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Quantitative Analysis of Acrolein in Heated Vegetable Oils by Liquid Chromatography with Pulsed Electrochemical Detection

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A sensitive and selective analytical method for the determination of acrolein in heated vegetable oils by liquid chromatographic separation with pulsed electrochemical detection is described. An optimized triple-step pulsed waveform, based on the formation/inhibition of PtOH species on the electrode surface, a consequence of the absence/presence of adsorbing analytes, is described for the sensitive detection of acrolein in acidic medium. Under these optimized experimental conditions the proposed analytical method allowed detection limits of 0.15 μM without pre- or postcolumn derivatization or tedious cleanup procedures. The proposed analytical method was successfully employed for the sensitive determination of acrolein in fresh and heated vegetable oils with good mean recoveries, selectivity, and analytical reproducibility.

KEYWORDS: Liquid chromatography; electrochemical detection; acrolein; vegetable oils

INTRODUCTION

Volatile aliphatic aldehydes are generally formed by lipid peroxidation and incomplete oxidation of many organic compounds and usually present in trace amounts in various matrices such as foods, tobacco smoke, air, water pollution samples, and physiological fluids (*1*). They are of increasing environmental concern because of their adverse effects on health.

Short-chain vinyl aldehydes, such as acrolein (1-propenal), have been found to be produced in polyunsaturated fatty acids during enzymatic and nonenzymatic maturation caused by lipid peroxidation processes (*2*, *3*). These molecules are known contributors to photochemical smog and irritants of the skin, eyes, and mucous membranes of the respiratory tract (*4*). Acrolein, in particular, has been associated with various human diseases such as atheroschlerosis, carcinogenesis pathologies, and aging. In addition, several studies show that acrolein concentration is increased in the brain in Alzheimer's disease (*5*). The cytotoxicity of acrolein is attributed to its reactivity toward sulfhydryl groups in proteins and glutathione, leading to interference with intermediary cell metabolism and modifying proteins by introducing intermolecular and intramolecular cross-linkages (*6*, *7*). Therefore, on account of the high toxicity of aldehydes, in particular, acrolein, for most higher organisms, their determination in foodstuff, biological samples, and exhaust gas emission sources is of major sanitary and environmental concern. In this respect, acrolein is considered a prioritary pollutant according to the U.S. Environmental Protection Agency (EPA). In addition, determination of acrolein levels in sensitive and resistant cancer cells, both drug treated and untreated, would provide further insight into the relationship between drug-induced oxidative stress, drug cytotoxicity, and resistance mechanisms.

Because of the environmental importance of these compounds, sensitive and selective analytical methods for the determination of vinyl aldehydes in exhaust gas and/or air, biological fluids, and foods are needed. Generally, determination of aliphatic aldehydes has been carried out by liquid chromatography with spectrophotometric detection (*8*−*11*) or gas chromatographic techniques (*12*−*17*) coupled with various detectors, such as thermionic, nitrogen−phosphorus, electron capture, or mass spectrometry. However, many analytical methods require derivatization procedures in order to increase the detection sensitivity. Although some of these derivatization methods are very sensitive, they, in general, sensibly increase the time of analysis and risk for systematic and/or casual errors. In addition, cleanup of the samples in order to remove excess derivatizing reagent and/or coexisting intermediate reaction products could be necessary. In this respect, analytical methodology that does not require derivatization schemes is generally preferred, when available, for convenience, rapidity, and analytical simplicity.

Electrochemical detection scheme following liquid chromatography represents an attractive analytical methodology for the direct quantitative determination of aliphatic organic compounds without the use of any derivatizing procedure. In this respect, electrochemical oxidation of unsaturated aliphatic alcohols and aldehydes can occur by a catalytic mechanism on various transition-metal oxides (*18*−*20*); thereby, these molecules may be suitable for analytical determination with electrochemical detection in batch or flow injection analysis. In addition, pulsed electrochemical detection (PED) overcomes fouling of noble-metal electrodes by combining amperometric detection with alternated anodic and cathodic polarizations to clean and reactivate the electrode surface. Thus, liquid chromatography

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coupled with pulsed electrochemical detection has become increasingly popular, and some interesting examples of quantitative determinations of aliphatic hydroxyl compounds in various analytical contexts have been successfully proposed (*21−26*). Recently, we demonstrated that pulsed electrochemical detection at platinum electrode substrates can be useful for the sensitive detection of aliphatic organic acids after ion-exclusion HPLC separations (*26*).

The aim of this paper is to investigate the possibility of using a polycrystalline platinum electrode as an amperometric sensor for the detection of acrolein, after liquid chromatographic separation, in virgin olive oil and other vegetable oil samples. Here, we demonstrate that combination of a chromatographic separation process based on the mixed steric exclusion and adsorption/partitioning scheme with the pulsed electrochemical detection allows a rapid, selective, and sensitive determination of acrolein in real matrices. The proposed method was successfully applied to quantification of the acrolein level occurring in the vegetable oils during thermal treatment. These analytical procedures can be used for routine analytical determination in order to check the oxidative degradation state of the vegetable oils.

MATERIALS AND METHODS

Chemicals. Acrolein (90% ww, stabilized with 0.1−0.2% ww hydroquinone) was purchased from Sigma-Aldrich (Steinheim, Germany) and used as received. Similarly, other chemicals were purchased from Sigma-Aldrich. All reagents used were of the highest purity available. Stock solutions of acrolein were prepared with pure water supplied by Milli-Q RG unit from Millipore (Bedford, MA). To prevent cyclic dimerization and microbial growth, the standard acrolein solutions were prepared fresh daily and diluted with pure water. Unless otherwise specified, experiments were performed by using nondeoxygenated 10 mM perchloric acid ($HClO_4$) as the mobile phase. All experiments were carried out at ambient temperature.

Apparatus. Amperometric measurements in flowing streams were performed by using a pulsed amperometric detector model ED 40 (Dionex, Sunnyvale, CA). A thin-layer electrochemical cell consisting of a 1.0 mm diameter platinum working electrode, an Ag/AgCl combined reference electrode, and stainless steel serving as the counter electrode was also purchased from Dionex. The polycrystalline platinum working electrode was weekly polished with 0.05 μm of alumina oxide powder on a microcloth using water as the lubricant. All experiments were performed using a metal-free pump, model PU-1580i (Jasco Corp., Tokyo, Japan), equipped with a programmable gradient module, model LG-1580−04 (Jasco), and a metal-free rotary injection valve, model 7125i (Rheodyne, Cotati, CA), with a 20 μL sample loop. A personal computer equipped with Kontron PC Integration Pack Software (Milan, Italy) allowed acquisition and processing of chromatograms. Unless stated otherwise, the pulsed amperometric detector settings were as follows: $E_{det} = 0.40$ V ($t_{det} = 340$ ms, $t_{int} = 60$ ms), $E_{ox} = 1.40$ V ($t_{ox} = 120$ ms), and $E_{red} = -0.40$ V ($t_{red} = 520$ ms). Currents are measured and integrated with respect to time (t_{int}) to give a Faradic charge (coulombs) for the detection cycle.

Chromatographic separations of acrolein from other compounds were achieved with an Aminex HPX-87H BioRad column (300 × 7.8 mm i.d.), packed with 9-μm spherical sulfonated polystyrene−divinylbenzene copolymer beads with 8% cross-linking and providing an ion-exchange capacity of 1.7 mmol/g.

Sample Treatment. Bottled, commercial vegetable oils (Desantis, S.p.A. Bari; Di Carlo S.r.L. Potenza) were purchased from local stores, protected from sunlight, and opened only just before the thermal treatment and/or analysis. A 10 mL amount of vegetable oil was mixed with 40 mL of distilled water in a 100 mL glass flask tightly closed and sonicated at 55 °C for 20 min in a water bath. The resulting emulsion was kept at room temperature for 5 min; then the aqueous phase was separated and filtered with nitrocellulose membrane 0.45 μm pores (Millipore, Bedford, MA), diluted 1:2.5 with distilled water, and injected into the column. Different temperatures and medium of extraction (i.e., distilled water and/or 10 mM $HClO_4$) were tested in order to obtain the best results in terms of recovery and reproducibility. The thermally treated oil samples were prepared by heating fresh vegetable oils placed in a glass flask tightly closed and conditioned at various temperatures and times. The temperature of the sample was measured by a mercury thermometer placed in the mineral oil bath containing the closed glass flask. After, the treated samples were allowed to stand for 30 min at ambient temperature and then analyzed as reported above. The determinations were performed in duplicate.

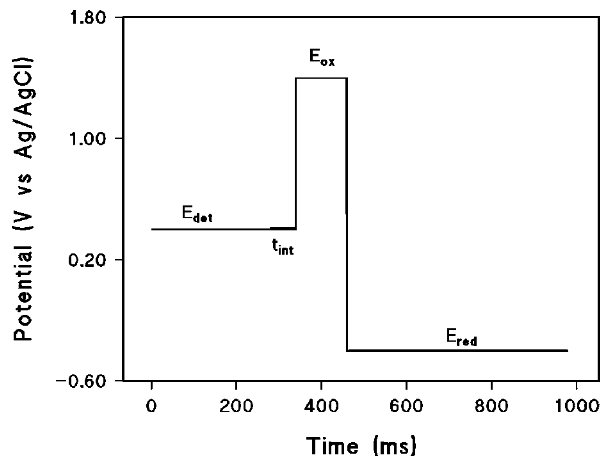

Figure 1. Potential–time waveform profile adopted for pulsed electrochemical detection: E_{det}, detection potential; t_{int}, integration time at E_{det}; E_{ox}, oxidation potential; E_{red}, reduction potential. The integration time is 60 ms.

RESULTS AND DISCUSSION

Optimization of PED Parameters. It is well known that adsorbed analytes at a reduced platinum electrode surface will affect formation of the platinum oxide species following a positive potential step (*25, 26*). In this respect, a triple-step pulsed waveform, based on the formation/inhibition of PtOH species on the electrode surface, a consequence of the absence/presence of adsorbing species, is used for sensitive detection of acrolein. Thus, depending on the choice of applied potentials and their relevant times of application, both "positive" and "negative" peak currents can be observed when adsorbing species were injected into a flowing stream. The optimized pulsed waveform used in this work is shown in **Figure 1**.

Pulsed amperometric detection thus employs a repeating of three potentials. Current from analyte oxidation is measured at the first potential (E_{det}). The second potential (E_{ox}) is more positive, oxidizes the platinum electrode, and cleans it of oxidation products. The third potential (E_{red}) reduces the platinum oxide on the electrode surface, thus permitting detection during the next cycle at E_{det}. The three potentials are applied for fixed durations referred to as t_{det}, t_{ox}, t_{red}, and t_{int}. The step from one potential to the next produces a charging current that is not part of the analyte oxidation current, so the Faradaic current is measured after a delay that allows the charging current to decay. The amperometric signal is measured by integrating the cell current after the delay (t_{det}). As a consequence, the current integrated over the selected t_{int} is expressed in coulombs. As can be seen, the platinum electrode is pulsed first at the detection potential of 0.4 V (E_{det}) for 340 ms and then to a positive potential of 1.4 V (E_{ox}) for 120 ms and back to a low potential of −0.4 V (E_{red}) for 520 ms. The integration time (t_{int}) induces a significant increase in the charge signal of the acrolein; when the t_{int} was changed from 10 to 100 ms, the relevant electrochemical signal increased by a factor

of 50. This electrochemical behavior is consistent with a time-dependent adsorption isotherm mechanism of acrolein on the platinum active sites. The selected triple-step PED waveform with an integration time of 60 ms was considered as an optimal compromise in terms of charge signal, rapid establishment of baseline values, low background level, and acceptable temporal stability. In addition, the detection potential of 0.4 V represents the best compromise between maximum analytical signal and a good level of selectivity. In fact, under the same experimental conditions, the hydrodynamic voltammograms of alcoholic (*21*) and acidic species (*26*) exhibit a maximum sensitivity between 0.2 and 0.3 V, while for higher detection potentials (i.e., 0.4 V), a marked diminution of the electrochemical signals was generally observed.

Optimization of Chromatographic Conditions. Ion-exclusion columns containing polystyrene–divinylbenzene copolymer fully sulfonated can be used for ion-exclusion separation of weak ionizable molecules in acidic medium. Under the experimental conditions used here, acrolein is not ionizable and the retention process is based mainly on the size exclusion mechanism of separation. Various concentrations of $HClO_4$ of the mobile phase were considered in order to evaluate the effect of acid on the retention time and electrode performance in terms of charge signal. In contrast with the behavior of the organic acids separated under the same chromatographic conditions, where retention times tend to increase with an elevation of the $HClO_4$ concentration (*26*), the retention times of smaller aldehydes, in particular, for the acrolein, are quite independent of the acidity level. In addition, the peak areas of acrolein (expressed in arbitrary units) tend to decrease slightly with the diminution of the $HClO_4$ concentration. On the contrary, the peak area of the saturated aldehydes, aliphatic alcohols, and saturated organic acids decreased markedly with decreasing $HClO_4$ concentration in the mobile phase. Therefore, for the analytical determination of acrolein, which is the focus of this work, a concentration of 10 mM $HClO_4$ was chosen as being a reasonable compromise between good selectivity and acceptable magnitude of the PED signal. In previous experiments we have shown that dissolved oxygen in the mobile phase markedly inhibits the detection of saturated aliphatic aldehydes alcohols (i.e., methanol, ethanol, propanol, etc.) and saturated organic acids (citric, formic, acetic acid, etc.). On the contrary, in this study we observed that the PED signal of the unsaturated aldehydes such as acrolein is nearly independent of the oxygen content in the mobile phase. This behavior may well be due to the selective exclusion of the saturated aldehyde molecules from the electrochemical active sites caused by a favorable and strong adsorption of dissolved oxygen or, more probably, its reduction products (i.e., adsorbed hydroxyl species) on the platinum surface. In this respect, using nondeaerated 10 mM $HClO_4$ solutions as eluent, the injection of solutions containing several saturated organic molecules, such as aliphatic aldehydes, alcohols, and acids (i.e., citric, tartaric, acetic, etc.), at relatively high concentrations (about 30 mM of each compound) did not produce any significant electrochemical signal in the chromatographic profile. **Figure 2** shows a typical chromatographic analysis of acrolein using an Aminex HPX-87H ion-exclusion column with PED accomplished under isocratic conditions using a mobile phase of non-deoxygenated 10 mM $HClO_4$ at a flow rate of 1.2 mL/min. Under these experimental conditions, the acrolein peak is present at a retention time of about 13.5 ± 0.2 min. Analytical quality parameters such as limits of detection (LOD), linearity, and precision were evaluated. The best-fit line for the graph of the regression analysis of calibration data has a slope of 23.5 ± 0.05 and an intercept of 0.015 ± 0.004. The inset in **Figure 2** shows the relevant calibration plot obtained for acrolein determination by liquid chromatography with pulsed electrochemical detection. The response of acrolein is linear from 75 μM to its limit of detection with a correlation coefficient always greater than 0.996, using 14 experimental data points. In all cases, at least three trials were run for each standard concentration. The LOD, evaluated experimentally as a signal-to-noise ratio of three at the lowest injected concentration with a 20 μL sample loop, was determined to be 0.15 μM. The precision, expressed as percent relative standard deviation (RSD %), was evaluated by analyzing two ultrapure water samples spiked with 15 and 30 μmol/L acrolein. The relevant precision was estimated on the same day from four replicate consecutive chromatographic injections of standard solutions (spanning over 4 h of operation time). Under these experimental conditions, the precision ranged from 3.8% to 6%, indicating good short-term stability of the amperometric sensor. The analytical performance in terms of LOD, linear range, and precision is generally comparable with that obtained in liquid chromatography using other detection strategies. In fact, fluorescent high-performance liquid chromatography studies on acrolein determination (*8, 10, 11*), reported limits of detection and precision were between 0.1 and 0.3 μM and 4% and 10%, respectively. However, the proposed electrochemical method of detection affords better selectivity (see below) and did not require any extraction, derivatization, or sample cleanup procedures.

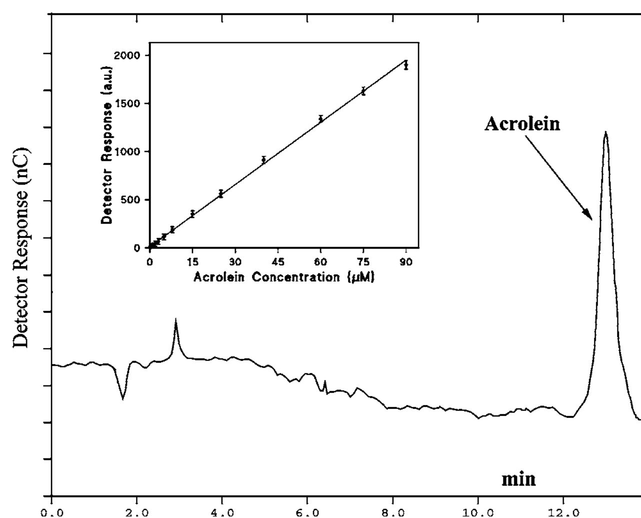

Figure 2. Liquid chromatographic analysis of 5.5 μM acrolein with pulsed electrochemical detection at a polycrystalline platinum electrode. Column: Aminex HPX-87H BioRad column (300 × 7.8 mm i.d.). Mobile phase: nondeaerated 10 mM $HClO_4$. Flow rate: 1.2 mL/min. Sample loop: 20 μL. Waveform: E_{det} = 0.40 V (t_{det} = 340 ms, t_{int} = 60 ms), E_{ox} = 1.40 V (t_{ox} = 120 ms), and E_{red} = –0.40 V (t_{red} = 520 ms). (Inset) Relevant calibration graph peak area vs concentration for the acrolein determination.

Quantitative Determination of Acrolein in Vegetable Oils. *Optimization of Extraction Procedure and Recovery.* Different parameters that impact the partition of acrolein between organic medium and aqueous solution, such as temperature and pH of the extraction medium, were tested. Fresh corn and sunflower oil samples were used as model matrices for optimization of the extraction procedure. The vegetable oil samples spiked with a known amount of acrolein (12.5 μM) were sonicated with distilled water or 10 mM $HClO_4$ aqueous solution at various temperatures. The effect of temperature during the extraction process was investigated, varying its value between 22 and 65

Table 1. Effect of the Extraction Temperature and Medium on the Precision and Recovery[a]

sample	extraction temp (°C)	medium	RSD %	recovery %
corn oil	22	H_2O	11	49
corn oil	55	H_2O	6	104
corn oil	65	H_2O	8	116
corn oil	55	10 mM $HClO_4$	7	108
sunflower oil (2)	55	H_2O	6	106

[a] Experimental conditions: Aminex HPX-87H BioRad column (300 × 7.8 mm i.d.). Flow rate: 1.2 mL/min. Sample loop: 20 μL. Mobile phase, 10 mM $HClO_4$. Waveform: $E_{det} = 0.40$ V ($t_{det} = 340$ ms, $t_{int} = 60$ ms), $E_{ox} = 1.40$ V ($t_{ox} = 120$ ms), and $E_{red} = -0.40$ V ($t_{red} = 520$ ms). The correlation coefficients of the regression plots for the standard addition (four additions) are generally >0.993.

°C. For all experiments an extraction time of 20 min was selected. The aqueous extract was filtered and diluted with distilled water before injection in the column. No chromatographic peak related to the acrolein molecule was observed in both unspiked fresh vegetable oils employed in these experiments. Peak identification of the acrolein was based on the retention time and confirmed by adding authentic standard to the diluted extract of the sample. Further support of acrolein identification was given by measuring the degree of peak asymmetry factor (*27*) as a function of the acrolein concentration. The observed peak asymmetry factor, evaluated under these optimum chromatographic conditions, was always between 0.92 and 1.07 and practically independent of the acrolein concentration in the extract sample. To avoid erroneous analytical quantification of acrolein caused by matrix effects, the relevant concentration in real samples was evaluated by ordinary linear least-squares regression by using the standard addition method. In particular, the quantitative determinations of acrolein were performed by spiking original sample extract (four additions) with standard additions of pure analyte containing about 10%, 20%, 30%, and 50% of the initial measured value of concentration. The mean recoveries were obtained by duplicate experiments of each spiked sample and determined as the percent difference between the determined amount and the concentration of acrolein added.

The effect of temperature and medium on the extraction efficiency of acrolein from vegetable oil matrices was evaluated. Relevant results are summarized in **Table 1**. As can be seen, employing an extraction time of 20 min at 55 °C and distilled water as extraction solvent, an excellent compromise in terms of time of analysis (i.e., extraction and chromatographic separation of acrolein was performed in less than 40 min of operation time), analytical precision (i.e., RSD 6%), and recovery (i.e., 104% − 106%) was obtained. The good mean recoveries of acrolein confirms the practical absence of interfering compounds and any matrix effect. In addition, considering that corn and sunflower oil samples showed similar degrees of recovery for the measured acrolein, it can be concluded that the matrix effect plays no important role in this specific analytical determination.

Analysis of Acrolein in Vegetable Oils. To demonstrate the analytical potentiality of the proposed method for practical applications, chromatographic analysis of various vegetable oils such as corn, peanut, sunflower, and olive oils is reported. Typical chromatographic separations of a commercially available olive oil before and after heating treatment at 145 °C for 2 h are compared in **Figure 3A** and **B**, respectively. As can be seen, heat treatment of the oil sample induces the formation of a well-resolved peak of acrolein species accompanied by three

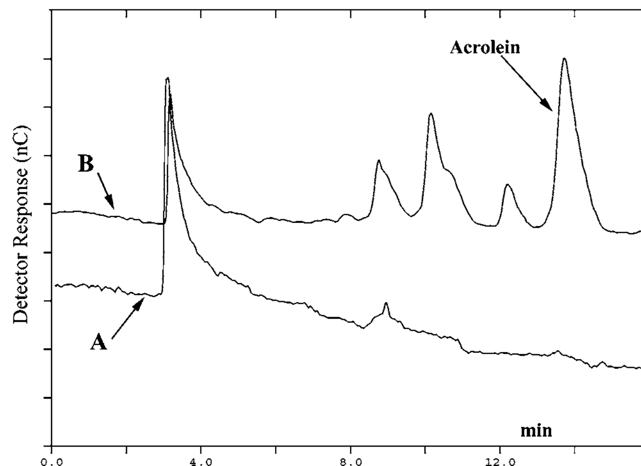

Figure 3. Chromatograms of olive oil sample (1) before (**A**) and after thermal treatment at 145 °C for 2 h (**B**). Experimental conditions as in **Figure 2**.

Table 2. Amount of Acrolein Contained in Various Fresh and Heated Vegetable Oils at 145 °C for 2 h[a]

sample	fresh (μm)	heated (μM)	iodine value (*29*)
corn oil	nd	4.3	105–128
sunflower oil (1)	1.1		120–134
sunflower oil (2)	nd	2.9	120–134
penaut oil	nd	2.7	88–98
olive oil (1)	nd	5.6	75–94
olive oil (2)	nd	9.3	75–94

[a] Experimental conditions as in **Table 1**. nd, not detected. Olive oil (1) is a commercial product (DeSanctis, 2003). Olive oil (2) is a local product (Potenza, Italy, 2003).

or four other unresolved and unidentified peaks. A similar chromatographic profile is seen when other vegetable oils such as corn, peanut and sunflower oil were analyzed. The simple extraction process used here apparently removes the bulk of the fat and potential interferences so that a fully sulfonated ion-exclusion column based mainly on the size-exclusion mechanism of separation is sufficient to provide good efficiency and resolution degree for the acrolein separation. As a comparison of this category of vegetable oils, **Table 2** reports the relevant analytical results in terms of acrolein concentration evaluated in several fresh and heated oils. As shown in **Table 2**, except for the sunflower oil sample that contains 1.1 μM acrolein, this compound was not detected at all in any of the examined fresh vegetable oils. On the other hand, the vegetable oils heated at 145 °C for 2 h show a level concentration of acrolein between 2.9 and 9.3 μM for sunflower and olive oil, respectively. Generally, saturated fatty acids are considered to be more resistant to thermal oxidation than unsaturated ones, and in particular, virgin olive oil has been shown to be more resistant to oxidation because of its low polyunsaturated fatty acid content. In addition, the presence of natural antioxidants, such as tocopherols and phenolic compounds (*28*), should increase the thermal stability of the olive oils. Nevertheless, under these experimental conditions, olive oils produced the greatest concentration of acrolein, while the sunflower oil produced the lowest concentration. In **Table 2** we also reported the iodine value (*29*), which is associated with the degree of unsaturation of the vegetable oils analyzed. It is interesting to observe that the amount of acrolein produced by thermal oxidation seems to correspond negatively to iodine values. A similar behavior of the saturated/unsaturated fatty acids compounds under

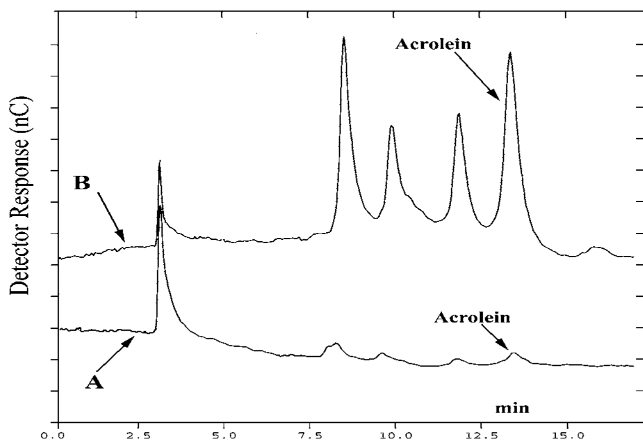

Figure 4. Chromatograms of a peanut oil samples treated thermally at 95 (**A**) and 200 °C (**B**) for 2 h. Experimental conditions as in **Figure 2**.

Table 3. Amount of Acrolein Contained in Peanut Oil Heated at Various Temperatures for 2 h[a]

temp (°C)	amount (μM)	temp (°C)	amount (μM)
95	0.2	145	2.7
110	0.2	200	24

[a] Experimental conditions as in **Table 1**.

oxidative thermal degradation was previously observed using gas-chromatographic methodologies (*16, 30*). In general, various pathways of acrolein formation can be considered, such as dehydration of glycerol species (*30, 31*), free-radical mechanisms involving homolytic scission of ester bonds (*30*), or direct condensation of formaldehyde and acetaldehyede species according to the general mechanism of aldol and croton condensation (*32)*. Nevertheless, partial inhibition of acrolein formation derived by thermal treatment of vegetable oils containing a high level of unsaturated fatty acids appears unclear at the present time, and further investigations regarding this specific point are necessary.

To check the kinetics of acrolein formation under thermal treatment of vegetable oil samples, peanut oil is used as a model sample. The selected oil sample is heated at various temperatures for 2 h and analyzed using the proposed analytical method. **Figure 4** shows an example of a typical chromatographic profile of the peanut oil sample thermally treated at 95 (**Figure 4A**) and 200 °C (**Figure 4B**), respectively. The relevant results are reported in **Table 3**. As expected, formation of acrolein from peanut oil increased markedly with increasing temperature, but it is interesting to underline that acrolein formation is observed at a relatively low temperature, such as 95 °C. Previous studies (*30*) showed that the acrolein species was formed by thermal oxidative degradation when vegetable oil samples were heated above 240 °C. In this respect, our proposed analytical methodology for acrolein determination in vegetable oils provides important evidence that this compound with marked cytotoxic activity can be produced at temperature as low as 100 °C. Although during thermal treatment of fatty acids containing samples the acrolein would be distributed into open ambient areas so that the actual concentration should be much lower, prolonged exposure of persons during vegetable oils distillation/extraction in industrial contexts and/or their use in kitchen may be hazardous.

The proposed analytical method is rapid, inexpensive, and reproducible for acrolein determination in routine contexts. The good level of sensitivity, selectivity, and recoveries without any complicated extraction procedure and chemical derivatization represent interesting advantages for determination of acrolein in various matrices and environments. To the best of our knowledge, this study represents the first example of the analytical determination of acrolein by an electrochemical detection mode using a polycrystalline platinum electrode as an amperometric probe.

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