**Detection of acrylamide using a biodegradable zein-based sensor with
surface enhanced Raman spectroscopy**

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Abstract

In this study, a biodegradable zein/gold SERS platform was tested as a potential tool to detect acrylamide for the first time. It was found that the presence of acrylamide resulted in a characteristic marker SERS peak that did not exist in the background SERS spectrum of the sensor. This unique peak was at 1447 cm^{-1} . This peak was used to detect and quantify acrylamide in aqueous solutions and a calibration curve was obtained using acrylamide solutions within the range of 10 mg/ml and 10 $\mu\text{g/ml}$. After 10 $\mu\text{g/ml}$ of concentration, it was not possible to make reliable measurements. The error or variation for each concentration in the range of 0.4 mg/ml (for 1 mg/ml) – 1.8 mg/ml (for 10 mg/ml) was observed. SERS was found to be an accurate technique for quantification purposes, in the concentration range studied. The calibration curve had an R^2 value of 0.93 and 0.97 (for log-log calibration curve). This was a proof-of-concept study to investigate the potential of a SERS sensor designed on a biodegradable platform and it was shown that this method can be potentially used with a careful design of acrylamide extraction from food samples.

1. Introduction

Acrylamide is a small organic molecule with the chemical formula of $\text{C}_3\text{H}_5\text{NO}$. It is an odorless, white compound, which is soluble in water and ethanol. It is generally used in its polymeric form, polyacrylamide, in the plastic industry (Tekkeli *et. al.* 2011). It has carcinogenic and toxic properties in animals, and can possibly be harmful to humans. For this reason, the level of acrylamide in water is regulated by Environmental Protection Agency in the US. Swedish scientists discovered in 2002 that certain foods contain acrylamide. The most common examples are French fries, potato chips and toasted bread (Mottram *et. al.* 2002).

Acrylamide is a by-product of Maillard reaction that occurs during high-temperature processing of starchy foods. The mechanism proposed is that the arginine amino acid goes under reaction with reducing sugars, i.e. glucose or fructose, and then the reaction further proceeds to form acrylamide (Mottram *et al* 2002). The cooking

temperature, cooking method, initial content of arginine and reducing sugar in the product, time and moisture of the cooking affect the amount of acrylamide formed in a food system (Arvanitoyannis and Dionisopoulou 2014).

In food products that are rich in proteins, after high-temperature processing the range of acrylamide was found to be moderate, between 5 – 50 µg/kg of food. On the other hand, for carbohydrate rich foods it ranged between 150–4000 µg/kg (Tareke 2002). In November 2013, FDA released a draft for acrylamide control and mitigation strategies for industry. Since the industry is trying to control the levels of acrylamide formation, there is a need for fast and routine quality control methods for detection of acrylamide. The current techniques used have the following steps: aqueous extraction of acrylamide from food, concentration of the sample and analysis by either gas/liquid chromatography-mass spectroscopy or high performance liquid chromatography (Bethke 2013). The whole process takes a considerable amount of time and requires both sophisticated equipment and trained personnel. For this reason, there is a need for a simple, robust and fast detection method for acrylamide. In this area, there has been some effort to develop alternative techniques such as the adsorption stripping voltammetry, capillary zone electrophoresis and none-aqueous capillary electrophoresis (Vesela and Sucman 2013, Bermudo et. al. 2006, Baskan and Erim 2007). To this date, detection of acrylamide by utilizing Raman spectroscopy has not been reported. In this paper, we investigated the use of Surface Enhanced Raman spectroscopy on a biodegradable platform as a potential detection platform for acrylamide.

2. Materials and methods

2.1. Preparation of acrylamide solution

Electrophoresis grade Acrylamide $\geq 99\%$, for biochemistry (Acros Organics) was used in this study. Acrylamide was stored at 4°C in a refrigerator to ensure stability. All solutions were freshly prepared before use. Distilled water was used as the solvent for the acrylamide solutions. The concentrations of the acrylamide solutions used on SERS-active zein substrates were as follows: 10 mg/ml, 7.5 mg/ml, 5 mg/ml, 2.5 mg/ml, 1 mg/ml, 100 µg/ml, 10 µg/ml. Environmental Protection Agency (EPA) suggests not

consuming more than 2 μg acrylamide per kg body weight of a person per day as a maximum reference threshold toxicity dose. In order to obtain a clear Raman signature of acrylamide we also conducted experiments on a glass slide. The concentrations of the acrylamide solutions used on glass were as follows: 100 mM and 10 mM (approximately 7 mg/ml and 0.7 mg/ml). The Raman signatures on glass provide the characteristic Raman spectrum of acrylamide that was used to compare with the zein based SERS substrate.

2.2. Preparation of SERS-active substrates

In this study, previously developed and optimized SERS-active zein substrates were used and the design and manufacturing method of the prototype laboratory sensor has been reported earlier (Gezer et. al. 2015). A brief summary of the method of preparation is given here for ease of reading of the paper. The fabrication process was as shown in Figure 1a. Polydimethylsiloxane (PDMS) with positive pyramid (pyramids sticking out) structures with 2 μm by 2 μm base and 2.1 μm height were used as a template. PDMS with positive pyramids were coated with 200 nm gold film with an e-beam evaporator. Zein solution which was poured on the gold film was prepared by dissolving zein in 75 % aqueous ethanol (1 g zein : 5 mL ethanol solution), adding oleic acid and emulsifier (1g zein: 1g oleic acid: 0.05 g emulsifier) and the mixture was stirred for 5 minutes on a hot plate at 70° C. The zein solution was exposed to ultrasound treatment in order to remove gas bubbles which occurred during the stirring process. The next step was for this solution to be poured and cast on the gold-coated PDMS template. After zein films were dried and set in a desiccator at room temperature, the films were cut and peeled off from the PDMS. The gold side of the zein sensor is shown in Figure 1b. Scanning electron microscopy images proved the successful transfer of 200 nm gold with inverted pyramid structures on zein films (Figure 1c). These photonic structures can also be recognized on SERS-active zein substrates inside the square area in Figure 1b, due to reflection of the light at various wavelengths generating a myriad of colors.

Figure 1.a) Schematic of fabrication process for zein-SERS sensors. PDMS with positive pyramid structures were used as a template. After being coated with gold by e-beam evaporator, zein solution was poured. Following the drying in a desiccator at room temperature, zein films were peeled off to give inverted pyramid structures with gold coating. b) Macroscopic image of zein-SERS sensor on gold-coated side. Squared area shows where the nanophotonic patterns are. c) Scanning electron microscopy images of the inverted pyramid structures.

2.3.Raman spectroscopy measurements

Samples for Raman spectroscopy were prepared by dropping 5 μ l of one of the acrylamide solutions at a given concentration on one of two platforms consisting of either a glass slide or a zein-SERS sensor described above. The drop of solution was allowed to dry at room temperature. Figure 2 illustrates the drop deposition on the zein-SERS sensor. The prototype simple laboratory sensor was immobilized onto a glass slide with tape in order to obtain a flat and stable surface. 5 μ l of acrylamide solution was dropped onto the region where the nanophotonic structures shown in Figure 1b are. Background

measurements were also done on glass and on the nanophotonic structures that did not have any solution for comparison.

Figure 2 Zein/gold-SERS sensor with a droplet of acrylamide solution, immobilized on a glass slide. The squared area in the middle has the nanophotonic structures.

Two Raman systems were used in this study: Nanophoton Raman-11 and Horiba LabRAM HR. In both systems, 532 nm laser was used. In the Nanophoton system, used with the glass slide, the excitation power was 5.35 mW (no filter was used). The objective used was 10 X and the grating was 600 lines/mm. The slit was 60 μm and the wavenumber range covered was 400 to 3000 cm^{-1} . The measurement was done in 'Fast x-y scan' mode with 1 second of exposure time. An area over the edge of the dried droplet was scanned with this system.

In the Horiba LabRAM HR confocal-Raman microscopy system, used for the zein/gold SERS prototype sensor the grating was 300 lines/mm the slit was 100 μm and the hole was 300 μm . 50 X objective and D 1 filter was used. The Raman spectra were collected in the wavenumber range of 500 to 1800 cm^{-1} . For quantification purposes, individual spectra over 5 different points were collected and the exposure time was kept constant at 1 second x 30 times.

2.3.1. Data analysis

Baseline correction was carried out to eliminate the fluorescence interference. A 5th order polynomial was used to fit a baseline using the 'baseline correction' option of the LabSpec 5 (provided by Horiba). Additional points were added to the baseline to ensure a perfect fit, if needed. This baseline was subtracted from the Raman spectrum.

For quantification purposes of the height of the acrylamide Raman intensity peaks, the Gauss-Loren peak-fitting function of the same software (LabSpec 5) was used. For each concentration the average of 5 points was used to calculate the peak intensity as well as its standard deviation.

The program in the Nanophoton Raman spectrometer, allows for averaging the spectra over a selected area. For measurements on the edge of the droplet, three different areas were selected: 1) outside the droplet (glass slide); 2) inside the droplet (glass slide + low concentration of acrylamide); 3) edge of the droplet (concentrated acrylamide).

3. Results and Discussion

3.1. Raman measurements of acrylamide on glass slide

When a volume of acrylamide solution is dropped on a surface and left to dry, it forms a coffee ring shaped residue. When a liquid with dispersed solids evaporates, the capillary forces bring most of the solids towards the edge of the droplet, causing a high concentration and leaving a low concentration inside the droplet (Figure 3). The different concentrations in these two regions were studied for acrylamide deposited on a glass slide.

Figure 3. Coffee-ring effect for acrylamide solution

Figure 4 Light Microscopy image of dried acrylamide solution on glass slide. Big rectangular area shows the region in which Raman measurements were done. The small green rectangle represents the interior of the droplet (low concentration), the blue rectangle represents the edge of the droplet (high concentration) and the grey rectangle represents the glass slide (no acrylamide). Color code represents the intensity of Raman signals over the region. Yellow color signifies higher concentration. Scale bar: 100 μm

Figure 4 shows the microscopy images of the edge of the droplet (100 mM acrylamide solution) and makes a distinction between glass slide without any acrylamide, inside of the droplet and the edge of the droplet. The Raman signatures of each of the regions are plotted in Figure 5. In this analysis, it was found that the glass slide had a background signal as shown in grey. Raman signals showed both glass and acrylamide peaks inside the droplet. The edge of the droplet showed high intensity, sharp Raman peaks as expected from the high concentration of acrylamide in this region. Some of the key characteristic peaks of acrylamide are reported in Table 1.

Figure 5 Overlay of the Raman spectrum of glass slide (grey), edge of the droplet (blue) and inside the droplet (green)

Table 1. Some characteristic peaks and vibration assignments of acrylamide (Gupta and Bansil, 1981)

Raman peaks (cm ⁻¹)	Bond	Type of vibration	Material
562	Si-O-Si	Bending	Glass slide
850	C-C side chain	Stretching	Acrylamide
966	CHHC	Wagging	Acrylamide
996	Si-ring	Breathing	Glass slide
1098	SiO	Stretching	Glass slide
1298	C-H bending	Bending	Acrylamide
1440	CH ₂	Bending	Acrylamide
1640	C-C stretching	Stretching	Acrylamide
1685	C-O stretching	Stretching (amide I)	Acrylamide

From the edge of the droplet experiment, it was concluded that for each concentration of acrylamide obtaining Raman spectra measurements on the edge of the droplet would be the most beneficial as the acrylamide concentration was the highest in this region as indicated above, consistent with prior studies of Sun and Wiederrecht (2007) who also benefitted from this phenomenon. Therefore, in the rest of the acrylamide Raman measurements spectra were collected from the edge of the droplet. Figure 6 shows the Raman spectra of both 100 mM and 10 mM acrylamide on glass slides with the Horiba

LabRAM Raman microscope. The 100 mM concentration in Figure 6a showed the expected characteristic peaks; however the 10 mM concentration of acrylamide spectra shown in Figure 6b were overwhelmed by the glass slide spectra and it was not possible to collect or identify any Raman peaks of acrylamide. Glass slides are known to give background signature, as this was the case for our situation. Table 1 summarizes the peaks that are coming from glass vs acrylamide. Glass slide gave peaks such as Si-O-Si bending at 562 cm^{-1} , Si-ring breathing at 996 cm^{-1} and SiO stretching at 1098 cm^{-1} . Acrylamide gave peaks C-C side chain stretching at 850 cm^{-1} , CH-HC wagging) at 966 cm^{-1} , C-H bending at 1298 cm^{-1} , CH₂ bending at 1440 cm^{-1} , C-C stretching at 1640 cm^{-1} and C-O stretching at 1685 cm^{-1} .

Figure 6 Raman measurements of acrylamide on glass slide with concentrations of 100 mM (a) and 10 mM (b)

3.2.SERS measurements of acrylamide on zein substrates

3.2.1.Identification of characteristic Raman peak of acrylamide

Since acrylamide is a small molecule and glass Raman spectra give overwhelming background signatures, it was not possible to measure low concentrations of acrylamide with normal Raman spectroscopy. In order to enhance the spectral intensities SERS-active substrates made out of zein were used. Zein-SERS substrates also give background signal and it was important to identify a characteristic peak of acrylamide that is different than the background. Figure 7 shows the comparison between background SERS spectra of the gold coated zein substrates and acrylamide with 10 mg/ml concentration on top of

the substrate. From this comparison, it was concluded that many of the Raman peaks of acrylamide overlapped with the background signal, such as 966 cm^{-1} (CH-HC wagging), 1298 cm^{-1} (CH bending), 1640 cm^{-1} (C-C stretching). However, the peak at 1447 cm^{-1} , which was CH_2 bending peak coming from acrylamide did not exist in the background signature. For this reason, this peak was used as the characteristic marker peak for quantification of the concentration of acrylamide.

3.2.2. Quantification of acrylamide on zein-SERS sensors

Different concentrations (10 mg/ml, 7.5 mg/ml, 5 mg/ml, 2.5 mg/ml, 1 mg/ml, 100 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$) of acrylamide on zein-SERS sensor are shown in Figure 7. As the concentration decreases, the intensity of the peaks decreased as well. When the parameters, such as laser power, laser area, conditions of the Raman instrument remained same, the intensity of the Raman peak is directly correlated with the concentration of the molecule of the interest. All the above-mentioned parameters kept the same and the intensity of the peaks were plotted against the concentration of known acrylamide.

Figure 7. Comparison of the background signature of zein-SERS sensor (green) with acrylamide on top of the sensor (blue). Red dotted square indicates the peak at the

wavenumber of 1447 cm^{-1} , which does not exist in the background, but exists in acrylamide signature. (A.u.: arbitrary units)

Figure 8. Surface enhanced Raman signatures of acrylamide with different concentrations on zein-SERS substrate

Subtraction of the Raman spectra from the background Raman spectra of the sensor might sometimes give desirable results in identifying the different peaks. It was found that the subtraction resulted in a too noisy spectrum. Thus, we decided to use the original spectrum in the calibration curve calculations.

3.2.3. Validation of sensor's ability to measure acrylamide

A calibration curve (Figure 9) was obtained using different concentrations of acrylamide ranging from 10 mg/ml to 10 $\mu\text{g/ml}$, specifically 10 mg/ml, 7.5 mg/ml, 2.5 mg/ml, 1 mg/l, 100 $\mu\text{g/ml}$, and 10 $\mu\text{g/ml}$. It was found that the intensity of the Raman peak at the location 1447 cm^{-1} increased with increasing concentration of acrylamide. Each value was obtained by averaging the intensity over at least 3 different locations on the edge of the formed 'coffee ring' structure. It was found that there was a certain amount of variation between different spots. Standard deviation was 403, 216, 66, 18, 94, 6 and 0 for concentrations of 10 mg/ml to 10 $\mu\text{g/ml}$, specifically 10 mg/ml, 7.5 mg/ml,

2.5 mg/ml, 1 mg/l, 100 µg/ml, and 10 µg/ml, respectively. The average of standard deviations of these data points were calculated to be 15%. This also gives us an extrapolation for the standard deviation of the detection limit. The slope of the calibration curve (Figure 9) was 0.12 and R^2 was 0.9325.

Figure 9. Calibration curve for different concentrations of acrylamide

On the other hand, when the intensity versus calibration was plotted on the log-log scale (Figure 10), the calibration curve obtained had a better R^2 value of 0.9687. This calibration curve was used to determine the concentration of several samples by using the average intensity of 1447 cm^{-1} peak over at least 3 points. We estimated a sample's concentration to be 5.2 mg/ml when it actually was 6 mg/ml by using concentration curve linear equation. There was a 0.8 mg/ml error. For another sample of 1 mg/ml concentration, calculated concentration was 0.6 mg/ml, which caused 0.4mg/ml error. When the intensity of a sample of 10 mg/ml concentration was used, the concentration was estimated to be 8.2 mg/ml. The variation was 1.8 mg/ml for 10 mg/ml sample.

Figure 10. Log-log calibration curve for different concentrations of acrylamide

This variation may be the result of several factors. First, Surface Enhanced Raman measurements are known to exhibit certain variability in the signal intensity due to the distribution of the so-called ‘hot spots’. Second, when working with a confocal microscope, the position, i.e. focus, of the objective affects the Raman intensity. When taking measurements at different spots, re-focusing was done. However, there might have been variation in the degree of focus that resulted in the intensity change observed in this study. Last, the local concentration of acrylamide molecules adsorbed to the surface while drying might differ at different locations and also in terms of orientation as well as thickness of the layers. Even though there was a certain variation at each point, which was unavoidable, there was a trend in which increasing concentration corresponded to increasing Raman peak intensity.

3.2.4. Limit of detection of prototype laboratory zein-SERS acrylamide sensor

At the concentration of 10 μg/ml, it was only possible to observe the characteristic Raman peak at one location among all the measurements taken on the sample. This means that the concentration was too low to form a clear coffee-ring structure and background signature became overwhelming, similar to the case of glass slide (Figure 6b). From this it was concluded that the limit of detection (LOD) for this set up was 10 μg/ml. This value was compared with the other LODs, which were reported in the literature for the conventional systems. In the literature, LODs are generally reported as

µg/kg food. In the conventional systems, acrylamide is extracted from a given amount of food and then concentrated before being injected into chromatography systems, such as gas or liquid chromatography in tandem with mass spectroscopy. The extent of extraction and concentration processes determines the final composition of acrylamide (mass/volume solvent) in the sample to be analyzed. The amount of acrylamide in foodstuffs ranges from 150 – 4000 µg/kg food (Keramat *et. al.* 2011). The LODs reported in the literature varies between 30 µg/kg and 0.1 µg/kg, depending on the technique used (Tekkeli *et. al.* 2012). For example, LC-MS-MS systems reported gave a sensitivity of 25 µg/kg for carbohydrate-based foods (Croft et al 2004), whereas the same system in another study was able to detect as low as 1 µg/kg for baby food/infant formula samples (Jiao et al 2005). On the other hand, GC-MS systems showed higher detection limits in general, such as 5 µg/kg for coffee and coffee products (Soares et al 2006), 5-50 µg/kg for potato chips (Fernandes and Soares 2007). However, in the cases of GC-MS-MS or GC was coupled with electron capture detector (ECD) it was possible to detect 0.1µg/kg acrylamide in potato crisps and French fries (Lee et al 2007). When zein-SERS sensor is directly compared with the conventional techniques, it can be seen that it is not as sensitive as the other techniques. Even though SERS is considered to be a very sensitive technique in the examples of silver nanoparticles or metallic platforms on non-biodegradable surfaces, for our zein-SERS system there was a trade-off between sensitivity and biodegradability. However, depending on the extraction technique, the calibration curve obtained in this study could be applicable. For instance, Rosén and Hellenäs (2002) reported sample preparation starting with 4 g of crispbread, which had 35 µg/kg acrylamide, and obtaining a 10 µl solution that is injected into a gas chromatograph. This protocol would result in having 14 µg/ml in the final solution. A concentration of this level could be detected with the zein-SERS sensor reported here. However, other extraction methods were such that the concentration in the final solution would be as low as 5 ng/ml, which would not be possible to detect with the zein-SERS sensor. Since these are extraction methods, they do not have sensitivity data and an optimization process is needed when the method is applied for food samples.

4. Conclusion

In this study, a zein/gold SERS platform was tested as a potential tool to detect acrylamide for the first time. Only 5 μl of acrylamide solution was used in detection. After the droplet was air-dried on the substrate, a 'coffee ring' structure was observed. The coffee ring method was utilized to detect acrylamide on the edge of the ring where the concentration is highest. It was found that the presence of acrylamide resulted in a SERS peak that did not exist in the background SERS spectrum of the sensor. This unique peak was at 1447 cm^{-1} . This peak was used to detect and quantify acrylamide in aqueous solutions and a calibration curve was obtained using acrylamide solutions within the range of 10 mg/ml and 10 $\mu\text{g/ml}$. After 10 $\mu\text{g/ml}$ of concentration, it was not possible to observe the ring structure due to low concentration and for the 10 $\mu\text{g/ml}$ concentration, it was only possible to observe 1447 cm^{-1} peak at one location despite of the multiple measurements taken on the ring structure. Further studies are needed to design a platform that has a better sensitivity. The error or variation for each concentration in the range of 0.4 mg/ml (for 1 mg/ml) – 1.8 mg/ml (for 10 mg/ml) was observed. Even though SERS was not found to be most accurate technique for quantification purposes, we still found that it gave reasonable approximation of the concentration in the given range with an R^2 value of 0.93 and 0.97 (for log-log calibration curve). This was a proof-of-concept study to investigate the potential of a SERS sensor designed on a biodegradable platform and it was shown that this method can be potentially used with a careful design of acrylamide extraction from food samples.

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References

Arvanitoyannis, I. S., & Dionisopoulou, N. (2014). Acrylamide: formation, occurrence in food products, detection methods, and legislation. *Critical reviews in food science and nutrition*, 54(6), 708-733.

Başkan, S., & Erim, F. B. (2007). NACE for the analysis of acrylamide in food. *Electrophoresis*, 28(22), 4108-4113.

Bermudo, E., Nunez, O., Puignou, L., & Galceran, M. T. (2006). Analysis of acrylamide in food samples by capillary zone electrophoresis. *Journal of Chromatography A*, 1120(1), 199-204.

Bethke, P. C., & Bussan, A. J. (2013). Acrylamide in processed potato products. *American journal of potato research*, 90(5), 403-424.

Croft*, M., Tong, P., Fuentes, D., & Hambridge, T. (2004). Australian survey of acrylamide in carbohydrate-based foods. *Food additives and contaminants*, 21(8), 721-736.

Gezer, P. G., Hsiao, A., Kokini, J.L., and Liu. G.L. (2015) "Simultaneous transfer of noble metals and three-dimensional micro-and nanopatterns onto zein for fabrication of nanophotonic platforms." *Journal of Materials Science* (2015): 1-11.

Granvogl, M., & Schieberle, P. (2006). Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *Journal of Agricultural and Food Chemistry*, 54(16), 5933-5938.

407

408 Gupta, M. K., & Bansil, R. (1981). Laser Raman spectroscopy of polyacrylamide. *Journal*
 409 *of Polymer Science: Polymer Physics Edition*, 19(2), 353-360.

410

411 Fernandes, J. O., & Soares, C. (2007). Application of matrix solid-phase dispersion in the
 412 determination of acrylamide in potato chips. *Journal of Chromatography A*, 1175(1), 1-6.

413

414 Jiao, J., Zhang, Y., Ren, Y., Wu, X., & Zhang, Y. (2005). Development of a quantitative
 415 method for determination of acrylamide in infant powdered milk and baby foods in jars
 416 using isotope dilution liquid chromatography/electrospray ionization tandem mass
 417 spectrometry. *Journal of Chromatography A*, 1099(1), 198-202.

418

419 Keramat, J., LeBail, A., Prost, C., & Soltanizadeh, N. (2011). Acrylamide in foods:
 420 chemistry and analysis. A review. *Food and bioprocess technology*, 4(3), 340-363.

421

422 Krishnakumar, T., & Visvanathan, R. (2014). Acrylamide in food products: a review.
 423 *Journal of Food Processing and Technology*, 5(7).

424

425 Lee, M. R., Chang, L. Y., & Dou, J. (2007). Determination of acrylamide in food by
 426 solid-phase microextraction coupled to gas chromatography–positive chemical ionization
 427 tandem mass spectrometry. *Analytica chimica acta*, 582(1), 19-23.

428

429 Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Food chemistry: acrylamide is
 430 formed in the Maillard reaction. *Nature*, 419(6906), 448-449.

431

432 Rosén, J., & Hellenäs, K. E. (2002). Analysis of acrylamide in cooked foods by liquid
 433 chromatography tandem mass spectrometry. *Analyst*, 127(7), 880-882.

434

435 Soares, C., Cunha, S., & Fernandes, J. (2006). Determination of acrylamide in coffee and
 436 coffee products by GC-MS using an improved SPE clean-up. *Food additives and*
 437 *contaminants*, 23(12), 1276-1282.

438

439 Suh, J. S., & Michaelian, K. H. (1987). Surface-enhanced raman spectroscopy of
 440 acrylamide and polyacrylamide adsorbed on silver colloid surfaces: Polymerization of
 441 acrylamide on silver. *Journal of Raman spectroscopy*, 18(6), 409-414.

442

443 Sun, Y., & Wiederrecht, G. P. (2007). Surfactantless synthesis of silver nanoplates and
 444 their application in SERS. *Small*, 3(11), 1964-1975.

445

446 Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002). Analysis of
 447 acrylamide, a carcinogen formed in heated foodstuffs. *Journal of agricultural and food*
 448 *chemistry*, 50(17), 4998-5006.

449

450 Tekkeli, S. E. K., Önal, C., & Önal, A. (2012). A review of current methods for the
 451 determination of acrylamide in food products. *Food Analytical Methods*, 5(1), 29-39.

452

453 Vesela, H., & ŠUCMAN, E. (2013). Determination of acrylamide in food using
 454 adsorption stripping voltammetry. *Czech J. Food Sci.* Vol, 31(4), 401-406.

455

456 Zhang, Y., Dong, Y., Ren, Y., & Zhang, Y. (2006). Rapid determination of acrylamide
 457 contaminant in conventional fried foods by gas chromatography with electron capture
 458 detector. *Journal of Chromatography A*, 1116(1), 209-216.

459