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The Inhibitory Role of Plant Extracts on Acrylamide Formation in Potato Chips

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Abstract: This paper investigated the efficiency of antioxidant of Thyme and extract of cumin on the reduction of acrylamide in potato chips and summarized the optimal levels of two additives. Seven experimental groups including a control group were organized for both of additives. Potato chips were made via traditional processing technology. The potato was mixed with different levels (0.001-3.5 g/kg flour) of Thyme and Cumin, respectively. The acrylamide level in potato chips was determined by Gas chromatography (GC/FID). Results showed that nearly 81.6% and 70.7% of acrylamide were reduced when the Thyme and Cumin addition levels were 1 and 0.1 g/kg, respectively. The elevated inhibitory effects of Thyme and Cumin on the acrylamide formation were achieved with an increase of additive levels unless the spiking levels of Thyme and Cumin extracts were greater than 1 and 0.1 g/kg, respectively. The present study indicated that both Thyme and Cumin could significantly reduce the acrylamide content generated in potato chips and keep original flavor and crispness of potato chips. This study could be regarded as an important contribution on the reduction of acrylamide by natural antioxidants.

Key words: Acrylamide, potato chips, reduction, antioxidant of thyme, extract of cumin.

1. 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: (1)

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Some key intermediates are eliminated under the change of reaction conditions; (2) Other vinylogous compounds instead of acrylamide are formed; (3) 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.. 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 Thyme, a Thyme powder extracted form Thyme leaves, was capable of blocking chain reactions of lipid auto oxidation, chelating metal ions of transient state, scavenging nitrite compounds and blocking the synthetic reaction of nitrosamine reported by our previous study [24]. Moreover, Thyme 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, Thyme was allowed to be added into puffed foods, meat products, fried foods and edible oils authorized by Ministry of Health, IRAN. The main functional components in Thyme are flavonoids, lactones and phenolic acids while flavone *C*-glucosides are a group of representative flavonoids in Thyme [26]. On the other hand, extract of cumin 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, epicatechin gallate, epigallocatechin, and epigallocatechin gallate are the main components in extract of cumin. Thyme and extract of cumin have been both approved as two kinds of food antioxidants in Iranian national standards. 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.

Potato chips, a kind of all-time favorite snacks for Iranian, have similar processing style with lots of Western fried foods. Studies conducted so far indicated that moderate protein and high carbohydrate foods such Asian potatoes substantially higher levels of acrylamide has formed under heating conditions [7, 8]. However, to our best knowledge, few published papers in peer-review journals reported the acrylamide content in potato chips. IRAN has a huge population and millions of people consume potato chips as their snacks 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: (1) to investigate the effect of extract of Thyme or cumin on the reduction of acrylamide in potato chips; (2) to summarize the optimal levels of two additives, which can be applied in this product.

2. Materials and Methods

2.1 Materials

The potato, vegetable oil used for the preparation of potato chips were purchased from a local supermarket in Qazvin (May 2010). Thyme was prepared from the Thyme leaves. Briefly, fresh cumin leaves were collected in Kerman and air dried. The coarse powder of cumin 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 obtained by membrane filtration to remove macro- and micro-molecular components such as polysaccharides and minerals. Finally, extract of Thyme or cumin was obtained after concentrating in vacuum and spray drying. The total content of four main flavanols (epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate) was approximately 98%.

2.2 Chemicals

Acrylamide (99%) and C_3-labelled acrylamide (isotopic purity 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively [13]. 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.

2.3 Preparation of Potato Chips and Addition of Extracts

The preparation of potato chips was conducted in the training kitchen of a restaurant according to the traditional technology and previous publication [30] with some modifications. As for the extract of Thyme or cumin test groups, a sequence of addition levels, i.e., 0.002, 0.05, 0.1, 1, 2.5 and 3.5 g/kg of extract of Thyme or cumin were mixed with the potato slice in advance. Therefore, extract of Thyme or cumin spiked potato were used in corresponding extract of Thyme or cumin test groups respectively while non-spiked potato was used in the control group. Then, a piece of potato chips in each group was prepared from selected potato, vegetable oil, additives and salt. Some of potato slices were dipped into a wok containing heated vegetable oil maintained at 180 ± 3 ℃ 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 ($n = 6$).

2.4 Determination of Acrylamide by GC/FID

Gas chromatography (GC) has been used to quantify acrylamide in a variety of industrial and environmental applications.

Column: Elite-Wax ETR-15 m, 0.53 ID, 0.50 μm film, Inj.: 1.0 μL, 0.5 min. hold, Liner: 2 mm splitless with wool, Injector temp.: 260 °C, Carrier gas: helium, constant pressure, Linear velocity: 62 cm/sec. @ 100 °C , Oven temp.: 100 °C (hold 0.5 min.) to 200 °C @ 15 °C/min., Detector: FID @ 260 °C.

2.5 Sample Preparation Procedure

The procedure we used in our analysis of potato chips was as follows: PerkinElmer Life and Analytical Sciences [31].

2.6 Sensory Evaluation and Statistical Analysis

Samples in each test group that contained different level of extract of Thyme or cumin 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.

3. Results

The correlation between acrylamide contents and different addition treatments of extract of Thyme or cumin in potato chips was shown in Fig. 1. Results

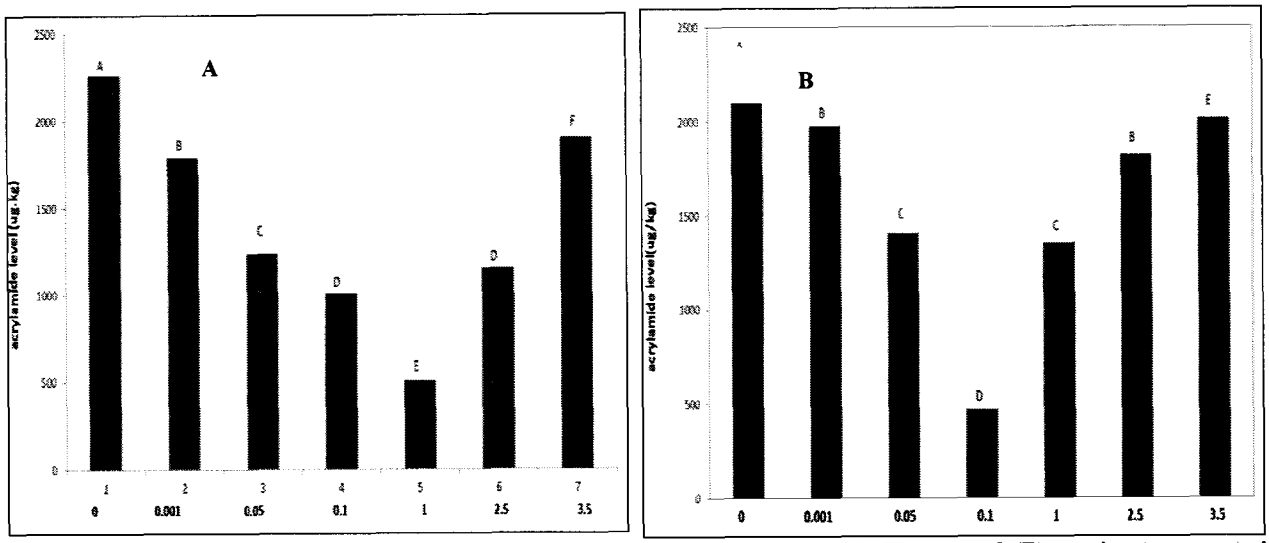

Fig. 1 The relationship between acrylamide levels and different conditions of (A) Thyme and (B) cumine treatments in potato chips (n = 6). Error bars designate standard deviation (SD) and different letters indicate significant differences via Duncan's multiple comparison test ($P < 0.05$).

indicated that potato chips with 0.001, 0.05, 0.1, 1, 2.5 and 3.5 g/kg potato chips of extract of Thyme treatments induced 8.9, 36.6, 68.4, 81.6, 60.0 and 28.2% reduction of acrylamide generation, respectively. Similarly, samples with the same addition levels of extract of cumin treatments incurred 26.3, 42.6, 70.7, 48.2, 25.8 and 7.0% reduction of acrylamide formation. The acrylamide contents in all of extract of Thyme or cumin treated groups were significantly different from that of the control group ($P < 0.01$). Results of Duncan's multiple comparison tests were also shown in Fig. 1 and $P < 0.05$ was regarded as the mark of significant difference.

4. Discussion

In the present study, a less content of acrylamide (nearly 1,500 μg/kg) in potato chips than French fried has been found. Addition of extract of Thyme or cumin greatly reduced the acrylamide content in the present food matrixes. Especially the acrylamide level in potato chips could be reduced below 400 μg/kg under the optimal addition level of extract of Thyme or cumin. Such reduction phenomenon may be due to the addition with other plants extracts before deep-frying and the use of antioxidants.

In the present study, a significant reduction effect of two antioxidants on the acrylamide formation was found. Results of acrylamide contents in potato chips showed the opposite concentration-dependent relationships in different ranges of extract of Thym or cumin 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" [32]. 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. [33] 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 [34]. 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 food system. In fact, both reduction and enhancement results of acrylamide formation via addition of different antioxidants were validated in different published researches, which suggested the dual effects of antioxidants on the generation of acrylamide [35]. In the present work, sufficient results demonstrated that potato chips with extract of Thyme or cumin treatments have a decreasing effect on the level of acrylamide compared to the food without any additive treatment.

Many research 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 [36, 37]. 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% KCl 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 [38].

5. Conclusion

In the present study, experimental results showed that the addition of extract of Thyme or cumin could effectively reduce the amounts of acrylamide in potato chips and snacks. The optimal way of extract of Thyme or cumin 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 by addition of natural antioxidants. However, the mechanism of acrylamide reduction by extract of Thyme 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 extract of Thyme or cumin after deep frying will be further considered and evaluated.

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