

SPE/HPLC/UV studies on acrylamide in deep-fried flour-based indigenous Chinese foods

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Abstract

A fast and cost-effective method using HPLC/UV has been developed for determination of acrylamide in deep-fried flour-based leaven dough foods available in Hong Kong. The samples were purified by a simple solid-phase extraction method which combined Oasis HLB and Bond Elut-Accucat cartridges. The aqueous sample solution was centrifuged at $14,500 \times g$ and 0°C for 15 min to successfully remove the fat in the samples. A gradient elution program and a mobile phase of 4.0% v/v acetonitrile in water allowed sufficient retention and well resolved acrylamide from the food matrices in the sample extracts. Acrylamide was detected at UV wavelengths of 210 and 225 nm. The amounts of acrylamide in eight food samples were 27–198 $\mu\text{g/kg}$ when 1-g samples were analyzed. The recoveries of acrylamide were larger than 78.0% and the precisions were 2.1–10.9% ($n=3$). Our proposed method is especially relevant for analyzing acrylamide in those oily food matrices.

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1. Introduction

On 24 April 2002, the Swedish National Food Administration and the University of Stockholm jointly announced that certain food products contain relatively high amounts of acrylamide [1]. Carbohydrate-rich foods such as French fries processed/cooked at high temperatures and under low moisture conditions were predominantly of concern. Few amounts of acrylamide were found in raw and boiled foods. These findings have attracted considerable interest worldwide since acrylamide is a known human neurotoxin and has been classified as a group 2A carcinogen (probably carcinogenic to human) [2]. The Swedish findings about the high level of acrylamide in heat-

treated foods were quickly confirmed by the UK Food Standards Agency through its official website notification on 17 May 2002 [3].

The US Environmental Protection Agency's (USEPA) limit for acrylamide in drinking water is extremely low ($0.5 \mu\text{g/kg}$) [4]. Intense research on this compound began in the US and Europe after the Swedish researchers found acrylamide in food at concentrations hundreds of times higher than what USEPA and the World Health Organization (WHO) consider safe for drinking water. In the past five years, over 200 scientific papers have been published on this chemical. Current researches mainly focus on methods of reducing acrylamide formation or controlling acrylamide level during cooking. The wave of research projects, across the Western hemisphere, in which analysis of acrylamide in foods was used to identify different food products and cooking processes so as to reduce the level of acrylamide and improve product safety. With the development of the state of affairs on such new contaminant, all available research data on acrylamide have been reviewed at an international level, e.g. by the WHO, the Food and Agriculture

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Organization of the United Nations [5], and Joint Institute for Food Safety and Applied Nutrition (JIFSAN)/National Center for Food Safety and Technology [6]. Meanwhile, JIFSAN has established a special website as a global resource and inventory of ongoing research on acrylamide in foods. This website includes formal research, surveillance/monitoring and industry investigations of acrylamide [7].

Numerous methods have in fact been developed in the past years to determine the acrylamide monomer, especially in water, biological fluids and food. The majority of them are based on liquid or gas chromatographic (GC) techniques [8–25]. However, these methods lack selectivity and the additional degree of analyte certainty required to confirm the presence of a small molecule such as acrylamide in a complex food matrix. To date, several analytical methods dealing with the analysis of acrylamide in cooked foods have been published. These methods are based mainly on mass spectrometry (MS) as the determinative technique, coupled with a chromatographic step either by GC [8–15], or LC [16–24], usually after derivatization of the analyte. The development of size-exclusion chromatography coupled with electrospray mass spectrometry for determination of acrylamide in fried foods was also reported [25]. In addition, current information of acrylamide research in food analysis, formation and control has been recently reviewed and discussed [26–30].

The expert Working Group on Analytical Methods in a recent JIFSAN meeting on acrylamide concluded [7] that the majority of laboratories use either GC/MS or LC/MS. The advantage of the LC/MS-based methods is that acrylamide can be analyzed without prior derivatization (e.g. bromination), which considerably simplifies and expedites the analysis. Due to the low molecular mass of acrylamide (71 g/mol) and thus also its low-mass fragment ions, confirmation of the analyte can be achieved with a two-stage mass spectrometer (monitoring of more than one characteristic mass transition) [13–24]. However, acrylamide is a very polar molecule with poor retention on conventional LC reversed-phase sorbents [13], and despite the use of tandem mass spectrometry more effort may need to be placed on efficient clean-up steps to avoid interference from co-extractives.

High-performance liquid chromatographic (HPLC) techniques possess the advantages of easy generalization, lower cost and strong maneuverability compared to those MS techniques. It was applied with an UV detector for systematically analyzing acrylamide [31–33]. Unfortunately, their systems are still complicated. Terada and Tamura have reported the use of two pumps, two 6-port-2-position valves, two columns and an UV detector for systematically analyzing acrylamide [31]. A method using normal phase HPLC with UV detection was developed for the analysis of acrylamide and methacrylamide [32]. This method relied on the chromatographic separation of these analytes on a polar HPLC column designed for the separation of organic acids. Hexane was added to the sample solution to extract remaining long chain fatty acids that could create problems in chromatographic analysis by giving peaks overlapping with the target analytes or block the polar column. Gökmen et al. [33] found that a conventional HPLC instrument

coupled to diode array detector (DAD) can also be used accurately and precisely as an alternative to tandem LC–MS methods for the determination of acrylamide in potato-based foods. This method entailed extraction of acrylamide with methanol, purification with Carrez I and II solutions, evaporation and solvent change to water. Whether using hexane or Carrez solutions to defat the sample solutions, it is a more complicated procedure.

The adventitious discovery of acrylamide in certain heat-treated foods has created a need to determine this compound in a wide variety of food commodities. Acrylamide levels were found highest in potato and cereal-based products subjected to heat processing such as frying, grilling or baking. Lower levels have also been found in some other heat-processed foods. However, most of the tested foods belong to the Western diet [5]. The range of foods investigated needs to be extended to include staple foods from different regions and diets. Recently, a determination of acrylamide in Chinese traditional carbohydrate-rich foods using GC with micro-electron capture detector (MECD) and isotope dilution LC combined with electrospray ionization tandem MS was reported [12]. The highest level of acrylamide has been found in Chinese corn crisps ($439.6 \mu\text{g kg}^{-1}$ by GC/MECD and $464.8 \mu\text{g kg}^{-1}$ by LC/MS/MS) and potato crackers ($771.1 \mu\text{g kg}^{-1}$ by GC/MECD and $734.5 \mu\text{g kg}^{-1}$ by LC/MS/MS) during the investigation. Since a large number of Chinese indigenous foods are deep-fried golden-colored flour-based products, very popular as breakfast foods and for festivals in Hong Kong, it is essential to acquire data on acrylamide in these food products. As such, the aim of our work is to develop a simple, fast and inexpensive SPE/HPLC/UV method to determine the acrylamide level in some deep-fried flour-based indigenous Chinese foods commercially available in Hong Kong.

In this work, seven traditional Chinese and Lunar New Year fried food samples including stuffed glutinous rice ball, ‘ox-tongue’ fritter, sesame ball, deep-fried sesame cookies, glutinous rice sesame ball, fried sweet dumpling, and fried egg pastry have been examined quantitatively using the reverse-phase HPLC with UV detection. These foods were mainly prepared by high-temperature cooking such as grilling, roasting, baking, frying, and deep-frying. In addition, another deep-fried crispy food (sweet potato crisps) was also analyzed for comparison purposes. The major attribute of our proposed method is that the preparation of sample is simple, fast and can cope with very oily starch-rich food products prior to HPLC analysis.

2. Materials and methods

2.1. Instruments and reagents

Acrylamide (>99%) was of electrophoresis grade obtained from Aldrich (Milwaukee, WI, USA). All organic solvents were of HPLC-grade unless otherwise stated. Water was purified by a Milli-Q-RO4 water purification system (Millipore, Bedford, MA, USA) with a resistivity higher than $18 \text{ M}\Omega\cdot\text{cm}$. All chemicals of analytical reagent grade or above were used as received. 50-mL polypropylene conical tubes with caps were from Nalge Nunc International (Rochester, NY, USA). Oasis

HLB 60 mg, 2 mL SPE cartridges were obtained from Waters (Milford, MA, USA). Bond Elut-Accucat 200 mg, 3 mL SPE cartridges were purchased from Varian (Chicago, IL, USA). Amber glass auto-sampler vials with septum screw caps were obtained from Agilent Technologies (Wilmington, DE, USA). The analytical column (Alltima C18 LC–MS, 150×2.1 mm i.d., 3 µm particle size) and syringe filters (0.45 µm PVDF) were from Alltech Associates (Deerfield, IL, USA).

A standard acrylamide stock solution (1.0 mg/mL) was prepared by dissolving 1.0 mg of acrylamide in 1.0 mL of Milli-Q water. An electronic analytical balance (FA 2004N, Shanghai Precision Scientific Instrument, Shanghai, China) was used to weigh accurately 1.0 mg of acrylamide. 1.0 mL of water was measured by a certified autopipette (Gilson, Paris, France). The acrylamide stock solution was diluted in amber glass volumetric flasks to prepare calibration standards at 50, 100, 200, 300, 400, 500, 1000 and 2000 µg/L, respectively. All standard solutions were stored in amber narrow-mouth bottles (Nalge Nunc International, Rochester, NY, USA) at 4 °C until further use.

2.2. Sample preparation

Food samples from the convenience stores and fast-food restaurants in Hong Kong were purchased and pulverized in an extra fine blade blender (Hitachi, Tokyo, Japan) prior to water extraction. The samples were extracted as described in literature [20] with minor modifications. Two 1.00-g solid samples were accurately weighed into separate 50-mL Teflon centrifuge tubes. To one centrifuge tube 10 mL of water was added. Similarly, 1.0 mL of 100 µg/L acrylamide standard was added to another centrifuge tube for recovery study. All the capped tubes were clamped in a rotating shaker (Flask Shaker SF1, Stuart Scientific, UK) and shaken for 30 min. The tubes were centrifuged at 6700 ×g for 10 min. A pipette was inserted through the top oil layer to the aqueous layer, avoiding the bottom solids with the pipette tips when 3.0 mL of aqueous phase was withdrawn. The aqueous sample solution was then centrifuged by a cyclone centrifuge with freezing (Eppendorf, Hamburg, Germany) at 14,500 ×g and 0 °C for 15 min to solidify and precipitate the oil residue in the aqueous sample solution. Then 2.0-mL aliquot of clarified aqueous layer was promptly removed and filtered through a 0.45-µm PVDF syringe filter to a vial.

Oasis HLB SPE cartridges and Varian Bond Elut-Accucat SPE cartridges were used to remove a number of early eluting co-extractives (i.e., the interferents) in the aqueous sample extract. It has been shown to effectively reduce the matrix effects in the analysis of cooked carbohydrate-rich foods [20]. Oasis and Elut-Accucat SPE cartridges were initially conditioned with 3.5 mL of methanol followed by 3.5 mL of water; the methanol and water portions were discarded. The filtered extract was allowed to successively pass through both SPE cartridges. All elutant from the Oasis and Elut-Accucat SPE cartridges was collected. The extract was then evaporated to almost dryness by a gentle stream of compressed air at room temperature. The resulting residue was re-dissolved in water and adjusted to 1.0 mL in a 1.5-mL amber glass vial. All clean sample extracts were stored at 4 °C until future use. Further

filtrations of the clean sample extracts through 0.2-µm PVDF syringe filters were performed prior to all HPLC–UV analyses.

2.3. HPLC–UV analysis

HPLC–UV analysis was done on an HP 1010 series HPLC instrument equipped with a vacuum degasser, a binary pump and a DAD (Hewlett Packard, Wilmington, DE, USA). The calibration standards and sample extracts were injected via a 20-µL sample loop, and detected simultaneously at two UV wavelengths of 210 and 225 nm. A gradient elution program was used at a flow rate of 0.10 mL/min, in which mobile phase A was consisted of acetonitrile and water 1:24 (v/v), and mobile phase B was pure acetonitrile. The elution program was applied as follows: 100% A (0% B) for 10 min; decreased to 20% A from 10 to 12 min; and kept at 20% A for 5 min; increased to 100% A from 17 to 19 min; and kept at 100% A for 21 min, so the total run time was 40 min. Under these chromatographic conditions, acrylamide and the food components in the tested samples were all baseline separated and eluted.

3. Results and discussion

3.1. Sample clean-up

Water was applied to extract our tested samples. At room temperature water has been frequently used to extract acrylamide from sample matrices because acrylamide is a good hydrophilic small molecule and this also minimizes the dissolution of hydrophobic compounds in the food products [25]. Furthermore, elution with water also has the advantage of eliminating desorption of all hydrophobic impurities that remains adsorbed on the SPE sorbent.

Some researchers defatted sample with hexane [34,35] while another purified sample with Carrez solutions [33]. However we found that when the temperature was 0 °C and the centrifugation speed was 13,000 ×g, the oil layer was solidified and could simply be removed by filtration, thus greatly reduced the interference effect.

A more extensive clean-up procedure has been proposed [20,21] which requires two SPE sorbents. Our approach was to combine Oasis HLB and Bond Elut-Accucat cartridges. Samples were directly passed through the two SPE cartridges and all elutant was collected in order to obtain high recoveries (Table 1).

Table 1
Recovery test of acrylamide for some tested food samples

Food sample	Mean acrylamide level (µg/kg)	Amount of acrylamide added (µg/kg)	Amount of acrylamide found ^a (µg/kg)	Recovery (%)
1	51	100	143±1.4	92.0
2	68	100	146±6.4	78.0
3	82	100	189±4.4	107
4	113	100	218±3.4	105

^a Average values and standard deviations are calculated on three replicates.

3.2. Optimization of the chromatographic conditions

When 100% water was employed as the mobile phase, excessive band broadening of the solutes was observed. As such, methanol/water and acetonitrile/water mixtures were evaluated as mobile phases for acrylamide. Fig. 1 displays the chromatograms obtained by various concentrations of methanol and acetonitrile. It seems that methanol and acetonitrile has similar elution effect on acrylamide. However, we observed that the baseline separation of acrylamide and the interferents of the food samples (stuffed glutinous rice ball, ‘ox-tongue’ fritter and sesame ball) was not satisfactory when 5.0% as shown in Fig. 2a or 10.0% v/v methanol as displayed in Fig. 2b were employed as the mobile phases. Fortunately, when 4.0% v/v acetonitrile was used as the mobile phase at a flow rate of 0.10 mL/min, acrylamide with a retention time of 7.0 min was satisfactorily separated from the food sample interferents. As such, 4.0% v/v acetonitrile was chosen as the optimal mobile phase for our HPLC/UV analysis of acrylamide in the tested samples.

Acrylamide is produced in food during cooking processes at high temperatures by means of the Maillard reaction [28,36–38]. The Maillard reaction is a related series of reactions which involve amino compounds and reducing sugars with acrylamide as one of its major products. Those unknown products from the food matrix can produce high interference background leading to difficulties for reverse-phase HPLC analysis of acrylamide. 4.0% v/v acetonitrile in water was chosen as the optimal mobile phase for our HPLC analysis because of three reasons. First, 4.0% v/v acetonitrile produced minimum interferent peaks at the shorter UV wavelength (210 nm) detection. Second, the column head pressure would be lower when the mobile phase was acetonitrile/water instead of methanol/water. Third, acry-

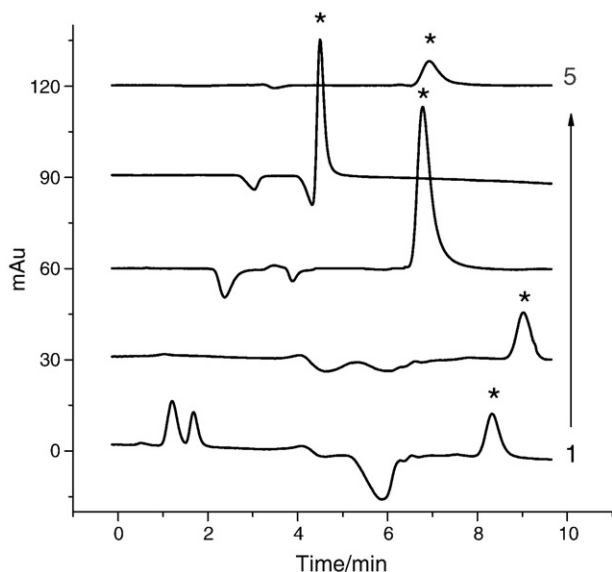


Fig. 1. Chromatograms of acrylamide when various mobile phases were used. (1) 10% v/v MeOH at 0.10 mL/min; (2) 5.0% v/v MeOH at 0.10 mL/min; (3) 5.0% MeOH at 0.15 mL/min; (4) 1.0% v/v MeOH and 0.09% v/v acetic acid at 0.20 mL/min; and (5) 4.0% v/v acetonitrile at 0.10 mL/min. The detection wavelength was 210 nm. Peaks with asterisks are acrylamide. The amount of the injected acrylamide standard was 10 ng. Chromatograms are offset for clarity.

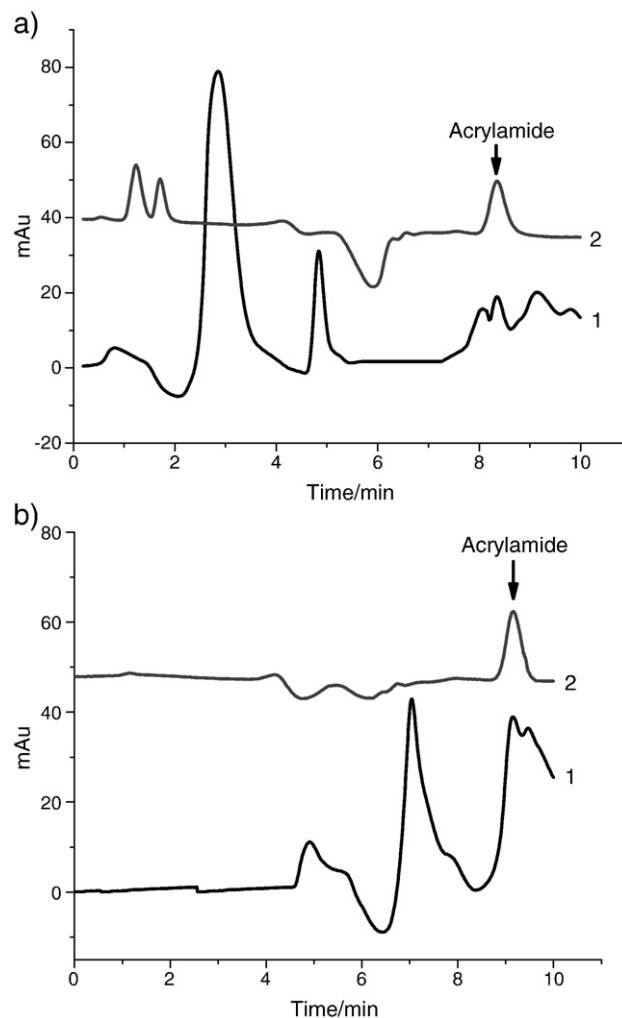


Fig. 2. Unsatisfactory separation of food samples using various mobile phases. (a) Mobile phase: 5.0% v/v MeOH and food sample: ‘ox-tongue’ fritter; and (b) mobile phase: 10% v/v MeOH and food sample: sesame ball. Traces 1 and 2 are the chromatograms of acrylamide standards and samples, respectively. The detection wavelength was 210 nm. Chromatograms are offset for clarity.

lamide could be baseline separated from the food sample interferents. Fig. 3 shows the analysis of acrylamide in an ‘ox-tongue’ fritter sample (Fig. 3a) and two fried sweet dumpling samples (Fig. 3b) by our optimal mobile phase and HPLC/UV method. Acrylamide was identified and well-separated from those interferents in the sample.

In this HPLC study, the gradient elution was primarily used for the column clean-up before injecting the next sample. For our tested samples, most interferents, especially the more hydrophobic ones, were eluted after the acrylamide peak. These are the typical situations for reverse-phase HPLC. When an isocratic elution was applied for HPLC separation, most interferents were eluted at retention times of 25–35 min. In contrast, their retention times could be shortened to less than 20 min when the solvent program was applied to the column. A higher% of acetonitrile in the mobile phase at the later elution program could be more efficient in cleaning up, lowering the column head pressure, and conditioning the column before the next sample analysis. However, when the acetonitrile % in the mobile phase was too

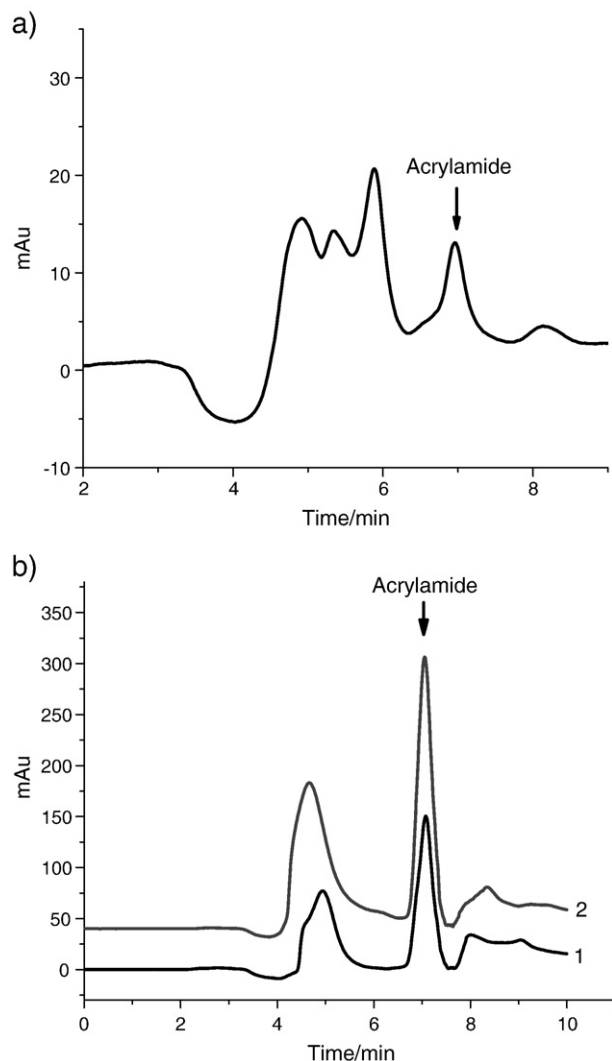


Fig. 3. Chromatograms of food samples in the first 9.0–10.0 min elution. (a) An 'ox-tongue' fritter sample, and (b) fried sweet dumpling samples 1 and 2. The mobile phase composition was 4.0% v/v acetonitrile for the first 10.0 min at 0.10 mL/min. The detection wavelength was 210 nm. Chromatograms are offset for clarity.

high (ca. 100%), some interferences in the samples would precipitate at the column head and build up very high column head pressure which would in turn make the analysis impossible. Hence, our applied gradient elution program is able to resolve acrylamide from the hydrophobic interferences as well as condition the column before the next sample analysis. The total run time for each sample was 40 min.

3.3. Identification of acrylamide

The determination of acrylamide in food samples was based on the peak area and retention time of acrylamide in a chromatographic run. In order to more accurately identify the acrylamide peak in a chromatogram, a known amount of acrylamide standard was pre-mixed with the food sample extract and then injected to the column. When the peak area of acrylamide in the spiked sample increased profoundly,

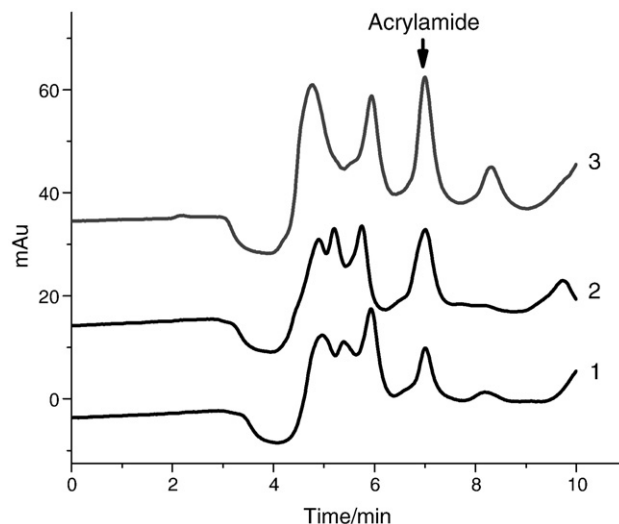


Fig. 4. Chromatograms of the spiked 'ox-tongue' fritter samples. (1) Original sample, (2) sample spiked with ca. 5 ng acrylamide, and (3) sample spiked with ca. 7.5 ng acrylamide. The detection wavelength was 210 nm. Chromatograms are offset for clarity.

acrylamide in the sample could be accurately located by comparing the peak area and retention time of the unspiked one as depicted in Fig. 4. This is a simple and quick way to semi-qualitatively determine the presence of acrylamide in a food sample. Once the acrylamide in the sample had been identified, the concentration of acrylamide could be calculated by using the calibration curves as shown in Fig. 5. Since the sensitivity was higher at detection wavelength of 210 nm than at 225 nm, those

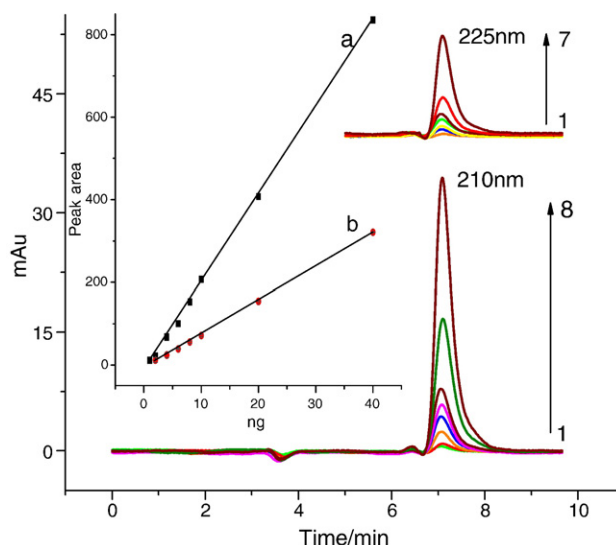


Fig. 5. HPLC chromatograms of acrylamide standards at detection wavelengths of 210 nm and 225 nm, respectively. At 210 nm, 1 to 8 were 50; 100; 200; 300; 400; 500; 1000; and 2000 $\mu\text{g/L}$ of acrylamide. At 225 nm, 1 to 7 were 100; 200; 300; 400; 500; 1000; and 2000 $\mu\text{g/L}$ of acrylamide. The mobile phase was 4.0% v/v acetonitrile at 0.10 mL/min. The inset displays the calibration curves at (a) 210 nm, and (b) 225 nm. These calibration curves are constructed by plotting the amount of acrylamide (in nanograms) against peak area of the standards. The linear fit equation for (a) is: $y = 25.49x + 9.119$, and (b) is: $y = 7.699x - 5.109$.

samples with lower concentrations of acrylamide would be detected at 210 nm.

3.4. Recovery test

Recovery determination can reflect the extraction efficiency and sample loss during sample preparation. Adding known amounts of acrylamide standards in the samples before sample extraction can be used to assess the recovery of acrylamide in real samples. Percentage recovery was determined from the amount of acrylamide added compared with the amount found. The experiments were repeated three times to obtain the recovery and the relative standard derivation (RSD) for each chosen sample. The results of recovery of the tested samples are summarized in Table 1. It was found that the recovery was 78–107% and this demonstrates that our sample preparation methodology and HPLC analysis is satisfactory.

3.5. Linear range, limits of detection and quantification, and reproducibility

The calibration curves were obtained by plotting the peak area against concentration of acrylamide. It has been reported that the concentrations of acrylamide in most food samples do not exceed 1000 µg/kg [39]. As such, aliquots of the stock solution of acrylamide were used to prepare working standard solutions at eight concentrations between 50 and 2000 µg/L with a detection wavelength of 210 nm. A linear response was obtained within the concentration range of 50–2000 µg/L with a correlation coefficient greater than 0.999 as depicted in Fig. 5. The limits of detection (LOD) and quantification (LOQ) of this method were determined with S/N equal to 3 and 10, respectively [40]. It was found that the LOD and LOQ were 6 and 23 µg/kg at a detection wavelength of 210 nm, respectively. Our LOQ is slightly higher than the literature values [20,21].

The intra-laboratory reproducibility of the method, expressed as the RSD ($n=3$), was determined at two-level concentrations 30 and 51 µg/kg of ‘ox-tongue’ fritter, and 27 and 52 µg/kg of stuffed glutinous rice ball. The RSD were found to vary between 2.1 and 10.9%.

3.6. The effect of cooking processes

The formation of acrylamide in a food product can be affected by the action of hot oil on the dough material. Hot oil heats the surface of the dough, initiating the formation of acrylamide, then other reaction products change the color of the dough to golden brown and at the same time the crust becomes crispy and hot oil will permeate into the interior food matrix. Maillard reaction in the interior of the dough continues with the direct heat of hot oil. Two samples of each ‘ox-tongue’ fritter and stuffed glutinous rice ball were determined for their acrylamide contents as displayed in Table 2. It was observed that the more brownish the sample, the higher the amount of acrylamide found. So the formation of acrylamide in the dough depends greatly on the temperature of hot oil during the deep-frying process. Table 2 illustrates that sweet potato crisps, fried sweet

Table 2

Analysis of acrylamide in various deep-fried indigenous Chinese foods available in Hong Kong

Food type	Concentration of acrylamide (µg/kg)
Stuffed glutinous ball (1) ^a	27±2.1 ^b
Stuffed glutinous ball (2) ^a	52±3.0 ^b
‘Ox-tongue’ fritter (1) ^a	30±4.1 ^b
‘Ox-tongue’ fritter (2) ^a	51±6.2 ^b
Sesame ball	68±10.9 ^b
Deep-fried sesame cookies	82±8.8 ^b
Glutinous rice sesame ball	113±3.2 ^b
Sweet potato crisps	134
Fried sweet dumpling	136±3.4 ^b
Fried egg pastry	198

^a Sample (2) looks more brownish than sample (1).

^b Average values and standard deviations are calculated on three replicates.

dumplings, and fried egg pastry contain higher levels of acrylamide. Wheat flour-based crisps are crispy and low in moisture content. They are in thin slice form with larger specific surface areas, and these account for the generally higher acrylamide levels. The results of some of our tested samples were comparable with a recent report using a more expensive LC/MS/MS technique [41].

Glutinous rice flour-based matrix (e.g. stuffed glutinous rice ball) was denser than wheat flour-based leaven dough (e.g. ‘ox-tongue’ fritter). Even though the color of the stuffed glutinous rice ball was slightly more brownish than ‘ox-tongue’ fritter, the amount of acrylamide in stuffed glutinous rice ball was more or less same as in ‘ox-tongue’ fritter. The fine and close structure of the glutinous rice dough can possibly reduce the diffusion rate of hot oil from surface to the interior part of the sample. But for the same type of glutinous rice food product, the amounts of acrylamide found in darker colored products are still higher than the lighter ones (Table 2). Thus, the formation of acrylamide appears to be a surface phenomenon associated with the browning reactions (i.e. Maillard reaction) when heating foods.

When starchy foods were heated to temperatures above 100 °C, a complex series of reactions ensued, known as caramelization, which gave rise to a wide range of flavor compounds as well as the brown pigments that were particularly associated with caramel [36]. If sugars were smeared on the surface of the food, the color would easier turn to dark brown. It has been reported that when starchy foods were cooked long enough and at high temperatures so they became very brown, they usually contained more acrylamide than the same food that had not acquired a dark color [42]. The results of our study conclude that controlling the color of food during frying is an important way to reduce or prevent acrylamide formation during cooking.

4. Conclusion

Our sample preparation comprising water extraction, low temperature centrifugation (0 °C) and SPE clean-up provides an effective method to extract acrylamide and remove most oily substances in a sample before HPLC analysis. Furthermore, the

applied gradient elution program is able to resolve acrylamide from the hydrophobic interferences as well as condition the column before the next sample analysis. It is anticipated that our proposed analytical technique is simpler, cost-effective, and can be adopted by food industry to monitor the by-product acrylamide during the manufacturing process. Although HPLC method is not as accurate as LC/MS/MS technique, it can be applied to screen food samples for acrylamide. Once the concentration of acrylamide found in food sample is high, more elaborate and expensive LC/MS/MS will be employed to determine accurately the concentration of acrylamide. Finally, continual effort is also required in improving food formulation, processing and cooking conditions in order to minimize the level of and possibly eliminate acrylamide in food products.

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References

- [1] Swedish National Food Administration, Acrylamide is formed during the preparation of food and occurs in many foodstuffs, 2002 <http://www.slv.se/>.
- [2] International Agency for Research on Cancer (IARC), IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: some industrial chemicals, vol. 60, Lyon, 1994, pp. 389–433.
- [3] UK Food Standards Agency, Food Standards Agency Study Shows Acrylamide in Food, 2002 <http://www.foodstandards.gov.uk/>.
- [4] World Health Organization (WHO), 2nd ed., Guidelines for drinking water quality, vol. 2, Geneva, 1996, pp. 940–949.
- [5] FAO/WHO Consultations on the Health Implications of Acrylamide in Food, Report of a Joint FAO/WHO Consultation, Geneva, Switzerland, 25–27 June, 2002.
- [6] JIFSAN/NCFST, Acrylamide in food workshop: scientific issues, uncertainties, and research strategies, Workshop on 28–30 October 2002, Rosemont, IL, USA, 2002, <http://www.jifsan.umd.edu/acrylamide2002.htm>.
- [7] JIFSAN, Acrylamide Infonet, 2002 <http://www.acrylamide-food.org/>.
- [8] C.F. Poole, W.F. Sye, A. Zlatkis, P.S. Spencer, Determination of acrylamide in nerve tissue homogenates by electron-capture gas chromatography, *J. Chromatogr.* 217 (1981) 239–245.
- [9] F. Andrawes, S. Greenhouse, D. Draney, Chemistry of acrylamide bromination for trace analysis by gas chromatography and gas chromatography–mass spectrometry, *J. Chromatogr.* 399 (1987) 269–275.
- [10] L. Castle, M.J. Campos, J. Gilbert, Determination of acrylamide monomer in hydroponically grown tomato fruits by capillary gas chromatography–mass spectrometry, *J. Sci. Food Agric.* 54 (1991) 549–555.
- [11] L. Dunovská, T. Čajka, J. Hajšlová, K. Holadová, Direct determination of acrylamide in food by gas chromatography–high resolution time-of-flight mass spectrometry, *Anal. Chim. Acta* 578 (2006) 234–240.
- [12] Y. Zhang, Y. Ren, H. Zhao, Y. Zhang, Determination of acrylamide in Chinese traditional carbohydrate-rich foods using gas chromatography with micro-electron capture detector and isotope dilution liquid chromatography combined with electrospray ionization tandem mass spectrometry, *Anal. Chim. Acta* 584 (2007) 322–332.
- [13] J.S. Ahn, L. Castle, D.B. Clarke, A.S. Lloyd, M.R. Phillo, D.R. Speck, Verification of the findings of acrylamide in heated foods, *Food Addit. Contam.* 19 (2002) 1116–1124.
- [14] M. Biedermann, S. Biedermann-Brem, A. Noti, K. Grob, P. Egli, H. Mändli, Two GC–MS methods for the analysis of acrylamide in foods, *Mitt. Lebensm.unters. Hyg.* 93 (2002) 638–652.
- [15] H. Ono, Y. Chuda, M. Ohnishi-Kameyama, H. Yada, M. Ishizaki, H. Kobayashi, M. Yoshida, Analysis of acrylamide by LC–MS/MS and GC–MS in processed Japanese foods, *Food Addit. Contam.* 20 (2003) 215–220.
- [16] H.Z. Senyuva, V. Gökmen, Survey of acrylamide in Turkish foods by an in-house validated LC–MS method, *Food Addit. Contam.* 22 (2005) 204–209.
- [17] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, Analysis of acrylamide, a carcinogen formed in heated foodstuffs, *J. Agric. Food Chem.* 50 (2002) 4998–5006.
- [18] K.S. Leung, A. Lin, C.K. Tsang, S.T.K. Yeung, Acrylamide in Asian foods in Hong Kong, *Food Addit. Contam.* 20 (2003) 1105–1113.
- [19] L. Peng, T. Farkas, L. Loo, J. Teuscher, K. Kallury, Rapid and reproducible extraction of acrylamide in French fries using a single solid-phase sorbent, *Am. Lab.* (October 2003) 10–14.
- [20] J.A.G. Roach, D. Andrzejewski, M.L. Gay, D. Nortrup, S.M. Musser, Rugged LC–MS/MS survey analysis for acrylamide in foods, *J. Agric. Food Chem.* 51 (2003) 7547–7554.
- [21] Y. Govaert, A. Ariseto, J. van Loco, E. Scheers, S. Fraselle, E. Weverbergh, J.M. Degroot, L. Goeyens, Optimisation of a liquid chromatography–tandem mass spectrometric method for the determination of acrylamide in foods, *Anal. Chim. Acta* 556 (2006) 275–280.
- [22] J. Rosén, K.E. Hellenäs, Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry, *Analyst* 127 (2002) 880–882.
- [23] P.C. Aguas, M.J. Fitzhenry, G. Giannikopoulos, P. Varelis, Analysis of acrylamide in coffee and cocoa by isotope dilution, *Anal. Bioanal. Chem.* 385 (2006) 1526–1531.
- [24] A. Becalski, B.P.-Y. Lau, D. Lewis, S.W. Seaman, Acrylamide in foods: occurrence, sources and modeling, *J. Agric. Food Chem.* 51 (2003) 802–808.
- [25] K. Inoue, Y. Yoshimura, H. Nakazawa, Development of high-performance liquid chromatography–electrospray mass spectrometry with size-exclusion chromatography for determination of acrylamide in fried foods, *J. Liq. Chromatogr. Relat. Technol.* 26 (2003) 1877–1884.
- [26] R.H. Stadler, G. Scholz, Acrylamide: an update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control, *Nutr. Rev.* 62 (2004) 449–467.
- [27] D. Taeymans, J. Wood, P. Ashby, I. Blank, A. Studer, R.H. Stadler, P. Gondé, P. Van Eijck, S. Lalljie, H. Lingnert, M. Lindblom, R. Matissek, D. Müller, D. Tallmadge, J. O'Brien, S. Thompson, D. Silvani, T. Whitmore, A review of acrylamide: an industry perspective on research, analysis, formation, and control, *Crit. Rev. Food Sci.* 44 (2004) 323–347.
- [28] I. Blank, Current status of acrylamide research in food: measurement, safety assessment, and formation, *Ann. N.Y. Acad. Sci.* 1043 (2005) 30–40.
- [29] T. Wenzl, M.B. de la Calle, E. Ankam, Analytical methods for the determination of acrylamide in food products: a review, *Food Addit. Contam.* 20 (2003) 885–902.
- [30] Y. Zhang, G.Y. Zhang, Y. Zhang, Occurrence and analytical methods of acrylamide in heat-treated foods: review and recent developments, *J. Chromatogr. A* 1075 (2005) 1–21.
- [31] H. Terada, Y. Tamura, Analysis of acrylamide, a carcinogen formed in heated foodstuffs, *J. Food Hyg. Soc. Jpn.* 44 (2003) 303–309 (in Japanese).
- [32] E.K. Paleologos, M.G. Kontominas, HPLC analysis of acrylamide. Determination of acrylamide and methacrylamide by normal phase high performance liquid chromatography and UV detection, *J. Chromatogr. A* 1077 (2005) 128–135.
- [33] V. Gökmen, H.Z. Senyuva, J. Acar, K. Sarioğlu, Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography, *J. Chromatogr. A* 1088 (2005) 193–199.
- [34] M. Murkovic, Acrylamide in Austrian foods, *J. Biochem. Biophys. Methods* 61 (2004) 161–167.
- [35] K. Hoenicke, R. Gatemann, W. Harder, L. Hartig, Analysis of acrylamide in different foodstuffs using liquid chromatography–tandem mass spectrometry and gas chromatography–tandem mass spectrometry, *Anal. Chim. Acta* 520 (2004) 207–215.
- [36] T.P. Coulter, Food the Chemistry of Its Components, The Royal Society of Chemistry, Cambridge, 2002, pp. 33–39.

- [37] D.S. Mottram, B.L. Wedzicha, A.T. Dodson, Food chemistry: acrylamide is formed in the Maillard reaction, *Nature* 419 (2002) 448–449.
- [38] R.H. Stadler, I. Blank, N. Varga, F. Robert, J. Hau, P.A. Guy, M.-C. Robert, S. Riediker, Food chemistry: acrylamide from Maillard reaction products, *Nature* 419 (2002) 449–450.
- [39] US Food and Drug Administration, Survey Data on Acrylamide in Food: Individual Food Products, 2006 <http://www.cfsan.fda.gov/~dms/acrydata.html>.
- [40] J.C. Miller, J.N. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education Limited, Essex, 2005, pp. 121–124.
- [41] Hong Kong Consumer Council, Determination of Carcinogen in Deep-fried Oil and Baked Foods: Acrylamide, *Choice*, October 13 2006, pp. 13–21 (in Chinese).
- [42] B. Hileman, No quick fix for acrylamide in food: intense scrutiny has provided methods of reduction but few answers about risk, *Chem. Eng. News* (August 14 2006) 43–44.