Determination of Carbon Monoxide in Tuna by Gas Chromatography with Micro-Thermal Conductivity Detector

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Abstract

The suitability of a portable gas chromatograph equipped with a micro-thermal conductivity detector for the head-space determination of carbon monoxide (CO) in tuna samples is evaluated; CO is estimated after its liberation from tissue by acidic treatment at 70°C. Using the tested technique, the CO contents in untreated and suspected treated samples are analyzed. A limit of detection of approximately 13 ng/g is reached. The results demonstrate that this apparatus has performances similar to more expensive and sophisticated instruments.

Introduction

Fish products treated with carbon monoxide (CO), not allowed by the current rules, are actually present on the European markets (1,2,3). Therefore, an analytical method for distinguishing the physiological CO content from that resulting from CO treatment is helpful for surveillance systems. Head-space sampling of CO followed by gas chromatography (GC) analysis, which has previously been used to determine CO in blood and tissues (4,5,6), was adopted for its determination in fish samples as well (7,8). A recent work states that better-releasing CO yields were obtained using a sulphuric acid solution instead of a potassium ferrycianide solution, and by direct treating of flesh homogenate instead of preliminary extraction of CO-myoglobin (7). According to the procedures introduced for blood and tissue analysis, different detection techniques have also been proposed for quantitating the CO liberated from the tuna muscle. The lowest detection limit was reached with a nickel catalyst system placed before flame ionization detection (8). On the other hand, an unsurpassable selectivity was achieved by mass spectrometry (MS) detection (7).

The thermal conductivity detector (TCD), which is the simplest and cheapest detection technique, was used to determine CO in blood only (4), and no studies on tuna are actually reported.

The aim of this work was to test the ability of a recently introduced micro-machined TCD on a portable GC apparatus (9) to reach the sensitivity of the more expensive MS detector for quantitating the endogenous CO content in tuna. The sample preparation already introduced for CO GC–MS analysis in tuna (7) has been followed with only slight modifications with a view to reduce the specimen amounts and reagent volumes used before GC–TCD. This new method was applied to the analysis of *Thunnus albacares* fillets.

Materials and Methods

Samples and materials

All tuna samples of *Thunnus Albacares* were purchased from local trade market. Three dorsal fillet aliquots from an entire subject were withdrawn and used as untreated samples. Suspected CO treated samples were vacuum-packed frozen fillets from Indian Ocean.

Head-space vials of 20-mL were purchased from National Scientific Company (Quakertown, PA). All reagents used were of analytical grade.

Apparatus and chromatographic conditions

The chromatographic system was from Varian (Palo Alto, CA) and composed of a Micro GC CP-4900 equipped with an automatic injection system, a micro TCD, a 10 m × 0.25 mm column containing the Molecular Sieve 5 Å stationary phase (MSA), and StarWS mod. 6.2 software. The injection time and temperature were 250 ms and 70°C, respectively, corresponding to a final injection volume of nearly 10 µL. The column temperature was maintained at 80°C.

Sample preparation

The recently introduced procedure (7) was modified by reducing 20 times both the sample weight and reagent volume.

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Briefly, approximately 100 g of frozen tuna specimen were homogenized for 30 s, then a 2 g aliquot was placed in a vial, followed by 4.2 mL of water and 5 µL of octanol. 0.5 mL of 5M sulphuric acid was then added to the vil, previously capped and shaken, using a syringe to reach a final volumne of 6.7m:. The vial was shaken again, heated at 70ºC for 1 h and allowed to cool at room temperature before the head-space was analyzed by the GC apparatus.

Calibration curve

The mixtures containing known amounts of CO were prepared according to the procedure of the previously cited work (7): first, pure CO was bubbled in vials by displacing water, and then stock gaseous mixtures were prepared by transferring pure CO aliquots by gastight syringe to closed vials containing air. The standard mixtures for the calibration curve were then obtained by adding stock mixture aliquots in closed vials containing a solution volume equal to that indicated in the sample preparation section. In fact, 2 mL of additional water were used instead of tuna homogenate, as in our preliminary experiments we measured the specific weight of tuna homogenate, which resulted in 1.00 g/mL ± 0.03 g/mL (n = 5, 25°C). Using this procedure, six different standard mixtures in the range 33.9 ng–6342.5 ng were prepared and analyzed in triplicate.

Results

In the tested range, the relationship between the peak areas (µvolt × s) and the CO amounts (ng) was linear (r^2 = 0.9999) with the following regression parameters: slope (b) = 0.1091 ± 0.0001, intercept = 0.0451 ± 0.2248, and residual standard deviation ($\sigma_{v/x}$) = 1.671.

According to a simple theoretical approach (10), the limit of detection (LOD = $3\sigma_{y/x}b^{-1}$) and limit of quantitation (LOQ = $10\sigma_{y/x}b^{-1}$) resulted as 13.28 ng and 44.28, respectively.

In Figure 1, the profiles corresponding to untreated and suspected treated samples are shown. The repeatability on a CO content of 72.8 ng/g was measured by analyzing untreated sample (RSD % = 11.8, n = 5). In Table I, the contents found in the analyzed suspected tuna samples are reported.

Discussion

Due to its low sensitivity, TCD has not been not used until now for the determination of CO in food. Recently, a new portable micro-GC equipped with a miniaturized TCD was introduced for gas analysis in environmental fields (9). The main novelties of this apparatus were the micro-machined injector, with no moving parts, the chip detector, with internal volume of 200 nL, and the new designed electronics, giving a very low electronic noise. In this work, a micro-GC was applied for the first time to the determination of CO in fish. Regarding the analytical conditions with respect to the most recently reported GC method on CO determination in tuna (7), the significant difference introduced was the use of the microTCD on the portable GC instead of the MS detector. In the described conditions, the detection suitability appeared satisfactory; in fact, only approximately 44 ng of CO were the theoretical LOQ, while the lowest quantitated amount in the already cited work (7) was 1 µL of CO corresponding to 1158 ng at 22°C. Thus it is not necessary to use an expensive GC–MS apparatus to reach the high sensitivity needed for regulatory purposes. The CO content found in the analyzed untreated tuna sample resulted, as expected, lower than the 200 ng/g value, which was considered the accepted limit for the CO physiological content (8). On the other hand, this value was clearly higher than the LOQ of the micro-TCD technique. Therefore, the method presented here appears suitable for distinguishing the untreated samples from the treated. In the analyzed samples of *Thunnus Albacares* from Indian Ocean, CO contents higher than 200 ng/g were found, confirming the frequent use of CO in those countries. In conclusion, considering its lower price and portability, the apparatus used is more convenient than GC–MS instruments for CO determination in either technological studies or inspection investigations.

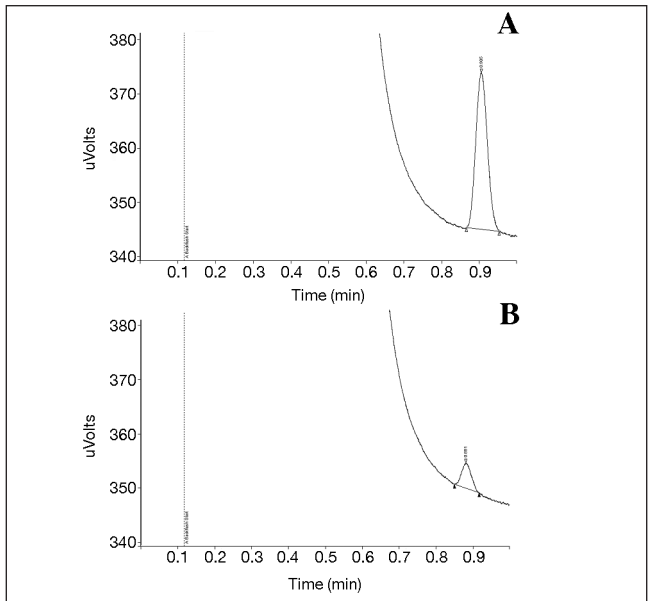

Figure 1. Chromatographic profiles corresponding to a sample from a vacuum packed frozen fillet (A) and to an untreated sample from an entire subject (B). The resulting CO contents were 559.5 ng/g and 72.8 ng/g, respectively.

Table I. CO Contents in *Thunnus Albacares* Samples*

Sample	CO content (ng/g)†
1	525.0
2	559.5
3	744.8
4	660.0
5	558.4
6	351.8

* All samples were vacuum-packed frozen fillets from the Indian Ocean.
† Mean of three determinations.

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