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Determination of Acrylamide in Selected Types of Iranian Breads by SPME Technique

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ABSTRACT: Acrylamide is a chemical compound that has been widely used since the last century for various applications. It is also formed from carbohydrate and amino acid containing foods by application of heat particularly in fried potato products, coffee and bread. Since bread is one of the primary foods of Iranian people, the determination of acrylamide content in bread might be considered important. This study is concerned with the qualitative and quantitative determinations of acrylamide in selected breads commonly consumed in Iran. The extraction level of acrylamide through three types of fibers, PEG, PDMS/DVB and PA, by DI-SPME-GC-FID in four selected types of baked breads were examined. The results of this study indicated that PEG fiber, as compared to the other fibers, was more reproducible and accurate. The concentration of acrylamide in Taftoon, Sangak, lavash and Barbari breads were, 146, 86, 26 and 135 (ppb) respectively.

Keywords: *Acrylamide, Flat Breads, GC/FID, Iranian Breads, SPME*

Introduction

Acrylamide is an organic compound that finds its way into many products in our everyday life. Acrylamide is a monomer of polyacrylamide. The monomer is regarded as a toxic substance affecting different system in human. The multiple unit or polymeric form is not known to be toxic (Otles, 2004).

Acrylamide was listed as a known carcinogen under proposition 65 on January 1, 1990 (the Safe Drinking Water and Toxic Enforcement Act of 1986, Health and Safety Code section 25249.5 etseq.). The No Significant Risk Level (NSRL) for acrylamide of 0.2 µg/day was also established in regulation in 1990 (Title 22, California Code of Regs. section 12705(c)).

In April 2002, the Swedish National Food Administration and a scientific group at the University of Stockholm jointly announced the formation of acrylamide during food preparation (Gokmen & Senyuva, 2006; Mottram *et al*., 2002). These findings clearly indicate the concern over acrylamide presence in some foods namely bread during the course of processing (Lingnert *et al.*, 2002). Therefore, development of an accurate analytical method to determine the traces of acrylamide in a variety of foods is quite indispensable (Lee *et al*., 2007).

Various techniques in chromatography are employed to determine the acrylamide concentration in bread (Hoeinck *et al*., 2004). Acrylamide was analyzed by GC with flame ionization detector (GC–FID) (Pedersen & Olsson, 2003). Acrylamide derivative was analyzed by GC with electron

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capture detection (GC–ECD), and limit of detection (LOD) was 0.032 g/L in aqueous matrices. Acrylamide was determined by LC with pulsed amperometric detection (LC–PAD), and LOD was 1.4g/kg in food. Lately, increased chromatographic methods based on mass spectrometry as the detection device were used to determine acrylamide (Lee *et al*., 2007). For the LC–MS method, the MS–MS mode is generally used to measure underivatized acrylamide, and LOD was 13µg kg−1 in food. Derivatized acrylamide was determined by GC–MS in the selected ion monitoring (SIM) mode, and LOD of 0.9 ppb was obtained in aqueous matrices. The preparation of the sample is the primary problem for trace acrylamide determined by MS (Lee *et al*., 2007; Wenzl *et al*., 2003).

The extraction methods of acrylamide consisted of soxhlet extraction, liquid–liquid extraction, solid-phase extraction (SPE). There are some disadvantages in these ordinary extraction methods such as, being time-consuming and requiring large volumes of organic solvents, especially, a large amount of solvent would influence trace analysis. Solid-phase microextraction (SPME) technology is a recent advance in sample preparation for trace analysis. In this solvent-free extraction technique, developed in 1990, the analytes are adsorbed directly from a gaseous or aqueous phase into a fused silica fibre coated with a polymeric phase. Therefore, sampling, extraction and concentration are accomplished in a single step. Collected analytes are then thermally desorbed directly to the gas chromatographic column and analyzed by mass spectrometry (Kataoka *et al*., 2000). SPME is useful in many diverse analyses, including characterization of flavor components in foods and beverages and fragrance compounds in a wide range of products (Vas & Vekey, 2004).

Considerable quantities of baked traditional breads consumed by the Iranian people are suspected of containing acrylamide. Studies have not been carried out on the acrylamide content on these bread extensively. Therefore the main object of this study was to evaluate the levels of acrylamide in selected breads in Kerman, Iran, and compare the effect of different processing procedure concerned with this chemical compound.

Materials and Methods

Acrylamide, methanol, sodium chloride, formic acid, aceton, n-hexane and dichloromethane in extra pure form were purchased from Merck Chemical Company (Darmstadt, Germany).

SPE cartridge (Oasis HLB, 3 ml) was obtained from Waters Company, USA.

Gas Chromatography, model 3420 equipped with Bpx70 capillary column from Beifen Company, China, (Varian 3400 technology).

Centrifuge equipped with refrigeration system, model U-320R from Boeco (Germany).

Filter 0.45µm, PTFE type was obtained from N.C.C Company (Germany).

Fiber retentive system for sample extraction and transfusion to GC port (57330U model) (Manual holder) was obtained from Supelco Company (USA).

Solid Microextraction fiberswas obtained from Supelco Company (USA):

PA (Polyacrylate), 85 µm. PDMS/DVB (Polydimethylsiloxane/Divenylbenzene), 65 µm.

PEG (Polyethylene Glycol), 60 µm.

Carrez I solution was prepared by dissolving 15 g of potassium hexacyano Ferrate in l00 ml of water).

Carrez II solution was prepared by dissolving 30 g of Zinc Sulphate in 100 ml of water.

In this study, four types of traditional flat breads of Iran (Taftoon, Sangak, Lavash and Barbary) were selected. Ten loaves of each bread types were purchased from bakeries of

Kerman city. The bread samples were dried at 50 ºC followed by milling, homogenization and mixing to provide uniform samples. The samples were kept at 2 ºC. Five grams of the homogenized sample was defatted with hexane and the residue, after filtration and drying, was mixed with water and centrifuged. The proteins were precipitated by application of Carrez solutions and then the solution passed through SPE Cartridge (3cc/60mg, oasis HLB) as directed through the instructions. After passing the solution through a 0.45 µm filter, extraction of acrylamide through PEG, PDMS/DVB and PA fibers by DI-SPME method three times for every one of the fibers extracted are as follows:

Six ml of the solution was transferred into a 10 ml brown glass vial and then mixed continuously at 1000 rpm at the time of extraction of analyte. The fibers were optimized according to the instruction of maker company (Supelco).

Results and Discussion

-Optimization GC/MS & GC/FID Conditions

Acrylamide standards were analysed on a Shimadzu 17A chromatograph equipped with Shimadzu mass spectrometer (QP 5050A, Japan). The column was a 50m×0.25mmi.d. fused-silica capillary column BPX70 and a stationary phase thickness of 0.25 µm. Helium carrier gas was at a rate of 3mL min−1 using an electronic pressure control. During the whole analysis, the injector was operated in the split less mode with an injector temperature of 230 ˚C. The oven temperature was initially set for 135 ˚C (5 min hold), followed by a linear temperature gradient of 10 ˚Cmin^{-1} upto 220 ˚C, and held at this temperature for 2 min. Under these conditions, acrylamide was eluted at 7.353 min (Figure 1).

Gas chromatography (GC) has been used to quantify acrylamide in a variety of industrial and environmental applications and with increasing interest in acrylamide analysis, we investigated the feasibility of using GC to determine this compound in food samples as GC is an efficient way to detect some compound. In this paper, the description of GC approach to analyse acrylamide, and introduce sample pretreatment using solid phase extraction has been described.

The following conditions were applied to GC to analyze acrylamide in food samples:

Column: DB-FFAP, 30 m, 0.25mm i.d., 0.25µm df.

Injector temp.: 210 °C.

Carrier gas: Nitrogen.

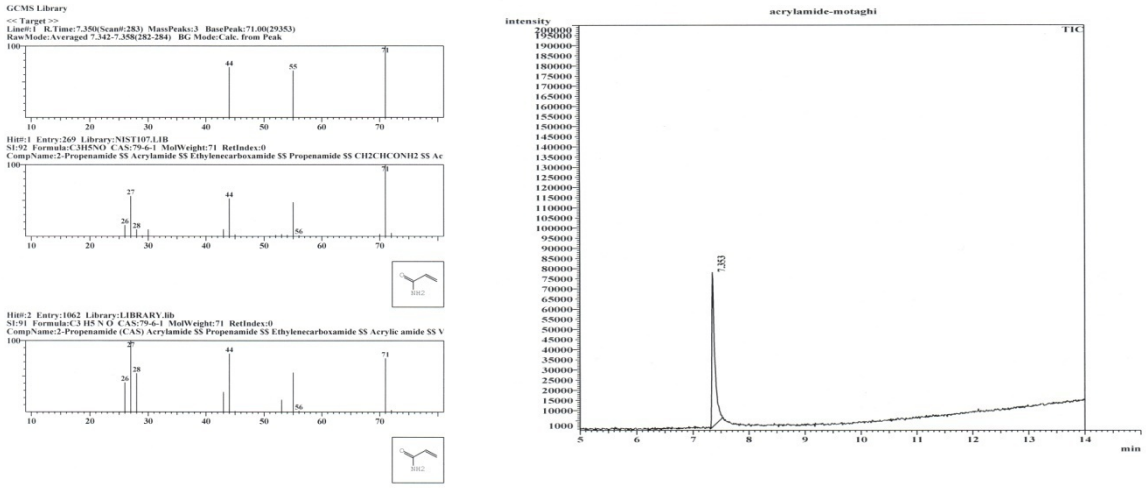

Fig. 1. Mass spectra & chromatogram of acrylamide (standard, 200ppb)

Oven temp: 90°C up to 225 °C at 10 °C/min.
Detector temprature 260 °C (Flame Ionisation Detector)
Fiber: PEG (Poly ethylene Glycol), 60 µm, (57355-u)
Extraction: Direct immersion, 30 min. at 25°C, stirred
Descrption: 5 min., 210°C

-Optimization of SPME Conditions

The extraction efficiency of the SPME technique is related to absorbent, extraction method, desorption temperature, absorption time, sample matrix such as, analyte and various other factors. Different types of coatings provide different absorption properties for different kinds of analytes. The selection of an appropriate coating for analyte is very important for the SPME method. Different fibers were tested under their optimum conditions by DI–SPME. The results in Figure 2 revealed that 60 µm PEG fiber was the best for acrylamide extraction. Because, PEG is a fully polar coating, and its polarity is similar to acrylamide.

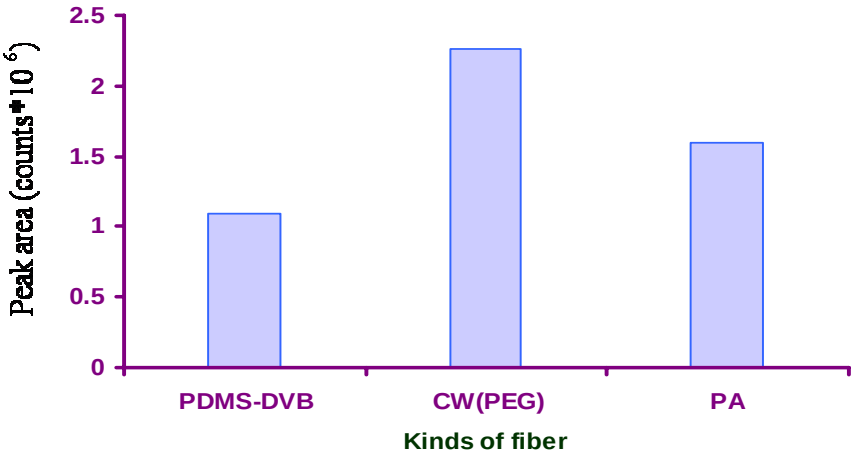

Fig. 2. Extraction efficiencies of peak areas acrylamide with different fibers

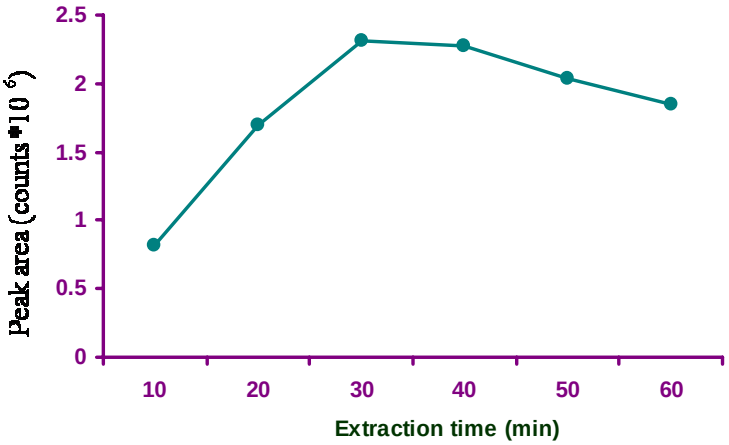

Fig. 3. Effect of extraction time on peak areas

Acrylamide is soluble in water and the volatility of acrylamide is very low, therefore, for extraction of acrylamide, DI-SPME method with higher efficiency instead of HS-SPME is used. One of the important principles of SPME is based on the balance between the density of the sample analyte and the absorbed analyte through the used fiber. Therefore this balance, mixing solution phase is quite important; for this reason, in all of the experiments, maximum speed (1000 rpm) of the mixer is applied.

The other important factor is the analyte extraction time from the solution by under test fiber. According to the experiments at different times and comparing the levels of under curve in various chromatographs, the optimal time was selected 30 min. Figure 3 shows that if the extraction time is long, then there is a possibility of analyte desorption from the surface of the fiber.

In this study the extraction temperature and the effect on the mass transfer rate of the analyte from liquid environment into under test fiber was investigated. Regarding acrylamide, if the temperature of the environment is increased, then the amount of analyte absorbed through the fiber is decreased. Therefore 25°C was selected as the chosen temprature (Figure 4).

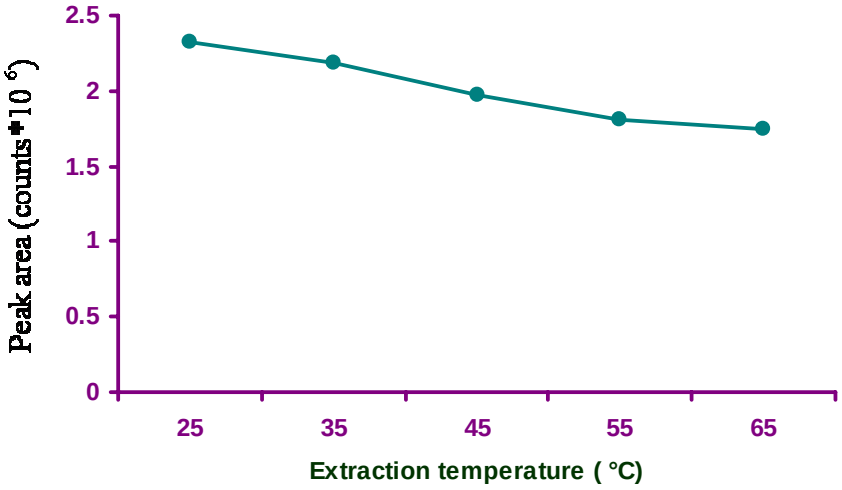

Fig. 4. Effect of extraction temperature on peak areas

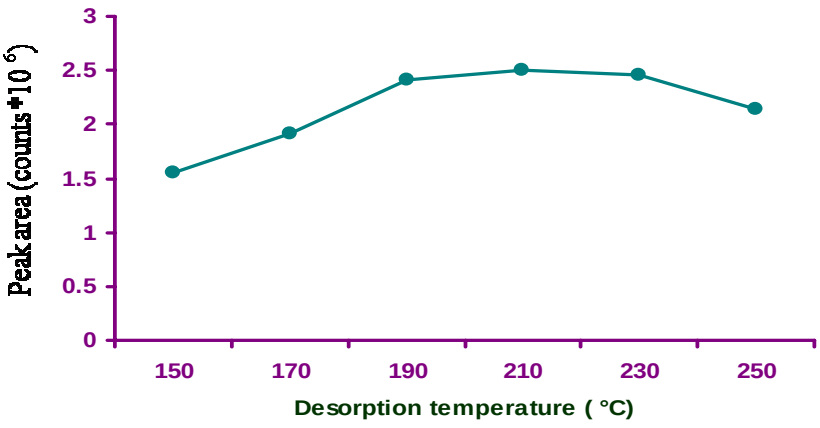

Fig. 5. Effect of desorption temperature on peak areas

Desorption temperature which is another efficient factor on quality of SPME technique was also investigated in this study. Desorption temperature was investigated within the range of 140-260 °C and 210 °C was selected as the optimum desorption temperature.

In Figure 5, the result indicates the peak area of acrylamide increased with the desorption temperature and then decreased slightly as the temperature increased further, reaching a maximum desorption at 210 °C.

The time of placing fiber into injector (desorption time) was investigated within a range of 0.5-6 min and 4 min was selected as the optimum time (Figure 6).

The pH is one of factors that has important effect on the signal intensity of analyte obtained by SPME with the changes in the sample matrix. In this study as indicated in Figure 7, the variation of pH from 3-9 was evaluated and the optimum pH for the extraction of acrylamide was determined.

Increasing of the sodium chloride can cause an increase in the samples absorption by the fiber coating which might be related to the polarity of analytes. If an analyte is non-polar, then adding sodium chloride into the sample solution might cause the environment polarity to increase and the analyte solubility to decrease. Therefore, reducing the samples solubilty in water can cause an increase in rate of absorption on the fiber coating. In this experiment, different concentrations (0-30%) were examined, and the most appropriate sodium chloride concentration (5%) was chosen (Figure 8).

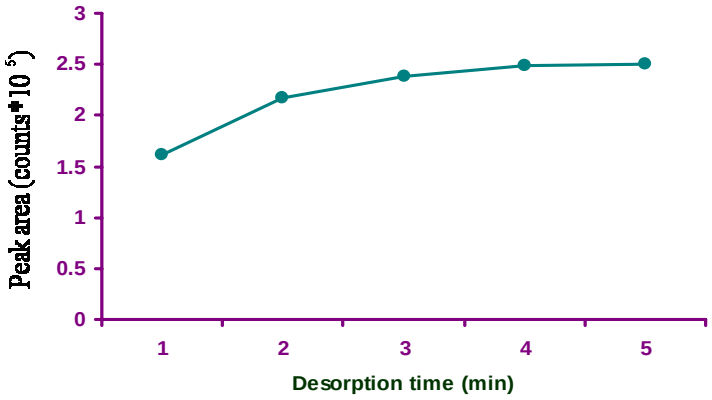

Fig. 6. Effect of desorption time on peak areas

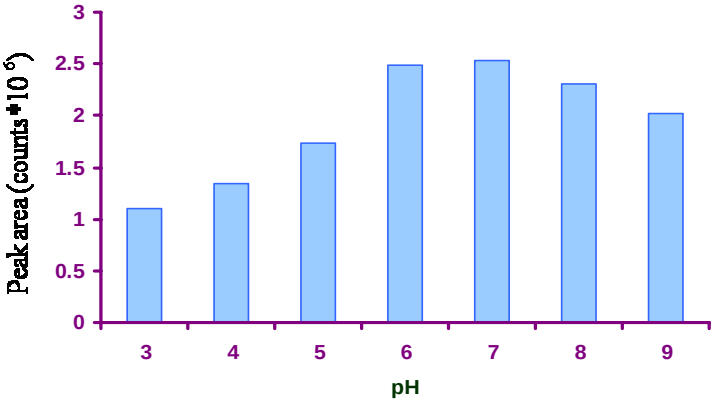

Fig. 7. Effect of pH on peak areas

The calibration graph was plotted based on linear regression analysis of peak area of the acrylamide (Y) versus concentration of acrylamide (X). The regression equation for acrylamide was $Y = 931.92 + 1720X$ and the correlation coefficient was $R^2 = 0.9986$. The limit of detection (LOD) was calculated to be 25µgkg−1 based on a signal-to-noise ratio of 5 (Figure 9).

In this study, four types of bread, Taftoon, Sangak, Lavash and Barbary were examined. The results indicated that Lavash bread with the highest percentage of salt and moisture had the lowest amount of acrylamide as compared to the others. Among the studied samples, Taftoon had the highest amount of acrylamide. Followed by, Barbary and Sangak (Table 1).

Various coatings were tested under their optimum conditions by DI–SPME. The results in Figure 2 revealed that 65 µm CW (PEG) fiber was considered best for acrylamide extraction.

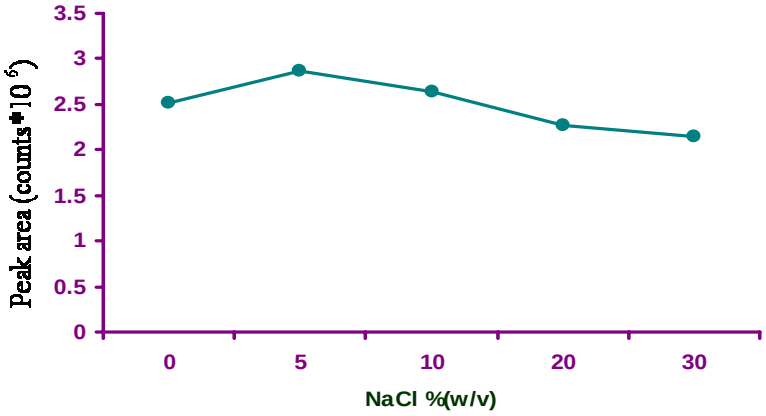

Fig. 8. Effect of addition of NaCl (%, w/v) on peak areas

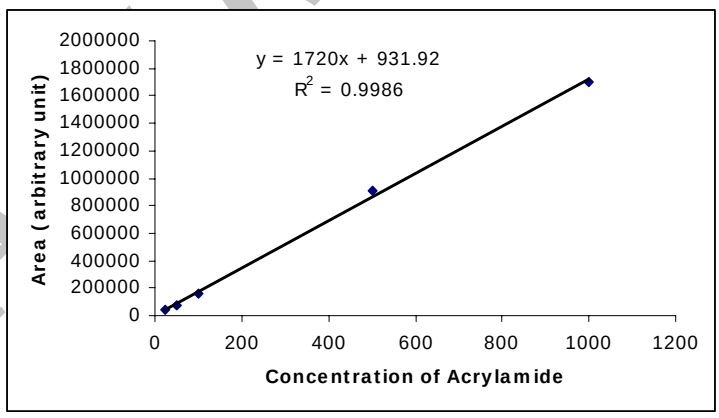

Fig. 9. Calibration curves of acrylamide in SPME technique

Table 1. Acrylamide contents (µg/kg) of flat breads

Bread	Number	Min.	Max.	Mean ± Std. Error
Taftoon	30	132	161	145.87 ±1.43
Barbary	30	119	142	135.04 ±1.05
Sangak	30	77	95	86.68 ±0.88
Lavash	30	18	28	26.43 ±0.34

The fact is related to the polarity of acrylamide (Vas & Vekey, 2004). CW (PEG) is a polar coating, and its polarity is similar to that of acrylamide. The relative area counts of 1mg L−1 acrylamide were compared by SPME with DI and headspace (HS) extraction. Knowing that in DI–SPME, the fiber is introduced in the aqueous phase, therefore the sensitivity of DI–SPME is higher than that of HS–SPME for acrylamide extraction. The absorption of HS–SPME is not suitable because acrylamide is soluble in water and the volatility of acrylamide is quite low. Therefore, acrylamide in water is easily extracted by DI–SPME (Kataoka *et al*., 2000).

Conclusion

Solid phase microextraction is a fast and might be regarded as an alternative to conventional sample extraction techniques. In SPME, analytes establish equilibria among the sample matrix, the headspace above the sample, and the stationary phases coated on a fused silica fiber, and are thermally desorbed from the fiber to a capillary GC column. Because solvent is not injected, and the analytes are rapidly desorbed onto the column, minimum detection limits are improved and resolution is maintained, therefore with the continued growth of SPME, it might expand into environmental, food, flavor, pharmaceutical, clinical, forensic, and reaction monitoring applications. The results from this study indicate that SPME coupled to GC or GC-MS is a precise method for analysing traces of acrylamide from aqueous samples.

References

Gokmen, V. & Senyuva, H. Z. (2006). A generic methods for the determination of acrylamide in thermally processed foods. *Journal of chromatography A*; 1120: 194-198.

Hoeinck, K., Gatermann, R., Harder, W. & Hartig, L. (2004). Analysis of acrylamide in different food stuffs using liquid chromatography - tandem mass spectrometry and gas chromatography - tandem mass spectrometry *Analytica Chemica Acta*; 520: 207-215.

Kataoka, H., Lord, H. & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of chromatography A*; 880: 35-62.

Lee, M. R., Chang, L. Y. & Dou, J. (2007). Determination of acrylamide in food by solid-phase microextraction coupled to gas chromatography - positive chemical ionization tandem mass spectrometry. *Analytica Chemica Acta*; 58: 219-23

Lingnert, H., Grivas, S., Jagerstad, M., Skog, K., Tornqvist, M. & Aman, P. (2002). Acrylamide in food: mechanisms of formation and influencing factors during heating of foods. *Scandinavia Journal of Nutrition*. 46(4): p. 159-172.

Luis, F. & Schmidt, P. (2007). A review of volatile analytical methods for determining the botanical origin of honey. *Food Chemistry*; 103: 1032–1043

Mottram, D. S., Wedzicha, B. L. & Dodson, A. T. (2002). Acrylamide is formed in Miallard reaction. *Nature*; (419): 448-449.

Otles, S. & Otles, S. (2004). Acrylamide in food. *EJEAF che* 3(5): 723-730

Pedersen, J. R. & Olsson, J. O. (2003). Soxhlet extraction of acrylamide from potato chips. *Analyst* 128:332-334.

U.S. EPA, (1990). *Assessment of health risks from exposure to acrylamide,* U.S. EPA, Office of Toxic Substances, Washington, D.C.

Vas, G. & Vekey, K. (2004). Solid-phase microextraction: a powerful sample prepration tool prior to mass spectrometric analysis. *Journal of mass spectrom*; 39: 233-254

Wenzl, T., De La Calle, M. B. & Anklam, E. (2003). Analytical methods for the determination of acrylamide in food products: a review. *Food Additives and Contaminants*; 20(10): 885-902.