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Determination of acrolein in french fries by solid-phase microextraction gas chromatography and mass spectrometry

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ABSTRACT

The frying of foods in the home can be a cause of indoor pollution due to the formation of acrolein. The emission of acrolein formed during frying in soybean, corn, canola, sunflower and palm oils was studied. A GC/MS method has been developed to determine acrolein in French fries using SPME as the sampling technique after derivatization with 2,4-dinitrophenylhydrazine (DNPH). Optimum SPME conditions included desorption at 250 °C for 2 min after an adsorption time of 10 min at room temperature. The method presented good resolution, repeatability, detection and quantification limits, and linearity of response. French fries were prepared in five different oils with four frying steps. The results showed that changes in acrolein concentration occurred after frying potatoes in different types of oil and at different frying cycles. Potatoes fried in soybean oil contained the lowest concentration of acrolein. Shoestring potatoes contained a lower concentration of acrolein than potato chips and French fries, respectively, because of the higher surface/volume ratio.

1. Introduction

Acrolein is a carbonyl compound (CC) that is also known as acrylic aldehyde or 2-propenal. Acrolein is introduced daily into the environment from many sources such as automobile emissions, cigarette smoke, forest fires, heated cooking oil, and industrial processes [1]. Acrolein is produced by the incomplete combustion of organic material, as well as by the oxidation of atmospheric chemicals such as 1,3-butadiene, a primary component of motor vehicle exhaust. It can also be formed by the oxidation of volatile organic compounds released by home furnishings, building materials, carpeting, wood finishes, glues, adhesives, and paints [2].

Acrolein is very toxic via all the routes of administration and may cause respiratory and ocular irritation. Contact with acrolein may produce necrosis of the skin or eye [3].

Because of its toxicity, the determination of acrolein residues in some foods, mainly fried foods, is important. Acrolein is released by frying foods. It is formed by the dehydration of glycerol, which is obtained from the hydrolysis of triglycerides. However, the development of analytical procedures for the determination of volatile chemicals such as acrolein in fatty samples is a challenge because it is highly volatile, highly reactive, and is capable of self-polymerization [4].

Known methods for determining carbonyl compounds require a derivatization step that usually involves agents such as 5,5-dimethyl-1,3-cyclohexanedione, N-methylbenzothiazolon-(2)-hydrazone (MBTH), pentafluorophenylhydrazine (PFPH), and 2,4-dinitrophenylhydrazine (2,4-DNPH).

For gas phase sampling, the derivatizing agent should not be very volatile [5]. For the determination of carbonyl compounds such as acrolein in complex matrices, 2,4-dinitrophenylhydrazine has been used as a derivatizing reagent because of its high reactivity. According to Veloso et al. [6], the production of hydrazones should occur in acid solution, Veloso et al. [6] concluded that the ideal pH range for an optimum yield of carbonyl derivatives was between 1.5 and 2.2. Perchloric or phosphoric acid can be used in the acid solution of 2,4-DNPH to permit the direct injection of the solution to increase the derivatization rate [7].

The DNPH derivatives of carbonyl compounds are usually determined in environmental samples such as beverages and food by HPLC or GC/MS. Some authors mention the advantages of HPLC analysis because of its robustness and good repeatability, but GC/MS has the advantage of furnishing a better separation from complex matrices than HPLC methods [8].

There are few studies in the literature that employ GC/MS for the analysis of acrolein in complex matrices such as food. Significant amounts of acrolein are produced from heated oils. Emissions of volatile organic compounds formed during heating of cooking oils such as coconut, sunflower, canola, and extra virgin olive oils were studied by Katragadda et al. [9]. The oils were heated at 180, 210, 240, and 240 °C for 6 h. The lowest amount of potentially toxic

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volatile chemicals was formed in canola oil. Acrolein formation was observed even at low temperatures, indicating that the use of frying procedures in the home has to be considered as an indoor pollution problem. In another study [10], Yasuhara and Shibamoto showed that the amounts of acrolein formed from heated lard, corn oil, cottonseed oil, and sunflower oil were 109, 164, 5.1 and 163 μg/L, respectively.

Two different studies [11,12] demonstrated that nearly 10-fold more acrolein (20.4 μg/L) was produced from reheated corn oil (190 °C for 30 min) than from fresh corn oil (2.9 μg/L). These workers analyzed acrolein vapor in the headspace of a corn oil sample that was heated for 2 h at temperatures that ranged from 180 to 320 °C. They also measured the acrolein content after heating the oil at 300 °C for periods that varied from one to 6 h. In both analyses, the authors concluded that the concentration of acrolein increased with temperature and duration of the heating period.

Yasuhara and Shibamoto [11] found significant amounts of acrolein in the outlet of a kitchen ventilator and concluded that one of the likely sources was the corn oil used in the kitchen for frying meat and fish. The author used an impinger as the sampling method and derivatization with N-methylhydrazine, followed by analysis by GC/NPD (Gas Chromatography with a Nitrogen–Phosphorus Detector). Saison et al. [13] determined 41 types of carbonyl compounds in beer by the headspace SPME-GC/MS method utilizing a PDMS/DVB fiber and (2,3,4,5,6-pentafluorobenzyl) hydroxylamide as the derivatizing agent. A good linearity (R^2 equal 0.997) was observed in the analysis of large concentration ranges (0.8–256 μg/L) of acrolein. The RSD was 5.09%, and the LD and LQ were 0.24 and 0.81 μg/L, respectively. Andreu-Sevilla et al. [14] identified and quantified acrolein in the vapors of extra virgin olive oil, sunflower oil and palm oil by GC/MS after heating the oils at 180 and 240 °C. They concluded that the formation of acrolein from cooking oils increased significantly when the cooking temperature was increased from 180 to 240 °C. Lane and Smathers [15] determined acrolein by reversed-phase liquid chromatography and by GC/MS, for confirmation, in fish fillet coatings fried at 182 and 204 °C in fresh and used oil and encountered 0.1 mg of acrolein per liter in both analyses.

Nevertheless, to our knowledge, no studies have been conducted that compared the impact of heating potatoes in oil on the acrolein content of the potatoes when the real cooking conditions used in homes, restaurants, and food industries were simulated. Therefore, the present study sought to develop an analytical method for determination of acrolein derivatized with 2.4-DNPH using solid phase microextraction and GC/MS for the analysis of French fries fried in different vegetable oils with four cycles of re-utilization of the oil.

2. Experimental

2.1. Sampling

The soybean, corn, canola, sunflower, and palm oils utilized for frying the potatoes were purchased in local supermarkets. Potatoes were also purchased in local supermarkets, manually peeled, cut into long, thick slices, washed, and fried. The fried potatoes were dried, packed in plastic bags after cooling and stored at 4 °C until the time for analysis. Commercial French fries, potato chips and shoestring potatoes were also analyzed by the same procedure.

2.2. Frying

The tests were performed in a home fryer containing 400 mL of oil at a temperature of 170 °C, measured with a thermometer. The frying time was observed and recorded, ranging from 12 to 15 min. After frying, each oil was cooled and stored for the next re-use until four frying cycles were completed.

2.3. Statistical methods of optimization

To optimize the analysis method, a study of the variables considered significant for the experiment was conducted using a factorial design studying 2^4 variables: extraction time, 10 min (−) and 40 min (+); temperature, 30 °C (−) and 50 °C (+); concentration of sodium chloride (C_{NaCl}), 0% (−) and 10% (+); and desorption time, 30 s (−) and 120 s (+). The signs (−) and (+) refer to the variables at the lower and higher levels, respectively. The central point was analyzed in triplicate with the following values for the variables: extraction time, 25 min; temperature, 40 °C; salt concentration, 5%; desorption time, 75 s. The Pareto chart was drawn for analysis of the significant variables so that the best working conditions for performing the analysis could be chosen. Some tests were conducted to validate the method in accordance with the guidelines of the Eurachem 1998 [16] for the validation of analytical methods. The parameters studied were linearity, detection limit, quantification limit, and precision.

2.3.1. SPME extraction method

The solid phase microextraction (SPME) was performed with a manual holder with 85-μm polyacrylate (PA) (purchased from Supelco, Bellefonte, PA, USA). Prior to use, the fibers were conditioned according to the manufacturer's instructions. The fiber was exposed to the sample for a suitable period (10 min) at room temperature and introduced into the GC injector (set at 250 °C and running in the splitless mode) for 2 min.

For the analysis of potatoes by GC/MS, 13.00 g of potato chips were weighed and placed in a beaker containing 80.0 mL of aqueous DNPH solution (0.006 g of DNPH in 80 mL of water) with 10% NaCl and 4 mL of acetonitrile. The pH was adjusted to 1.0 with a solution of 15 mol/L of phosphoric acid. The solution was stirred for 30 min to complete the reaction. Aliquots of 17 mL were introduced into 20-mL Pyrex vials, and the vials were immediately sealed with aluminum caps containing Teflon septa. For the blank sample, the same procedure was performed without the addition of acrolein to the vial.

2.4. Instrumentation

GC/MS analyses were conducted on a Trace GC Ultra chromatograph equipped with a POLARIS Q model ion trap mass spectrometer (Thermo Electron, San Jose, CA). An HP-5MS (Agilent, Santa Clara, CA) capillary column (30 m × 0.25 mm id × 0.25 μm film) containing 5% diphenyl and 95% dimethylpolysiloxane was used. The inlet was adjusted to operate in the splitless mode for 2 min at a temperature of 250 °C. The temperature of the oven was programmed as follows: 120 °C (holding time, 2 min) with an increment of 10 °C/min up to 290 °C (holding time, 5 min), terminating with an increase of 10 °C/min up to 300 °C for 2 min. The total run time was 27 min, and helium was used as the carrier gas at a constant flow of 1.5 mL/min. The following parameters were used for operating the mass spectrometer: electron ionization at 70 eV, full scan mode with a mass range of 50–650 m/z, and a source temperature of 200 °C.

2.5. Fatty acids analysis

The fatty acid compositions of the oils were determined as the methyl esters by GC/FID. Analyses were performed on an HP5890 Gas Chromatograph equipped with a flame ionization detector. A Econocap carbowax column (Alltech) 30 m × 0.32 mm × 0.20 μm was used. The temperature program began with the oven

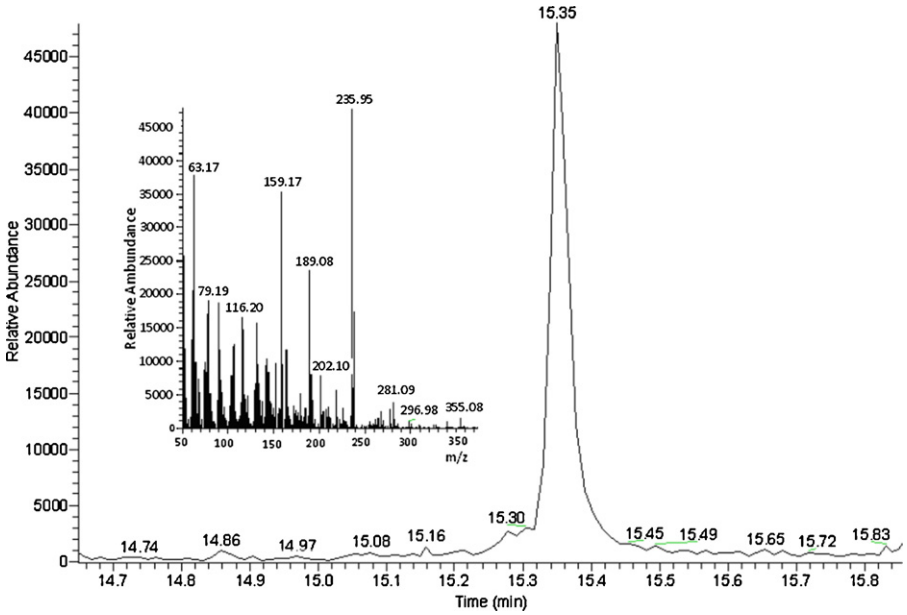

Fig. 1. GC/MS chromatogram in the Full/scan mode and mass spectrum at *m*/*z* 236 corresponding to an acrolein-DNPH standard sample at 18 μg/L with in-solution derivatisation (PA fiber, 10 min extraction, 25 °C and 10% NaCl). Chromatographic conditions in text.

temperature at 120 °C for 1 min; the oven was heated at a rate of 7 °C/min to 240 °C and held for 3 min. The flow rate of the hydrogen carrier gas was 2 mL/min. The injector was maintained at 250 °C in split mode at the ratio of 1:50, and the temperature of the detector was 250 °C. The identification of peaks was achieved by comparison with a Supelco37 (Supelco) fatty acid methyl ester standard mix.

3. Results and discussion

A chromatogram and mass spectrum of the acrolein standard are presented in Fig. 1, demonstrating the fact that the method is selective and efficient. A 2^4 factorial design (conditions and parameters studied are described in Section 2) was proposed to optimize the SPME parameters. A Pareto's plot was constructed (Fig. 2) to determine the most significant effects with a confidence interval of 95%. This plot shows that the extraction temperature (B) was significant at the lowest temperature utilized and the salt concentration (C) and desorption time (D) were significant at the highest values, considering a 95% confidence level.

The Pareto's graph indicates whether the effects of the individual variables and interactions have a higher significance (95% confidence level) for the method. The extraction temperature, sodium chloride concentration (C_{NaCl}), and desorption time presented significant effects, and the extraction time did not show a significant response since the corresponding bar in the graph did not exceed the vertical line at the 95% confidence level. The lower extraction temperature and higher C_{NaCl} furnished the best results. The desorption time was significant for higher values, and the extraction time did not show a significant response.

With regard to the interactions of two variables, the interaction of the extraction time with the desorption time and the extraction time with the extraction temperature were significant at lower values, indicating that they should furnish better results at levels closer to the lowest values chosen in the planning stage. The opposite occurred with the interactions of extraction time with

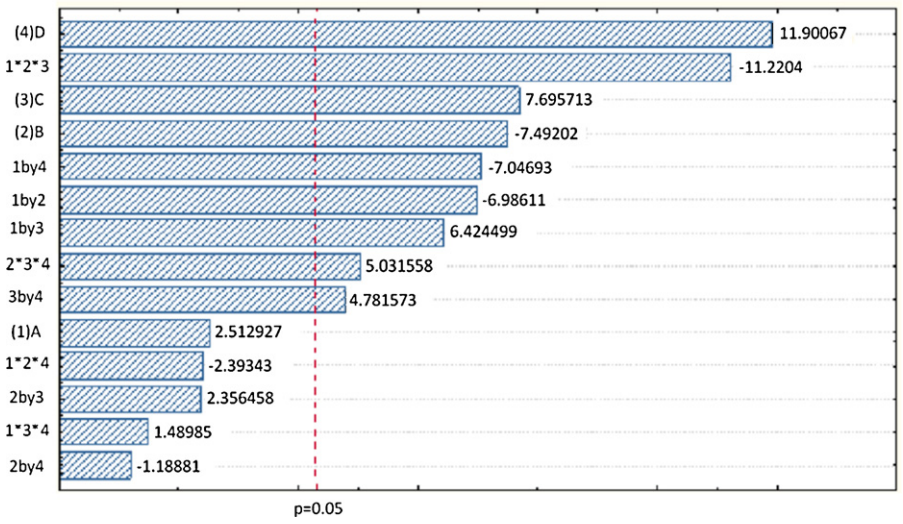

Fig. 2. Pareto's graphs related to optimization of the SPME parameters: extraction time (A) extraction temperature (B), salt concentration (C) and desorption time (D). The plot was constructed based on the results achieved from the factorial design.

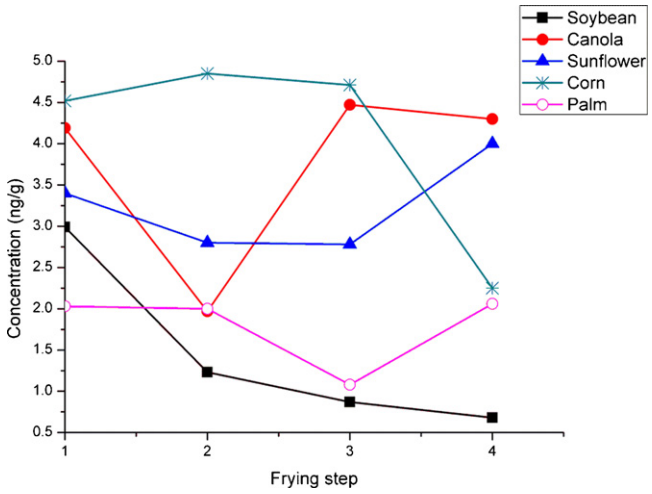

Fig. 3. Concentration of acrolein in French-fried potatoes fried in soybean, canola, sunflower, corn and palm oil. Each oil was reused in four frying cycles.

the salt concentration and the salt concentration with the desorption time, which were significant for higher levels, i.e., the working range should be close to the highest levels specified in the plan, 40 min for the extraction time, C_{NaCl} of approximately 10%, and a desorption time of 120 s. The interaction between extraction temperature and C_{NaCl} was not significant at the 95% confidence level.

The interaction of three variables, extraction time, extraction temperature, and C_{NaCl} was significant, trending to better results at the higher values (ca. 40 min, 50 °C and 10%). The interaction between extraction temperature, C_{NaCl}, and time of desorption proved to be significant for lower values, 30 °C, 5% and 30 s, respectively. The interactions that presented no significance were extraction temperature with extraction time and desorption time and extraction time with C_{NaCl} and desorption time.

Based on this result, the extraction time chosen was 10 min, which permitted faster analyses. The desorption time and C_{NaCl} variables were significant for higher levels. Therefore, the desorption time of 120 s and the 10% C_{NaCl} were selected. The extraction temperature was significant for the lowest level so a temperature of 30 °C was utilized. The effect of extraction time was not relevant.

Using the optimized conditions, the analytical curve was constructed with six analyte concentrations and three steps for each concentration. For acrolein, the curve ($y = 7407x - 317.19$) presented a linear range of 1.0–18.0 μg/L, $R^2 = 0.994$. The limits of detection (LOD) and quantification (LOQ) were calculated by the expressions:

$$\mathrm{LOD} = X_b + 3S$$

$$\mathrm{LOQ} = X_b + 10S$$

according to the recommendation of the Eurachem Guide [16], where X_b and S are the mean area and standard deviation, respectively, of ten consecutive measurements of the blank. The LOD was 0.84 ng/g, and the LOQ was 1.40 ng/g.

In assessing the intra-assay precision (repeatability), ten replicates at the 9.08-μg/L concentration were analyzed on the same day. The coefficient of variation (RSD) was of 9.7%. Five replicates at three concentration levels were analyzed on three consecutive days to assess the intermediate precision. The RSD was of 9.6%. The high quality of the parameters of merit obtained by this method are comparable to the results obtained by Saison et al. [13], even though they utilized PFBH as the derivatizing agent and SPME in the headspace mode.

3.1. Application of proposed method to the analyses of French fried potatoes

A study with fried potatoes was performed using assorted oils to determine whether fresh and re-heated oil can contaminate fried food with acrolein. The results of the determination of acrolein in French fries using five types of oil with four frying cycles are presented in Fig. 3.

The results show that the level of acrolein in potatoes fried in soybean oil had an average concentration of 1.44 ng/g, with a range from 0.68 to 2.99 ng/g. In addition, the concentration of acrolein in the fried potatoes significantly decreased with number of times that the soybean oil was recycled.

Jorge et al. [17] concluded that the potatoes absorbed a smaller quantity of soybean oil than sunflower and corn oils. Soybean oil has a larger amount of saturated fatty acids, which decreases the absorption by the food [18]. These factors may have contributed to the values found in this study, since the absorption of a smaller amount of oil can affect the amount of acrolein retained in food. Another factor that explains the result obtained in this study for soybean oil is that the amount of glycerol decreases with the reuse of oil, thereby decreasing the acrolein concentration.

The average concentration of acrolein in potatoes fried in sunflower oil was 3.25 ng/g with a range from 2.78 to 4.00 ng/g. An average concentration of 4.08 ng/g with a range from 2.25 to 4.85 ng/g was observed for the potatoes fried in corn oil; and a mean of 3.73 ng/g with a range from 1.97 to 4.47 ng/g was obtained for the potatoes fried in canola oil.

In the case of the sunflower, canola and corn oils, the concentration of acrolein remained almost constant for the potatoes fried in the three types of oil during the four frying cycles. However, a significant increase in the acrolein concentration occurred in the last frying step in the case of the sunflower oil, while a decrease occurred in the second and fourth frying steps in the canola and corn oils, respectively. The acrolein concentration was higher in the potatoes fried in the three types of oil mentioned above than when soybean oil was used. The concentrations of unsaturated fatty acids presented in Table 1 show that sunflower, canola and corn oils contain higher concentrations of unsaturated fatty acids than does soybean oil. This factor would lead to the absorption of a larger quantity of oil by the food. This fact explains why the acrolein concentrations found in potatoes fried in sunflower, canola and corn oils are higher than those found in potatoes fried in soybean oil.

The content of unsaturated fatty acids is higher in sunflower oil (87%) than in corn oil (84%), as is shown in Table 1. This fact does not justify the higher concentration of acrolein in the potatoes fried in corn oil. The temperature during the frying process is another factor that may have affected the results obtained for these two types of oil.

In a study conducted by Pozo-Díez [19], the absorption of the oil by food was not directly related to the number of times in which the oil was re-used, but was affected by the fact that temperature changes during the frying process may affect the absorption of oil by food.

Small variations in temperature were observed during the experiments, which may explain the fluctuation in acrolein concentration observed in potatoes fried with canola, corn, and palm oils. Finally, the results for the analysis of acrolein in potatoes fried in palm oil had an average concentration of 1.93 ng/g, with a range from 1.08 to 2.06 ng/g. The acrolein concentration was similar throughout almost all the frying cycles. The factor that justifies the different concentrations of acrolein encountered is the same as that mentioned above for the other oils; hence, changes in temperature during the frying process can be expected to cause a change in the absorption of oil by the food.

Table 1
Range for unsaturated fatty acid composition and viscosity values of samples of vegetable oils (soybean, canola, corn, sunflower and palm oil).

Unsaturated fatty acid	Soybean	Canola	Corn	Sunflower	Palm
Values expressed in % p/p of methyl esters					
C18:1	22.07	56.72	34.69	28.12	36.72
C18:2	53.70	23.31	47.91	58.66	28.10
C18:3	6.31	8.47	1.63	0.52	2.54
Total	82.71	88.50	84.23	87.30	67.36
Values expressed in cP					
Viscosity	53.60	65.10	56.40	50.00	99.50

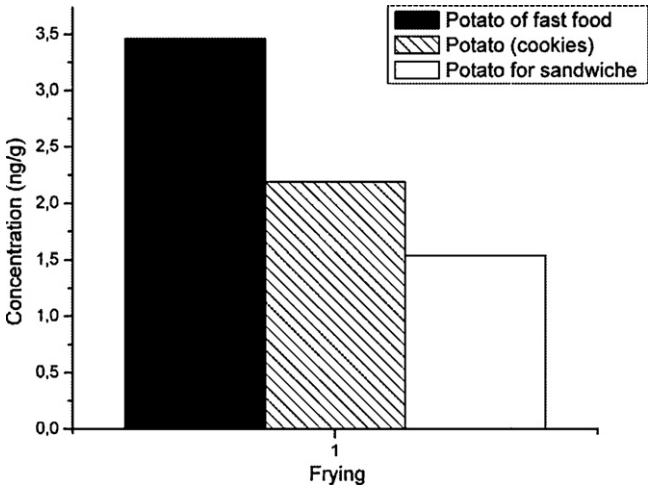

Fig. 4. Concentration of acrolein in commercial potatoes.

The potatoes fried in palm oil contained lower concentrations of acrolein after the first frying cycle (Fig. 3). According to Paul and Mittal [20], several factors affect the penetration of oil into the food, one being the viscosity of the frying oil. Palm oil presented the highest viscosity of the oils studied. This factor may have contributed to the finding that the potatoes fried in palm oil contained a lower concentration of acrolein after the first frying cycle.

The concentration of acrolein in commercial potato sticks (French fries) purchased in a fast food restaurant, shoestring potatoes for sandwiches, and potato chips were similarly determined. The results are represented in Fig. 4. The concentrations of acrolein in potato chips and shoestring potatoes were much lower than that observed in French fries. One factor that explains this difference is that the surface/volume ratio is much higher for potato chips and shoestring potatoes than for French fries. According to Paul and Mittal [20], the surface/volume ratio of food in contact with the frying oil is an important contributing factor for determining the amount of oil absorbed by food. The absorption of the oil by food depends mainly on the distance from the center to the surface of foods such as the potato chips and French fries used in these experiments.

4. Conclusions

This work describes an alternative SPME-GC/MS method for the determination of acrolein in fried potatoes. This procedure was validated and found to be precise, sensitive and linear in the range of interest. SPME represents a good alternative method for the quantitative analysis of acrolein in fried foods. The acrolein concentration varied with the type of oil employed for frying and with the number of times in which the oil was reused. However, the values were below the limit recommended by the World Health Organization (2002), which specifies that the acrolein concentration should be a maximum of 40 μg/g in foods.

Acknowledgements

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