and 0.1 g/kg, respectively. The elevated inhibitory effects of AOB and EGT on the acrylamide formation were achieved with an increase of additive levels unless the spiking levels of AOB and EGT were greater than 1 and 0.1 g/kg, respectively. Sensory evaluation results showed that the flavor and texture of fried bread sticks processed by AOB and EGT had no significant difference compared to normal food matrixes ($p>0.05$) when both AOB and EGT addition levels were no more than 1 g/kg. The present study indicated that both AOB and EGT could significantly reduce the acrylamide content generated in fried bread sticks and keep original flavor and crispness of fried bread sticks. This study could be regarded as an important contribution on the reduction of acrylamide by natural antioxidants.

Key Words: acrylamide, fried bread sticks, reduction, antioxidant of bamboo leaves, extract of green tea

Introduction

Acrylamide, a potential genetic and reproductive toxin, was detected in carbohydrate-rich fried or baked food samples by the research groups from Swedish National Food Administration (SNFA) and University of Stockholm in 2002.[1] Such novel finding has attracted wide attention throughout the world. Earlier toxicological studies demonstrated that acrylamide is carcinogenic to experimental rats and mice, causing tumors at multiple sites in both species.[2,3] The International Agency for Research on Cancer (IARC) has therefore classified it as "potential carcinogenic to humans".[4] However, epidemiologic studies of possible health effects from exposures to acrylamide have not produced consistent evidence of increased cancer risk, in either occupationally exposed workers or the general populations of several countries.[5,6] Nevertheless, considering the acknowledged neurotoxicity of acrylamide, recent investigation of considerable acrylamide levels in fried foods still evoked an international health alarm. After the discovery of acrylamide formation in Maillard reaction,[7,8] several hypotheses on its formation mechanisms were recommended at very early stages of investigations. Initially, mechanistic studies mainly focused on the acrylamide formation in vegetable oils or lipids since the problem primarily encompassed carbohydrate-rich foods that are fried or baked. To date, researches demonstrated some important intermediates including 3-aminopropionamide,[9] decarboxylated

Schiff base,[10] decarboxylated Amadori products,[11] acrylic acid,[12] and acrolein,[13] which play key roles in the final generation of acrylamide.

Based on current knowledge, acrylamide may be reduced under the following situations: (i) Some key intermediates are eliminated under the change of reaction conditions; (ii) Other vinylogous compounds instead of acrylamide are formed; (iii) Some key pathways such as the formation of Schiff base, Strecker type degradation, *N*-glucoside pathway and β-elimination reaction of the decarboxylated Amadori compounds are blocked.[10–11,14] During these years, various effective methods for the reduction of acrylamide in the actual food matrixes have been found such as prevention of reducing sugar liberation during the storage period of food materials,[15] change of heat processing methods,[16] optimization of suitable cultivar and storage temperature of food materials,[17] fermentation,[18] modification of pH,[19] reduction of ammonium bicarbonate,[20] addition of competing amino acids,[21] etc.

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However, few studies reported the effective way to reduce acrylamide by the addition of food antioxidants. For instance, a weak reduction effect on the acrylamide formation was found via the addition of ascorbic acid in a potato-based model.[22] Furthermore, nearly 50% reduction of acrylamide occurred after the addition of a flavonoid spice mix.[23]

Antioxidant of bamboo leaves (AOB), a pale brown powder extracted form bamboo leaves, was capable of blocking chain reactions of lipid autooxidation, chelating metal ions of transient state, scavenging nitrite compounds and blocking the synthetic reaction of nitrosamine reported by our previous study.[24] Moreover, AOB was testified to be a strong antioxidant activity and inhibitory effect on transition metal ion and free radical induced deterioration of macromolecules *in vitro*.[25] In addition, AOB was allowed to be added into puffed foods, meat products, fried foods and edible oils authorized by Ministry of Health, P. R. China. The main functional components in AOB are flavonoids, lactones and phenolic acids while flavone *C*-glucosides are a group of representative flavonoids in AOB.[26] On the other hand, extract of green tea (EGT) is also demonstrated as a strong antioxidant applied in many food matrixes.[27] It is well known that four kinds of flavanols, i.e. epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) are the main components in EGT. AOB and EGT have been both approved as two kinds of food antioxidants in Chinese national standards (*i.e.* GB-2760). Meanwhile, the safety of both natural antioxidants was systematically demonstrated in previous studies.[28,29] Addition of edible plant extracts in various products to exert their special effects is a potential technique for reduction of acrylamide in corresponding foods.

Fried bread sticks, a kind of all-time favorite cereal breakfast food for Chinese and Asian people, have similar processing style with lots of Western fried foods. Studies conducted so far indicated that moderate protein and high carbohydrate foods such as potatoes developed substantially higher levels of acrylamide under heating conditions.[7,8] Therefore, fried bread sticks probably have high risk exposure of acrylamide under heating conditions. However, to our best knowledge, few published papers in peer-review journals reported the acrylamide content in fried bread sticks. China has a huge population and hundreds of millions of people consume fried bread sticks as their breakfast in everyday life, so it is very indispensable to find a feasible way to reduce acrylamide levels in this product.

The aims of this study were
(i) to investigate the effect of AOB and EGT on the reduction of acrylamide in fried bread sticks; (ii) to summarize the optimal levels of two additives, which can be applied in this product.

Materials and methods
Materials
The wheat flour, yeast and baking soda used for the preparation of fried bread sticks were purchased from a local supermarket in Hangzhou (April, 2005). AOB was prepared from the bamboo leaves of *Phyllostachys nigra* var. *henonis* identified by Research Institute of Subtropical Forestry of the Chinese Academy of Forestry (Hangzhou, China). Briefly, fresh bamboo leaves were collected during the autumn season in Anji district (Zhejiang, China) and air dried. The coarse powder of bamboo leaves was obtained by crashing into the size of 20–40 mesh and 10 g powder was extracted with the time of 1 h by 100 ml 30% (v/v) ethanol aqueous solution using the hot reflux method. The filtrate was then isolated by membrane filtration to remove macro- and micro-molecular components such as polysaccharides and minerals. Finally, AOB was obtained after concentrating in vacuum and spray drying. EGT was a gift from Professor Xianqiang Yang in Department of Tea Science (Zhejiang University, Hangzhou, China). The total content of four main flavanols (EC, ECG, EGC and EGCG) was approximately 98%.

Chemicals
Acrylamide (99%) and $^{13}C_3$-labelled acrylamide (isotopic purity 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Formic acid (96%) was obtained from Tedia (Fairfield, OH, USA) while methanol (HPLC-grade) was purchased from Merck (Whitehouse Station, NJ, USA). All of other solvents and chemicals used for the determination of acrylamide were of analytical grade.
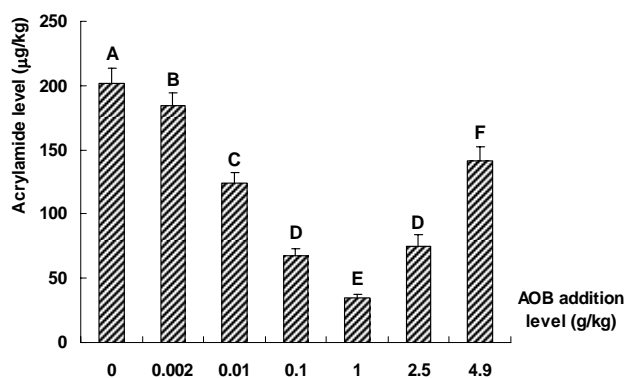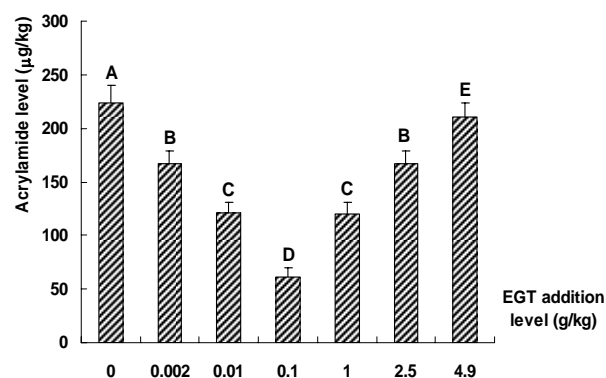

Figure 1A

Figure 1B

Figure 1. The relationship between acrylamide levels and different conditions of (A) AOB and (B) EGT treatments in fried bread sticks (*n* = 6). Error bars designate standard deviation (SD) and different letters indicate significant differences via Duncan's multiple comparison test (*p*<0.05). AOB, antioxidant of bamboo leaves; EGT, extract of green tea.

Table 1. The sensory evaluation results of fried bread sticks with AOB treatments in different groups ($n = 10$)†

	Addition level of AOB (g/kg)						
	Control	0.002	0.01	0.1	1	2.5	4.9
Color							
Mean score	4.7	4.4	4.1	4.3	4.2	4.1	ND
p value	–	0.193	0.081	0.269	0.052	0.051	ND
Flavor							
Mean score	4.1	3.5	3.6	3.5	3.6	2.5	ND
p value	–	0.313	0.138	0.217	0.212	0.013*	ND
Texture							
Mean score	4.0	3.8	3.6	3.4	3.3	3.2	ND
p value	–	0.591	0.373	0.111	0.132	0.003**	ND

† ND, the fried bread sticks treated with 4.9 g/kg of AOB were not evaluated because their sensory attributes were thoroughly unacceptable. Full score: 5.0; * $p<0.05$, ** $p<0.01$.

Preparation of fried bread sticks

The preparation of fried bread sticks was conducted in the training kitchen of a restaurant according to the traditional technology and previous publication[30] with some modifications. As for the AOB and EGT test groups, a sequence of addition levels, *i.e.* 0.002, 0.01, 0.1, 1, 2.5 and 4.9 g/kg of AOB and EGT were mixed with the wheat flour in advance. Therefore, AOB and EGT spiked wheat flour were used in corresponding AOB and EGT test groups respectively while non-spiked wheat flour was used in the control group. Then, a piece of dough in each group was prepared from selected wheat flour, yeast, baking soda and salt. The prepared dough was then mixed with water and set for about 8 h. After yeast fermentation, the dough was stretched on a flat board and cut into 2×15 cm strips. Two of the strips were combined and stretched to about 25 cm long. About 15 of such combined strips were dipped into a wok containing heated vegetable oil maintained at 180±3 ºC and deep-fried to generate the product. The oil temperature was monitored by a hand-held thermocouple probe. After a certain period, final products were retrieved and set aside to cool and drain away the excess oil before packing for subsequent laboratory testing. All of fried products were then submitted for quantitative analysis of acrylamide. The experiments in each test group and control group were performed in sextuple repeats ($n = 6$).

Determination of acrylamide by LC-MS/MS

The quantitative analysis of acrylamide was based on the pretreatment of selected final products and performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray positive ionization (ESI+) using a Micromass Quattro Ultima mass spectrometer coupled to Waters 2695 HPLC chromatograph. The separation of acrylamide was achieved with an Atlantis dC$_{18}$ column (150 mm × 2.1 mm, 5 μm; Waters, Milford, MA, USA) according to the optimized LC and MS/MS conditions from our previous published methods.[31] The acrylamide levels in selective samples were quantified by the internal standard ($^{13}C_3$-acrylamide) method. The limit of quantification (LOQ) for acrylamide (10 μg/kg) was estimated by the MassLynx v4.0 software (Micromass, Manchester, Lancashire, UK).

Sensory evaluation and statistical analysis

Ten randomized volunteers were invited to score samples from the control and test groups in terms of color, flavor, texture and overall acceptability. Samples in each test group via coating with the different level of AOB or EGT were compared with samples in the control group. The sensory analysis was performed in double blind manner in order to eliminate the effect of subjective prejudice. Experimental data from acrylamide analysis were shown as mean ± SD while statistical analysis was performed by Duncan's multiple comparison tests and paired Student's *t*-test to determine the significance differences for treatment means of acrylamide formation and sensory estimation results in different treatments, respectively.

Results

The correlation between acrylamide contents and different addition treatments of AOB and EGT in fried bread sticks was shown in Figure 1. Results indicated that fried bread sticks with 0.002, 0.01, 0.1, 1, 2.5 and 4.9 g/kg wheat flour of AOB treatments induced 8.5%, 38.4%, 66.4%, 82.9%, 63.0% and 29.9% reduction of acrylamide generation, respectively. Similarly, samples with the same addition levels of EGT treatments incurred 25.1%, 45.7%, 72.5%, 46.1%, 25.3% and 6.0% reduction of acrylamide formation. The acrylamide contents in all of AOB and EGT treated groups were significantly difference from that of the control group ($p<0.01$). Results of Duncan's multiple comparison tests were also shown in Figure 1 and $p<0.05$ was regarded as the mark of significant difference.

The sensory evaluation results of fried bread sticks treated with AOB and EGT in different groups were shown in Table 1 and 2, respectively. The flavor and texture of fried bread sticks processed by AOB and EGT treatments had no significant difference compared to normal food matrixes ($p>0.05$) when both of AOB and EGT addition levels were no more than 1 g/kg. The color of samples could be regarded as no significant change ($p>0.05$) when the AOB and EGT treatment levels ranged 0.002–2.5 g/kg and 0.002–1 g/kg, respectively. However, the color of samples in the 2.5 g/kg of EGT treatment group showed great discrepancy compared to the control ($p<0.01$). Meanwhile, the sensory attributes of samples

Table 2. The sensory evaluation results of fried bread sticks with EGT treatments in different groups ($n = 10$)†

	Addition level of EGT (g/kg)						
	Control	0.002	0.01	0.1	1	2.5	4.9
Color							
Mean score	4.9	4.6	4.4	4.6	4.4	2.7	ND
p value	–	0.081	0.096	0.193	0.052	0.002**	ND
Flavor							
Mean score	4.5	4.0	4.0	3.7	3.0	2.3	ND
p value	–	0.397	0.138	0.070	0.138	0.003**	ND
Texture							
Mean score	4.4	3.7	3.7	3.7	3.8	2.0	ND
p value	–	0.066	0.088	0.132	0.111	0.001**	ND

† ND, the fried bread sticks treated with 4.9 g/kg of EGT were not evaluated because their sensory attributes were thoroughly unacceptable. Full score: 5.0; ** $p < 0.01$.

with 4.9 g/kg of AOB and EGT treatments could not be acceptable.

Discussion

In the present study, a less content of acrylamide (nearly 200 μg/kg) in fried bread sticks than other deep-fried cereal-based foods has been found. Addition of AOB and EGT greatly reduced the acrylamide content in the present food matrixes. Especially the acrylamide level in fried bread sticks could be reduced below 50 μg/kg under the optimal addition level of AOB or EGT. Such reduction phenomenon may be due to the fermentation with yeast before deep-frying and the use of antioxidants.

The extensive fermentation with yeast may be one of possible ways to reduce acrylamide content in products of the present work. Free asparagine and sugars are both important precursors for acrylamide formation in cereal-based products. In general, most of the asparagine was utilized after 2 h of fermentation with yeast. Sourdough fermentation, on the other hand, did not reduce the content of free asparagine as efficiently but had a strong negative impact on asparagine utilization by yeast. Such results indicated that this type of fermentation may result in breads with higher acrylamide content than in breads fermented with yeast only. Compared with short fermentation time (30 min), longer fermentation (6 h) reduced acrylamide content in bread made with whole grain wheat 87%. For breads made with rye bran, the corresponding reduction was 77%.[32] Nevertheless, Baardseth *et al.*[33] suggested that the lactic acid fermentation process of potato rods with *Lactobacillus plantarum* NC8 could also effectively reduce the acrylamide formation during production of French fries. Surprisingly, the contents of sugars (*i.e.* glucose, fructose and sucrose) declined greatly while the content of free asparagine remained largely unaffected during fermentation. Such observations indicated that the reduction of acrylamide was due to decreasing level of sugars rather than reduction of free asparagine. Unfortunately, an inevitable controversy about the effect of fermentation on the acrylamide generation made the researchers feel confused which factor induces the reduction of acrylamide during the fermentation process, *i.e.* via decreasing the content of free asparagine or sugars.

In the present study, a significant reduction effect of two antioxidants on the acrylamide formation was found. Results of acrylamide contents in fried bread sticks showed the opposite concentration-dependent relationships in different ranges of AOB and EGT treatments (Fig. 1). Such reverse tendency on the reduction of acrylamide may relate to the inherent property of these two antioxidants and the antioxidant activity of food matrixes, which is so-called "antioxidant paradox". In other words, it does not mean that an inevitable forward or backward relationship presents between the reduction efficiency of acrylamide and the antioxidant activity of deep-frying systems. First, previous study indicated the enhancement of acrylamide level dramatically with an increase of the antioxidant activity of frying foods. Summa *et al.*[34] found a direct correlation between the concentration of acrylamide and the antioxidant activity in self-prepared cookies and demonstrated that the combined conditions including long baking time, high protein content in samples and low moisture could simultaneously increase the acrylamide level and the antioxidant activity. Second, the acrylamide content could be reduced via the effect of antioxidants on preventing the occurrence of acrolein reaction pathway. Actually, the possible effect of lipids used for deep-frying systems in acrylamide formation has induced an intensive controversy. Acrolein reaction pathway initially originates from the degradation of lipids and promotes the oxidation of fatty acids or glycerol. It is well known that lipids heated at high temperature can lead to the formation of acrolein.[35] Acrolein can further react via oxidation to generate acrylic acid or by formation of an intermediate acrylic radical. Both of the intermediates could then induce acrylamide formation in the presence of a nitrogen source under favorable reaction circumstance.[13] Addition of antioxidants could block the oxidation of acrolein to a certain extent and further mitigate the generation of acrylamide. Therefore, the fact whether acrylamide could be reduced via addition of antioxidants should be confirmed by taking both of abovementioned factors into consideration and judging alternatively which factor plays a predominant role in the food system. In fact, both reduction and enhancement results of acrylamide formation via addition of different antioxidants were validated in differ-

ent published researches, which suggested the dual effects of antioxidants on the generation of acrylamide.[36] In the present work, sufficient results demonstrated that fried bread sticks with AOB or EGT treatments have decreasing level of acrylamide compared to the food without any additive treatment.

Many researches found effective ways to reduce the acrylamide content during heat processing but their sensory evaluation was not reported or not very reasonable, even not acceptable.[37,38] For instance, the largest decrease of acrylamide content (90%) in crisps was obtained when potato slices were soaked in acetic acid solution for 60 min at 20 °C, and a large reduction of acrylamide content (74%) was also observed after soaking of potato slices in 1% NaOH solution. However, a sour and acerbic taste from both of treatments greatly influenced the appearance as well as the taste and flavor of crisps, which were not sensorially acceptable.[39] In the present work, although the optimal inhibitory rate of acrylamide in fried bread stick samples was a bit lower than some other published methods, the balance between reduction effects and sensory acceptance was well achieved.

Conclusion

In the present study, experimental results showed that the addition of AOB or EGT could effectively reduce the amounts of acrylamide in fried bread sticks. The optimal way of AOB or EGT treatments could not only effectively achieve the reduction of acrylamide but also remain reasonable sensory attributes. This study could be regarded as an important contribution on the reduction of acrylamide formation in deep-fried foods and Asian cereal breakfast by addition of natural antioxidants. However, the mechanism of acrylamide reduction by AOB still remains to be clarified and will be conducted in due course. The effects of acrylamide reduction on other fried or baked products via the addition of these two antioxidants are necessarily studied further. Meanwhile, the safety of polyphenol components in both AOB and EGT after deep frying will be further considered and evaluated.

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