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# Determination of Arsenic in Edible Fats and Oils by Focused Microwave Digestion and Atomic Fluorescence Spectrometer

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## ABSTRACT

A method for the determination of arsenic in edible fats and oils was developed. A half-gram sample was digested with 10 mL sulfuric acid and 5 mL nitric acid by focused microwave digester, and then analyzed by atomic fluorescence spectrometer (AFS). The detection limit of the instrument was at 50 pg/mL. The recovery studies were performed at 0.05, 0.10 and 0.20 ppm spike levels of arsenic in palm oil, olive oil and lard. The recoveries were in range of 89.8 94.7, 86.4 96.9 and 86.9 91.5%, respectively. Twenty-one market samples of edible fats and oils were analyzed to contain 0.005 0.027 ppm of arsenic.

Key words: focused microwave digestion, atomic fluorescence spectrometer, edible fats and oils, arsenic

## INTRODUCTION

Arsenic is a hazardous heavy metal. Signs and symptoms of intoxication appear even when the amount of exposure is minute. The major mechanism of toxicity is inhibition of enzyme activities upon its binding. The toxicity of arsenic species relies on their forms. Trivalent arsenic, the more toxic form, accumulates more in the tissues and slowly excretes from the body[1]. The form of arsenic commonly seen in food is organic arsenic, which is absorbed rapidly and excreted rapidly mostly in urine. Because arsenic accumulated in the body has a higher affinity to keratin, hair and nails tend to store more arsenic than other tissues. The maximum permissible concentration of arsenic in Taiwan, R.O.C. is 0.1 ppm according to the revised "Hygienic Standard of Edible Fats and Oils" published on January 4, 1993 (Ordinance No. 8189322) by the Department of Health, Executive Yuan[2].

Methodologies for detection of arsenic include colorimetry[3], atomic absorption spectrometry[4], and hydride generation-atomic absorption spectrometry[5]. All of these require the digestion pretreatment of samples before instrumental determination. Digesting by dry ashing procedure that involves the use of high temperature (approximately 450˚C) would result in volatile loss of the analyst; therefore, it is necessary to add assistant. Digestion with wet ashing procedure would prevent this volatile loss due to the lower temperature of digestion (approximately 200˚C). Generally, perchloric acid and nitric acid are employed as the digesting acids for wet ashing digestion to pre-treat fats and oils, but the time required for digestion is extended. Microwave digestion is widely utilized for the pretreatment of heavy metal analysis because microwave irradiation has a more direct, thus controllable, heating mechanism. It therefore shortens the time of digestion and provides a rapid and complete digestion compared with conventional methods[6,7]. Microwave digestion includes closed or open systems, depending on the designs of the digestion vessels. The closed vessel system[8] is widely applied because the efficiency of digestion can be improved by elevating the pressure of digestion. However, the sample volume is usually smaller to prevent pressure buildup. On the other hand, the open vessel system[9] is operated under ambient atmosphere. It thus has the advantage of being capable of a larger sample volume because the formation of high pressure as a result of acid heating is avoided. The atomic fluorescence spectrometer detects the intensities of specific fluorescent bands emitted from gaseous atoms that have been excited to higher energy levels by absorption of electromagnetic radiation. Quantitative determination with this method demonstrates the advantages of background interference reduction, sensitivity enhancement, and linear range spanning. As a consequence, the present study employed the hydride generation-atomic fluorescence spectrometer to investigate the optimal conditions for microwave digestion and atomic fluorescence spectroscopic detection of arsenic in various fat and oil samples, to report the detection limits and recoveries of this method. Ultimately a detection method was established that is rapid with good precision and accuracy, and requires only traces of arsenic for analysis.

## MATERIALS AND METHODS

### I. *Reagents*

Sulfuric acid, sodium hydroxide and potassium iodide used were suprapur grade. Hydrochloric acid and nitric acid were tracepure grade. Hydrogen peroxide, sodium borohydride and arsenic standard (1000 mg/L) were reagent grade. These chemicals were all obtained from E. Merck Co.

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(Germany). Deionized water with resistivity equaling or greater than 18 MΩ-cm was used for preparation of solutions. Standard stock solution was prepared by diluting 1 mL of standard arsenic to 100 mL with 1% (v/v) hydrochloric acid. The standard stock solution was diluted with 30% (v/v) hydrochloric acid to make standard working solutions of 250, 500, 1000, 2000, and 3000 pg/mL. Hydrochloric acid (30%, v/v) was prepared by adding 30 mL of hydrochloric acid into deionized water and adjusting the volume to 100 mL. One percent (w/v) sodium borohydride solution was made by adding sodium hydroxide (0.1 N) into 10 g sodium borohydride and increasing the volume to 1000 mL. Preparation of 40% potassium iodide solution was made by dissolving 10 g potassium iodide with deionized water and diluted to 25 mL. Standard working solutions, 1% sodium borohydride solution, and 40% potassium iodide solution were all freshly prepared prior to use. Volumetric flasks were used for the measurement of all solutions to ensure precision.

II. *Equipments and Labwares*

The water purification system Milli-Q model was manufactured by Millipore Co. (USA). The focused microwave digester Microdigest 3.6 with maximum microwave output 300W was purchased from Prolabo (France). Instrumental parameters for microwave digestion are shown in Table 1. The atomic fluorescence spectrometer Excalibur was made by PS Analytical (England). Operation settings were as following: Carrier and dryer gas, argon; flow rate, 300 and 2500 mL/min, respectively; duration time, 10, 20, 30 and 30 sec for delay, rise, analysis and memory, respectively.

Funnels, volumetric flasks, and pipettes were Pyrex® brand. All were brushed with detergent, rinsed with water, immersed in mixture of $HNO_3/H_2O$ (1:1) overnight, rinsed again (with tap water and deionized water), and dried before use.

III. *Methods*

The completeness of different digesting acids was compared by detecting the arsenic concentration in olive oil (0.5 g) digested with 15 mL of sulfuric acid, sulfuric acid-nitric acid (2:1), sulfuric acid-nitric acid (1:2), respectively, followed by an identical microwave digestion procedure, with hydrogen peroxide as the assistant. To study the effect of various concentrations of sodium borohydride, a standard arsenic working solution of 500 pg/mL and 30% hydrochloric acid were reacted with 0.5, 1.0, 1.5, 2.0, or 2.5% (w/v) sodium borohydride. Comparison of various concentrations of hydrochloric acid on the efficiency of digestion was made by reacting 500 pg/mL arsenic standard and 1% (w/v) sodium borohydride with 20, 25, 30, 35, or 40% (v/v) hydrochloric acid. Emission data of the corresponding reagent blanks were deducted before comparison. The effect of flow rates of the carrier gas was tested by detecting 500 pg/mL arsenic standard using argon as the carrier gas at flow rates of 100, 150, 200, 250, 300, 350, or 400 mL/min. All of these protocols were performed in triplicate and the emitted fluorescence intensities were taken as the basis of comparison.

**Table 1.** Experimental scheme for the digestion of edible fats and oils by microwave digestion system

| Step | Reagent | Vol.(mL) | Temp. (˚C) | Time(min.) |
|---|---|---|---|---|
| 1 | $H_2SO_4$ | 10 | | |
| | $HNO_3$ | 5 | 100 | 15 |
| 2 | | | 120 | 10 |
| 3 | $H_2O_2$ | 2 | 170 | 5 |
| 4 | $H_2O_2$ | 2 | 190 | 5 |
| 5 | $H_2O_2$ | 2 | 200 | 5 |
| 6 | | | 210 | 5 |

The standard calibration curve was calculated by linear regression of the fluorescence intensities emitted from arsenic standards of 300, 500, and 700 pg/mL. The precision of the atomic fluorescence spectrometer was determined by the relative standard deviation derived from forty replicates of arsenic standard of 500 pg/mL.

One milliliter of arsenic standards of various concentrations (0.025, 0.05, or 0.1 μg/mL) was each individually spiked into 0.5 g of palm oil, olive oil and melted lard. The blanks were the respective lipid samples without the addition of arsenic standards. These lipid samples, with or without arsenic standards, were digested, and their arsenic contents determined, by the optimized analytical and instrumental operation parameters for the calculation of the recoveries.

IV. *Analysis of Arsenic Content in Edible Fat and Oil Samples*

A half-gram of fat or oil sample was weighed into the digestion vessel to which 10 mL of sulfuric acid and 5 mL of nitric acid were added. Hydrogen peroxide was added to assist digestion. After the completion of the digestion programs, indicated by turning clear, the digests were diluted to 25 mL in volumetric flasks with water. One milliliter of potassium iodide (40%) was added to 10-mL aliquots of the diluted digests. The digests were left to stand for 30 min before reacting with hydrochloric acid (30%) and sodium borohydride (1%) solutions to form the volatile hydrides. Arsenic contents of the hydrides were quantitatively determined by the atomic fluorescence spectrometer.

## RESULTS AND DISCUSSION

I. *Effects of Acids on Microwave Digestion*

Microwave digestion employed sulfuric acid or mixtures of sulfuric and nitric acids as the digesting acid and hydrogen peroxide as the assitant to break down the bonds in lipid molecules. It was more time-consuming to reach complete digestion, as indicated by the clarification of the reaction solutions, when sulfuric acid (15 mL) was used exclusively. A large amount of dark supernatants was formed after adding potassium iodide into the digested sample solution. The fluorescence intensity under this reaction condition was 57.7 ± 5.1. On the other hand, the reaction was vigorous,

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sometimes splattering during the heating processes, when the digestion was carried out in the mixture of sulfuric acid (5 mL) and nitric acid (10 mL). The fluorescence intensity was 60.8 ± 4.7 under this condition. The incompleteness and vigorousness of digestion were prevented when the digestion was performed in the mixture of sulfuric acid (10 mL) and nitric acid (5 mL). The rate of digestion is also accelerated with the latter digesting mixture. The fluorescence intensity was 60.9 ± 3.1. These data indicate that sulfuric acid *per se* was unable to completely digest lipids. Mixtures of sulfuric and nitric acids in either ratio presented comparable digestive efficiencies; however, the former required longer time for a complete digestion. As a consequence, sulfuric acid-nitric acid in 2:1 (10 mL: 5 mL) ratio was selected as the digesting reagent.

### II. *Optimization of Atomic Fluorescence Spectrometer Conditions*

The principle of arsenic detection is to determine the atomic fluorescence emission intensities of gaseous arsenic hydrides, the reaction products of acidified arsenic and sodium borohydride. The efficiency of arsenic hydride formation significantly affects the analysis. To compare the effect of sodium borohydride, the reactions of arsenic (500 pg/mL) and hydrochloric acid (30%) with various concentrations of sodium borohydride solutions were performed. The fluorescence intensities were 40.3 ± 5.6, 96.3 ± 3.2, 93.4 ± 4.5, 91.5 ± 5.9, 70.3 ± 6.5 for 0.5, 1.0, 1.5, 2.0, 2.5% of sodium borohydride, respectively (Figure 1). As the data shown, reacted with 1% sodium borohydride emitted the strongest fluorescence. Although reacted with 1.5 and 2.0% sodium borohydride also provided strong fluorescence signals, the accuracy of the analysis was interfered by the strong diffusion flame formed.

To determine the effect of hydrochloric acid, arsenic (500 pg/mL) and sodium borohydride (1%) was reacted with various concentrations of hydrochloric acid. The fluorescence intensities were 92.6 ± 3.1, 98.7 ± 2.9, 53.7 ± 5.2, and 51.3 ± 6.7 for 25, 30, 35, and 40% hydrochloric acid (v/v), respectively (Figure 2). The results indicated that reacting with 30% hydrochloric acid emitted the strongest fluorescence. Reactions with 35 and 40% hydrochloric acid were too vigorous to reflect accurate analyses.

Argon is used as the carrier gas for atomic fluorescence spectroscopic detection. The major role played by this carrier gas is to deliver the volatile arsines to the burner head of the atomic fluorescence spectrometer for analysis. Therefore, the flow rate of the carrier gas directly affects the analytical results. Arsenic hydrides generated from the reaction of arsenic (500 pg/mL) with hydrochloric acid and sodium borohydride was transported to the burner head by argon flowed at various rates. The fluorescence intensities were 43.2 ± 3.1, 64.2 ± 3.3, 80.6 ± 3.7, 96.5 ± 2.5, 94.4 ± 4.1, 85.0 ± 3.6, and 75.4 ± 3.4 for argon at 100, 150, 200, 250, 300, 350, and 400 mL/min (Figure 3). The results showed that the highest fluorescence intensity was reached when the flow rate was at 250 mL/min. Carrier gas with this flow rate also exhibited the most stable diffusion flame so that it was the

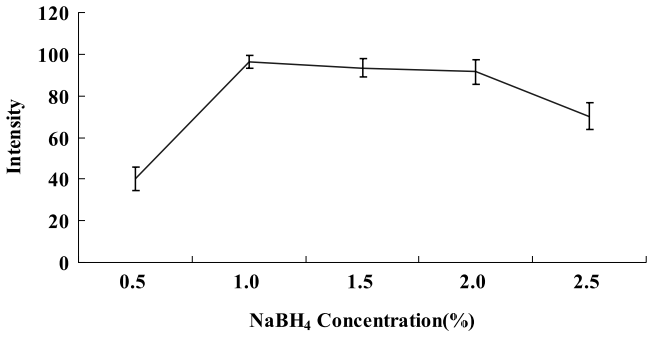


**Figure 1.** The effects of $NaBH_4$ concentration on the arsenic measurement by AFS.

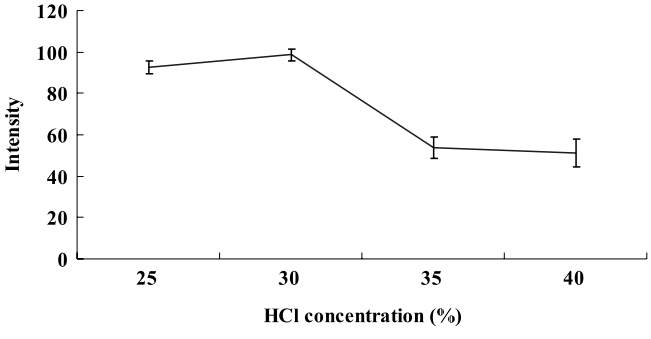


**Figure 2.** The effects of HCl concentration on the arsenic measurement by AFS.

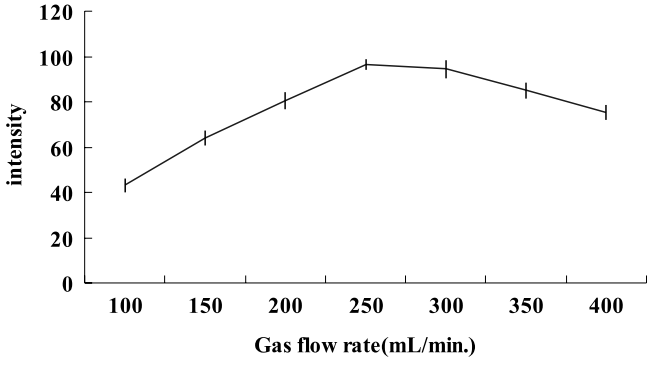


**Figure 3.** The effects of gas flow rate on the arsenic measurement by AFS.

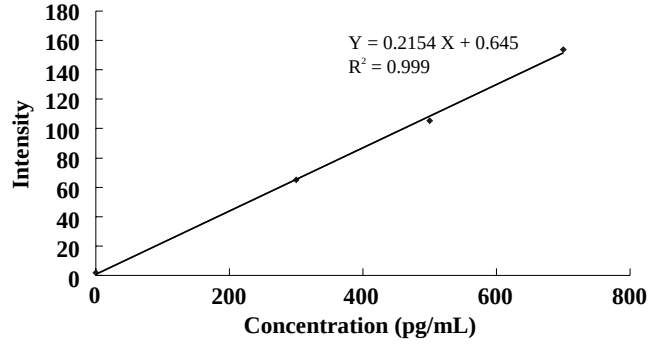


**Figure 4.** Calibration curve of arsenic .

selected flow rate for later analysis.

Standard arsenic working solutions of 300, 500 and 700 pg/mL were determined by atomic fluorescence spectrometer. The standard calibration curve plotted concentration verse fluorescence intensity was regressed to be Y=0.215X+0.645 with a square correlation coefficient of $r^2$=0.999 (Figure 4), indicating great linearity. The fluorescence intensity of standard arsenic working solution (500 pg/mL) underwent consecutive analyses for forty times and resulted in intensity of 106.6 ± 2.4 with a relative standard deviation of 2.3%, reflective of great precision. The instrumental detection limit was determined to be 50 pg/mL, which was calculated based on three times of the standard deviation of twelve replicates of the reagent blanks.

III. *Recoveries of Arsenic Spiked in Edible Fats and Oils*

One milliliter of various concentrations (0.025, 0.05, and 0.1 μg/mL) of arsenic standard were separately spiked into 0.5 g of palm oil, olive oil, and melted lard and digested with the optimized analytical conditions determined above, including the digesting acid mixture, the analytic reagents (reducing agent and acid solution), and the flow rate of the carrier gas argon. The digested samples were then analyzed for their arsenic contents to calculate the respective recoveries. Recoveries obtained from palm oil spiked with arsenic standards of 0.025, 0.05, or 0.1 μg/mL were 89.8 ± 3.5, 93.0 ± 3.2, and 94.7 ± 2.9%, respectively. Recoveries obtained from olive oil spiked with arsenic standards of 0.025, 0.05, or 0.1 μg/mL were 86.4 ± 4.1, 92.0 ± 3.7, and 96.9 ± 3.5%, respectively. Recoveries obtained from lard spiked with arsenic standards of 0.025, 0.05, or 0.1 μg/mL were 86.9 ± 4.1, 91.2 ± 3.5, and 91.5% ± 3.3, respectively (Table 2). These data suggest that the recoveries were excellent using the analytical conditions selected by the present study.

**Table 2.** Recoveries of arsenic spiked in palm oil, olive oil and lard

| Added(ppm) | Recovery(%) | | |
|---|---|---|---|
| | Palm oil | Olive oil | Lard |
| 0.025 | 89.8[a] ± 3.5[b] | 86.4 ± 4.1 | 86.9 ± 4.1 |
| 0.050 | 93.0 ± 3.2 | 92.0 ± 3.7 | 91.2 ± 3.5 |
| 0.100 | 94.7 ± 2.9 | 96.9 ± 3.5 | 91.5 ± 3.3 |

[a] Average of three determinations. [b] Average ± CV (%).

**Table 3.** Contents of arsenic in edible fats and oils

| Sample | No. of samples | As (ppm) |
|---|---|---|
| Peanut oil | 2 | 0.006, 0.027 |
| Sesame oil | 2 | 0.006, 0.011 |
| Olive oil | 2 | 0.006, 0.012 |
| Sunflower oil | 2 | 0.005, 0.006 |
| Salad oil | 3 | 0.006, 0.007, 0.012 |
| Teaseed oil | 1 | 0.006 |
| Butter | 1 | 0.009 |
| Lard | 1 | 0.007 |
| Corn oil | 1 | 0.011 |
| Winter oil | 1 | 0.011 |
| Canola oil | 1 | 0.013 |
| Vegetable oil | 1 | 0.013 |
| Palm oil | 1 | 0.025 |

IV. *Arsenic Content in Edible Fat and Oils*

Edible fats and oils on the market were analyzed for their arsenic contents by atomic fluorescence spectrometer with conditions optimized as described. The quantitative results detected in the samples were as following: 0.006 and 0.027 ppm for peanut oil, 0.006 and 0.011 ppm for sesame oil, 0.006 and 0.012 for olive oil, 0.005 and 0.006 ppm for sunflower oil, 0.006, 0.007 and 0.012 ppm for salad oil, 0.006 ppm for teaseed oil, 0.009 ppm for butter, 0.007 ppm for lard, 0.011 ppm for corn oil, 0.011 ppm for winter oil, 0.013 ppm for canola oil, 0.013 ppm for vegetable oil, and 0.025 ppm for palm oil (Table 3). These were all below the maximum permissible concentration (0.1 ppm), and were in agreement with Tsai *et al.*[10] who reported 0.02 (0.01-0.03) ppm for soybean oil, 0.02 (0.01-0.02) ppm for corn germ oil, 0.01 (N.D.-0.02) ppm for peanut oil, and 0.01 (N.D.-0.02 ppm) for sesame oil.

## CONCLUSIONS

Taken together, the established operation protocol for arsenic analysis for edible fat and oil samples were as following: weigh 0.5-1.0 g of sample to digestion vessel and add 10 mL of sulfuric acid and 5 mL of nitric acid into the sample-containing vessel, followed by heat digesting the sample with microwave digester. Add hydrogen peroxide midway to assist digestion until the digestion mixture turns clear. Dilute the digest to 20 mL with water using a volumetric flask. Add 1 mL of 40% KI to 10-mL aliquot of the diluted digest and let stand for 30 min. React with 30% (v/v) hydrochloric acid and 1% (w/v) sodium borohydride. Finally perform the analysis for arsenic determination by atomic fluorescence spectrometer.

Digesting edible fats and oils with focused microwave digester has the advantages of time-saving and more complete digestion. In addition, the atomic fluorescence spectrometer has high sensitivity and it only takes approximately 5 min for a complete analysis of an individual sample. The method described herein coupled the focused microwave digester and atomic fluorescence spectrometer, which demonstrated features of a rapid, safe and accurate analysis of arsenic in edible fats and oils. The arsenic contents of market-available fats and oils analyzed by the present study were minimum, all below the maximal permissible standard of 0.1 ppm for edible lipids.

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# 以聚焦式微波消化法及原子螢光光譜儀分析食用油脂類中砷含量

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## 摘　要

本研究探討以聚焦式微波消化器（Focused Microwave Digester）前處理，及原子螢光光譜儀（Atomic Fluorescence Spectrometer）檢測食用油脂類中砷含量。將檢體0.5 g加硫酸10 mL與硝酸5 mL，以聚焦式微波消化器加熱消化，再以原子螢光光譜儀檢測，儀器檢出限量為50 pg/mL。添加砷0.05，0.10及0.20 ppm於棕櫚油中，其回收率分別為89.8、93.0及94.7%，於橄欖油中回收率為86.4、92.0及96.9%，於豬油中其回收率為86.9、91.2及91.5%。分析市售食用油脂21件，檢出範圍為0.005　0.027 ppm，均低於食用油脂類衛生標準之最大容許量（0.1 ppm）。

關鍵詞：聚焦式微波消化法，原子螢光光譜儀，食用油脂類，砷