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Evaluation of Arsenic Concentration in Poultry and Calf Meat Samples by Hydride Generation Atomic Fluorescence Spectrometry

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Highlights

- Determination of arsenic at trace level by simple and cost effective method.
- Arsenic concentration was significantly higher in calf meat samples than poultry samples.
- Daily intake of total arsenic via calf meat was almost two times higher than via poultry meat.

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Abstract

A simple, cost effective hydride generation atomic florescence spectrometry (HG-AFS) method was used for determination of total arsenic (As) in poultry and calf meat samples. The samples were digested in long necked glass digestion tubes using concentrated HNO_3, $HClO_4$ and H_2SO_4 as a mixture. The volume of acids (HNO_3, $HClO_4$) and the amount of sample to be used for digestion were optimized to achieve appropriate digestion. The accuracy of the proposed HG-AFS method was tested with certified reference material (DOLT 3 Dogfish Liver, NRC, Canada) and obtained results were in good agreement with certified value. The method limit of detection (LOD) value was calculated as 0.3 ng/g and dynamic range was 25 – 5000 pg/ml. Arsenic concentrations of poultry and calf meat samples were determined accurately by using aqueous calibration standards. Totally 31 samples (calf, chicken and turkey) obtained from local markets were analyzed. It was found that the average As concentration in calf meat (12.1 ± 3.9 ng/g) was significantly higher than the poultry samples whereas the arsenic concentrations were similar in turkey (3.1 ± 1.2 ng/g) and chicken (2.8 ± 1.1 ng/g) samples. In addition, dietary intake estimation of arsenic through consumption of calf and poultry meat was calculated and according to the gathered results daily intake of arsenic via calf meat was almost two times higher than poultry meat.

1. INTRODUCTION

Heavy metals are toxic substances for living organisms and with the influence of industrialization, the risk of heavy metal exposure for humans' increases rapidly. Amongst the heavy metals, arsenic naturally occurs in the environment, especially in water and soil [1]. Thus, human exposure to arsenic generally occurs via consuming drinking water and foods. It is a well-known fact that arsenic is toxic to humans and arsenic toxicity is related with its chemical form and organic arsenicals are less toxic than inorganic arsenic (i-As) species [2]. In addition, oxidation state is an important parameter since toxicity depends on the oxidation state of arsenic which +3 oxidation state arsenicals are more toxic than +5 oxidation state arsenicals.

The absorption of inorganic arsenic species takes place via the gastrointestinal path, and the metabolism of i-As species occurs through methylation. Methylated arsenicals such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are formed as the result of methylation process [3]. In 2014, European Food Safety Authority (EFSA) evaluated dietary exposure to i-As and a benchmark dose level (BMDL): 0.3–8 µg/kg body weight per day was designated for an increased risk of various types of cancer as well as for skin lesions [4, 5]. Under these circumstances, to determine arsenic at ppb or sub ppb concentration is important in consumer products, especially in food products. Roxarsone (ROX) which is a phenylarsenic compound, had been used in poultry production in a widespread manner as a feed additive, for the purpose of preventing diseases and increasing weight gain [6]. ROX is a compound that has low toxicity; however,

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the studies showed that its continuous usage could lead toxicity since it is transformed into i-As in chicken tissues [7, 8].

The atomic techniques such as inductively coupled plasma mass spectrometry (ICP-MS) [9-15], hydride generation atomic absorption spectrometry (HG-AAS) [16-21] and hydride generation atomic fluorescence spectrometry (HG-AFS) [22-25] are widely used to determine arsenic in various samples. It is well known that the use of HG can improve the sensitivity and the selectivity in arsenic determination. In addition, atomic techniques coupled with HG allow either total arsenic determination or speciation of arsenic species. Amongst the atomic techniques, the sensitivity of HG-AFS is similar to ICP-MS and better than HG-AAS. Also, simplicity and low operational costs of HG-AFS compared to ICP-MS results in widespread usage of this technique for arsenic determination at trace levels. The drawback of ICP-MS is the polyatomic interferences due to matrix components of the sample. Conversely, hydride forming analytes such as arsenic, selectively separated from matrix components during hydride generation step, as a consequence interferences related to matrix components are avoided at HG-AFS technique. On the other hand, arsenic speciation analysis can be achieved by coupling high performance liquid chromatography (HPLC). In a recent study, Saucedo-Velez et al. [26] used microwave assisted extraction method and determined phenyl arsenicals in feed samples by HPLC-HG-AFS. According to their results, only *p*-arsanilic acid (0.72 - 12.91 mg/kg) was determined among the analyzed arsenic species in feed samples. Nachman et al. compared i-As concentrations in various chicken samples and emphasized that conventional chicken samples had higher arsenic concentrations than the other samples. Also according to their results, ROX concentrations decreased and i-As concentrations increased when the samples were cooked which showed that the cooking process had influence on arsenic forms [27, 28]. Hu et al. used HPLC-ICP-MS for speciation analysis of inorganic and organic arsenic species in chicken meat and both roxarsone and inorganic arsenic were detected at notably high amounts in chicken samples [29]. In an another study, cigarette filter was utilized as an adsorbent and coupled with HG-AFS for determination of total arsenic in various food samples such as vegetables, rice, chicken and fish [20]. The limit of detection (LOD) of the method in food samples was differed in the range of 2.5 to 9.9 ng/g. Perello et al. examined the influence of cooking processes on the concentration of arsenic by using ICP-MS and according to the results, As concentration in meat samples decreased after cooking processes while the concentration of As in chicken samples does not differ [30].

In this study it was aimed to determine total arsenic concentrations in poultry and calf meat samples obtained from commercial markets in Turkey. With this purpose, cost effective and an easy analytical procedure based on HG-AFS was used. In addition, the amount of total arsenic intake from consumption of meat products was estimated.

2. MATERIAL METHOD

2.1. Instrumentation

Millennium Excalibur atomic fluorescence spectrometer coupled with continuous flow hydride generation system (PS Analytical Ltd., United Kingdom) was used for arsenic determination. An arsenic boosted-discharge hollow cathode lamp was used and the fluorescence signal intensity of As was measured at 193.7 nm.

2.2. Reagents and Materials

Unless otherwise stated, ultra pure water was used for the preparation of the solutions. All the chemicals used throughout the experiments were of analytical reagent grade. Arsenic species in the samples were oxidized to arsenic (V) during the digestion step therefore, calibration standards (0, 25, 50, 100, 250, 500, 1000 pg/mL) were prepared from 1000 mg/L As (V) standard (CertiPUR Merck, Germany) daily by appropriate dilutions. As a consequence, the hydride generation efficiency of the samples and calibration standards were identical. The final concentration of HCl (Merck) and H_2SO_4 (Merck) in calibration standards were 1.5 mol/L and 0.45 mol/L, respectively so that final acid concentrations in the samples and the calibration standards did not differ. The reducing reagent $NaBH_4$ (min. 96% purity, Aldrich) was prepared in 0.4% (m/v) NaOH (Aldirch) for hydride generation. The validation of the method was performed by using certified reference material (CRM) DOLT 3 Dogfish Liver (NRC, Canada). In order to

transport generated arsenic hydrides from gas-liquid separator to the atomizer argon (Ar, high-purity grade) was used as carrier gas and to avoid moisture accumulation nitrogen (N_2) was used as dryer gas at HG-AFS instrument.

2.3. Determination of Arsenic by HG-AFS

The oxidation state of arsenic affect the sensitivity because the hydride generation efficiency of arsenic species are different from each other and the sensitivity of As (III) is higher than As (V). Therefore, in order to achieve lower LOD values, As (III) is more convenient than As (V). Nevertheless, when calibration standards were prepared from As (V) the sensitivity was still sufficient to determine arsenic at trace level in meat samples. Thus, the samples were analyzed without using a pre-reduction step and the sample preparation step was simplified.

Arsenic hydride was generated with continuous flow hydride generation manifold by using $NaBH_4$ at a concentration of 1.4 % (m/v) as a reducing agent and 1.5 mol/L HCl as a carrier solution. The sample, carrier and reducing solutions were pumped (4.0 mL/min) simultaneously into the sample valve for mixing. Depending on the valve position, whether sample or carrier solution was mixed with $NaBH_4$ via using a PTFE tubing (0.8 mm i.d., 20 cm in length) and the other goes off to waste. After mixing with $NaBH_4$, gaseous and liquid phases were separated at gas-liquid separator, where the generated arsenic hydride was transferred to the diffusion flame atomizer (H_2-Ar). The peak area of fluorescence signal was used for quantification and the optimized parameters for HG-AFS system are summarized in Table 1. The quantification of arsenic concentration in meat samples was achieved with aqueous calibration standards.

Table 1. *HG-AFS method parameters*

Hydride generation	
$NaBH_4$ concentration, m/v	1.4 % in 0.4 % NaOH
HCl concentration, mol/L	1.5
Solution flow rate, mL/min	4.0
Mixing coil length, cm	20
Atomic fluorescence spectrometer	
Carrier gas flow rate, L/min	0.25
Dryer gas flow rate, L/min	2.5
Wavelength, nm	193.7
Acquisition time, s	30
Signal integration	Peak area

3. RESULTS AND DISCUSSION

3.1. Optimization of Acid Concentrations and Sample Amount for Open Vessel Digestion

Digestion of samples was achieved by slight modification of the open vessel digestion procedure which was proposed by Welz et al. [31]. Before digesting the poultry and calf meat samples, DOLT 3 CRM was used in order to find out the optimum acid volumes for digestion procedure. Thus, to achieve complete digestion, volume of concentrated HNO_3 and $HClO_4$ was varied between 2.0 - 5.0 mL and 0.5 - 1.5 mL, respectively. As shown in Figure 1 the volume of HNO_3 is more important than $HClO_4$ volume for digestion of the samples. Also at least 3.0 mL of concentrated HNO_3 is needed for complete digestion. However, when 3.0 mL HNO_3 was used for digestion, the accuracy of the digestion was appropriate but the precision was not proper. Therefore, 4.0 mL HNO_3 was used throughout the experiments. The effect of $HClO_4$ was significant when HNO_3 was used less than 3.0 mL, but when HNO_3 was used either 4.0 or 5.0 mL the effect of $HClO_4$ volume was insignificant, only the standard deviation values were improved. Thus, 1.0 mL $HClO_4$ was chosen as the optimum value for the digestion procedure.

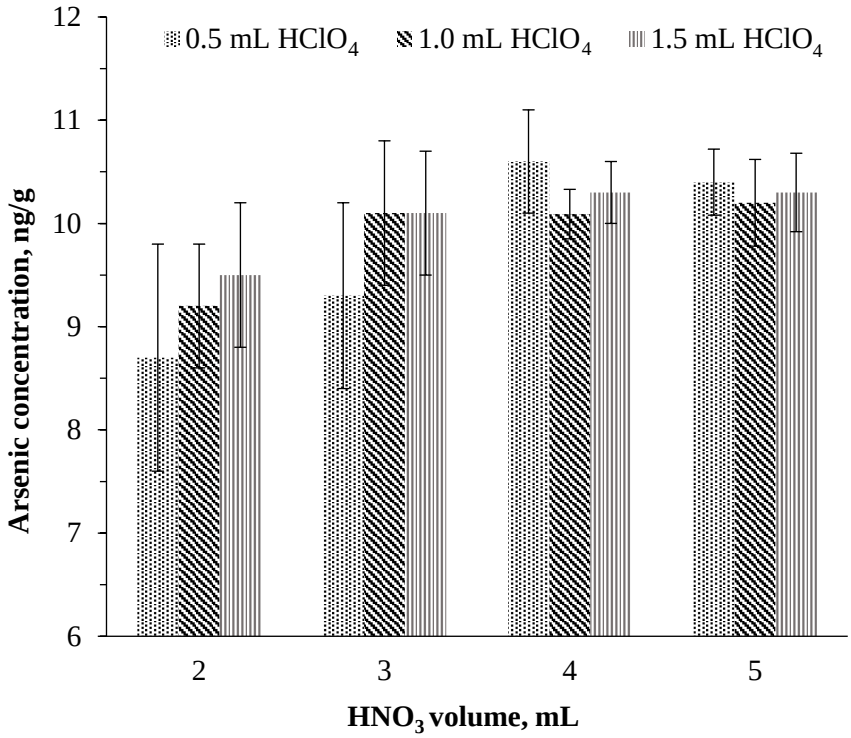

Figure 1. *Optimization of HNO_3 and $HClO_4$ volume for digestion of samples, (n=3)*

Since the amount of poultry and meat samples used for digestion were higher than DOLT 3 CRM; different weighed amounts (100, 300, 500, 700 mg) of a randomly selected poultry sample was weighed and the same digestion procedure was applied to ensure that the samples were digested properly. According to the obtained results, arsenic concentration was consentient for 100, 300, 500 and 700 mg weighed samples (Figure 2). Therefore, it was concluded that the amount of acids and the digestion procedure was adequate to digest the samples.

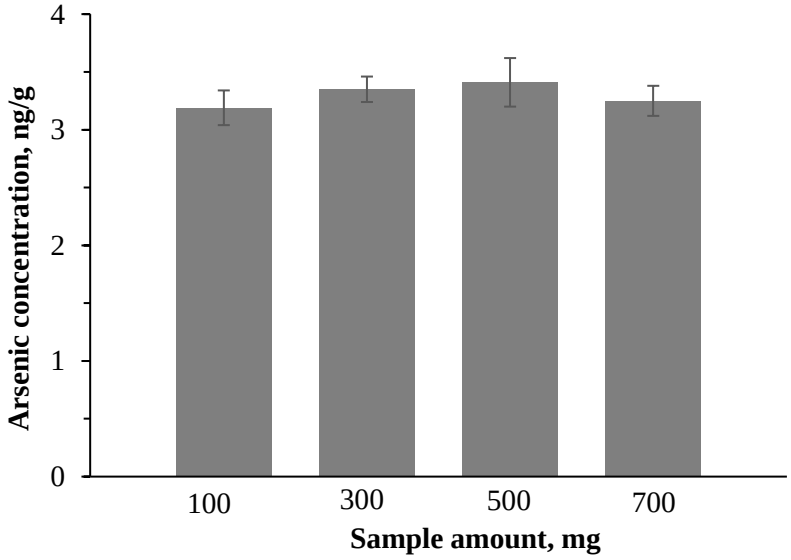

Figure 2. *The effect of sampling weight amount for digestion procedure, (n=3)*

3.2. Sample Preparation and Open vessel Digestion Procedure

The meat samples were placed in long-necked glass digestion tubes and digested in a thermostatic heating block, (Grant Instruments, United Kingdom). Each sample was homogenized and 300-600 mg was weighed from each homogenized sample and transferred into long-necked digestion tubes and 4.0 mL HNO_3 was added. Temperature was increased to 80°C and stayed constant for 15 min., thereafter temperature was raised to 130°C and remained 15 min. Then, 1.0 mL $HClO_4$ was added and heated up to 170°C for 15 min. After cooling down, 0.5 mL H_2SO_4 was added and the temperature raised to 230°C. The temperature stayed constant at 230°C for 15 min then, the temperature raised to 300°C and stayed for 30 min thereby, HNO_3 and $HClO_4$ were evaporated. After cooling to room temperature, digested samples were diluted to 10 mL with ultrapure water. From this digested sample, 5 mL was taken and after adding 1.25 mL concentrated HCl, it was diluted to 10 mL. So that the final HCl and H_2SO_4 concentrations of the digested samples were 1.5 mol/L and 0.45 mol/L, respectively. All the samples were digested in triplicate.

In order to digest DOLT 3 CRM, the same digestion procedure that was described above was applied with slight modifications such as, reducing the weighed amount (100 mg) and increasing the dilution volume because of its high arsenic concentration.

3.3. Analytical Performance Parameters of HG-AFS Method

The analytical performance parameters of HG-AFS method were shown in Table 2. The method limit of detection (LOD) and limit of quantification (LOQ) values were calculated as 3.3 and 10 times the standard deviation (SD) of 10 consecutive measurements of the sample blank signal, respectively and divided by the slope of the calibration curve. The LOD and LOQ value of the method was 0.3 ng/g and 1 ng/g, respectively. The repeatability of the method was determined by using the calibration standard with the lowest arsenic concentration (25 pg/mL), and in terms of RSD, repeatability was calculated less than 3%.

Table 2. *Analytical performance parameters of HG-AFS*

Parameter	**Value**
Dynamic range, pg/mL	25 – 5000
Calibration equation	y = 8.89[As] + 138.4 (R^2 = 0.9996)
RSD, %	1 – 3
LOD, ng/g	0.3
Sampling frequency, h^{-1}	50

Certified reference material (DOLT 3 Dogfish Liver) was used in order to check the accuracy of HG-AFS. The CRM was digested in triplicate with the same procedure, which was described previously, and aqueous calibration standards were used to calculate the arsenic concentration. When the certified and the calculated concentrations were compared, the results were in good agreement with the certified value at 95 % confidence level where the certified value was 10.2 ± 0.5 mg/kg and the calculated value was 10.3 ± 0.4 mg/kg.

3.4. Determination of Arsenic in Samples

Arsenic concentration of 31 poultry and calf meat samples which were purchased from local stores was determined with the proposed HG-AFS method. Among the samples 11 of them was calf, 14 of them was chicken and 6 of them was turkey samples. As shown in Table 3, As concentration in calf meat is significantly higher than the poultry (chicken and turkey) samples and the arsenic concentration in turkey and chicken samples are almost the same. Amongst the published studies, determination of arsenic concentration in chicken samples obtained from Turkish market is very limited. Kaya et al. [32] evaluated seasonal variations of arsenic concentration in chicken samples and arsenic concentration in breast tissues was found 3-14 ng/g. In another study, arsenic concentration was varied in between 60-100 ng/g in various parts of chicken samples [33]. Yaman and Akdeniz determined arsenic in poultry samples and arsenic concentration was found to be 290 ± 30 ng/g [34].

Table 3. *Arsenic concentration levels of poultry and calf meat samples, (n=3)*

Sample	Concentration, ng/g	Concentration, ng/g	Concentration, ng/g
	Calf	**Chicken**	**Turkey**
1	16.1 ± 1.5	2.7 ± 0.2	4.9 ± 0.7
2	14.2 ± 2.0	3.5 ± 0.3	3.8 ± 0.8
3	4.8 ± 1.1	2.4 ± 0.3	3.3 ± 0.2
4	11.1 ± 0.9	2.4 ± 0.2	2.0 ± 0.5
5	15.1 ± 1.2	1.1 ± 0.5	3.4 ± 0.5
6	11.3 ± 0.6	2.3 ± 0.6	1.7 ± 0.1
7	5.5 ± 0.9	2.9 ± 0.1	
8	15.3 ± 0.8	5.6 ± 0.7	
9	14.1 ± 0.6	1.5 ± 0.6	
10	15.3 ± 2.5	3.7 ± 0.6	
11	11.1 ± 0.8	3.7 ± 0.4	
12		3.0 ± 0.1	
13		1.4 ± 0.2	
14		3.1 ± 0.1	

3.5. Dietary Exposure Estimation of Arsenic

According to the official OECD/FAO report, which includes the average consumption of meat products per person in Turkey stated that poultry and calf meat consumption is 53 g and 23 g per day, respectively [35]. Thus, the estimation of arsenic dietary intake via meat products was calculated by using the data given in OECD/FAO official report and multiplying the mean As concentration of each food by its mean consumption in Turkey. According to our results, daily intake of total arsenic via calf meat is almost two times more than via poultry meat (Table 4).

Table 4. *Daily intake of arsenic by consumption of calf and poultry products*

Product	Consumption (g/day)	Arsenic intake (ng/day)
Calf meat	23	279
Poultry meat	53	155

4. RESULTS

In this study, determination of total arsenic concentration in poultry and calf meat samples was achieved with a cost effective and an easy HG-AFS method. Since the sensitivity of HG-AFS is high, LOD value at ng/g level can easily be achieved. Therefore, after the samples were digested, total arsenic concentration could be determined directly by appropriate dilution of the samples without using any preconcentration step. Depending on the results, the arsenic concentrations of calf meat samples were significantly higher than the poultry samples, whereas the concentrations in turkey and chicken samples were almost the same.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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