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Determination of Formaldehyde and Methanol Contents in Canned Food and Drinks Samples: Application of Headspace Gas Chromatography and Industrial Reliance

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Abstract

This study presents a headspace gas chromatographic (HS-GC) method for the simultaneous determination of formaldehyde and methanol in canned food and drinks samples, such as pickle, fresh meat or stored fruit juices etc. The method was based on the reaction between formaldehyde and sodium borohydride in a concentrated potassium carbonate solution, in which formaldehyde was quantitatively converted to methanol and determined by HS-GC. By measuring the methanol content in the sample before and after the reaction with borohydride, both methanol and formaldehyde contents can be determined. By spiking a given amount of isopropanol in the tested sample solution, the uncertainty in above two separate measurements by HS-GC can be significantly reduced when using isopropanol signal as a reference in the internal standard calibration. Results showed that the present method had a good accuracy (with a recovery ranging from 94 – 103 %). The limit of quantification (LOQ) of the method is 0.835 mmol/kg. This method is straightforward, reliable, and suitable for the high-throughput monitoring of these common water pollutants, offering a valuable tool for environmental quality control and pollution assessment.

Introduction

Canning is one of the popular methods in food preservation. The basic process of canning consisting of placing food in sealable containers, closing, heating and cooling to destroy essentially all micro-organisms present and to prevent re-contamination. So, head space in a canned product plays an important role in proper food preservation. Most fruits, vegetables, a wide variety of meat and meat products, fish products, soup and many other items are all canned.

In normal canning, heat transfer from the outside of the container to the inside will require many minutes or even hours depending upon the container size to reach the sterilization temperature. It can be shortened to second or even fraction of a second in aseptic canning. Canned food may be spoiled due to improper temperature for sterilization, inappropriate time of heating, sealing is not done properly, rusting and damage may have taken place, corrosion by a food may lead to leakage or infected cooling water may result in contamination. As a result, different types of spoilage like Flat sour, Toxic Spoilage, H_2 - smell, Botulism, Metallic salt may occur in canned foods. Nutritive value of canned food is almost similar to that of fresh foods. Except there are some nutrient losses like - canned meat losses Vit B complex when storage temperature reaches 28°C or higher, during blanching Vit. C may be lost, when the sterilization temperature is very high, protein quality of meat and fish may be denatured.

The enhancing of shelf life canned food can be done by proper sterilization with temperature, hermitic sealing, and lowering water activity in canned food product, proper storage conditions and temperature.

In the era of modernization and busy hectic life and work schedules of civilians. To cope up with its canned food products have gained much more importance and are playing a crucial role nowadays.

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Formaldehyde and methanol are prevalent environmental contaminants frequently detected in various food items, including industrial food colors, frozen meat, and even in cold drinks [1, 2]. Formaldehyde is extensively used as a chemical intermediate in the production of resins, adhesives, and industrial disinfectants, leading to its potential misuse into canned food items for long shelf life [3]. Methanol is commonly employed as an industrial solvent, antifreeze agent, and a component in various fuel additives, making its way into the environment through similar pathways [4, 5]. Due to their toxicity and potential health impacts, the presence of these compounds in canned food is a significant concern. Formaldehyde is classified as a probable human carcinogen [6], while methanol exposure, though primarily a risk through ingestion, indicates general food quality deterioration and can be harmful to hu-man health [7, 8]. Consequently, regulatory agencies worldwide have established guidelines limiting their concentrations in food items [9, 10]. For instance, the World Health Organization (WHO) recommends a guideline value for formaldehyde as strictly prohibited in food items as preservative and environmental protection agencies often set stringent limits for industrial effluents.

Formaldehyde is an instable species in gas phase at temperatures < 150°C, because the polymerization between formaldehyde molecules can be easily taken place to form its dimers or trimers, or it can react with moisture in air to form methylene glycol [11]. These chemical reactions are neither predicable nor complete, making it impossible to indirectly quantify the formaldehyde based on the resulting species. For these reasons, the derivatizing formaldehyde with either hydrazine or other reagents is widely used in many analytical methods [12-16]. The best-known method applied to the aqueous environmental products is prob-ably the one based on the reaction of formaldehyde with acetylacetone and ammonia to form a coloring compound, which is measured by the colorimetry at 411 nm [17]. The major problem of the method is that it is not suitable for the samples having colors (e.g. colored drinks), be-cause the measurement error cannot be simply offset by running a separate blank testing [18-20]. Therefore, the separation-based technique such as high-performance liquid chromatography (HPLC) [12-14] and gas chromatography (GC) [15, 16] are the typical method used in the formaldehyde analysis, after sample pretreatment including the use of derivatizing agent, e.g., dinitrophenylhydrazine.

For methanol analysis, many methods such as chronotropic acid Colori-metric [21], titrimetric [21], enzymatic [22], biosensor [23], near-infrared spectroscopy [24], HPLC [25], and GC [10] methods have been developed. Because of the complicated sample compositions in aqueous environmental products, only the separation based HPLC and GC methods can provide the accurate analysis in methanol quantification. Similar to those met in the formaldehyde analysis, the sample pretreatment procedures such as distillation, membrane filtration and/or derivatization must be applied [10, 24]. Obviously, these pretreatment procedures involved in both formaldehyde and methanol analysis are very complicated and time-consuming, which not only make the meth-ods less efficient but also introduce significant experimental errors in the testing.

Because of requiring little or no sample pretreatment, headspace gas chromatography (HS-GC) becomes an efficient tool that has been widely applied to the analysis of volatile species in samples with com-plex matrices [26]. Previously, we have successfully developed a number of HS-GC methods for the determination of methanol in pulp mill effluents and paper materials [27, 28]. Recently, we have developed a HS-GC method [29-32] for indirect determination of formaldehyde content in paper materials [33], based on the reaction between formaldehyde and sodium borohydride (in an alkaline medium) to form methanol [34], i.e.,

$$4CH_2O + BH_4^- + 2OH^- + H_2O = 4CH_3OH + BO_3^{3-} \qquad (1)$$

The converted methanol from the above reaction can be easily deter-mined by HS-GC.

It is expected that the above method can be also simply applied to the analysis of not only methanol but also formaldehyde by measuring the methanol contents in the sample solutions before and after Reaction (1). However, because the methanol content in aqueous environmental products could be much higher than that of formaldehyde (e.g., > one magnitude), the measurement accuracy is a major problem in the formaldehyde analysis.

In this paper, we proposed a reaction-based HS-GC technique coupled with an internal standard calibration (using isopropanol) to determine the contents of both formaldehyde and methanol in aqueous environ-mental samples. The major focuses were to investigate the sample ex-traction conditions, establish an internal standard calibration for minimizing the measurement error. The accuracy of the present method was also evaluated.

Experimental

❖ Samples

All chemicals used in the experiment were reagent-grade purchased from commercial sources, including methanol, formaldehyde and iso-propanol. A standard methanol solution (1000 mg/L) was prepared by adding 20 μL of methanol to 20 mL of distilled water. The concentration of formaldehyde in a commercial canned food derived aqueous solution (~37% by weight) was accurately determined by iodometry. A standard formaldehyde solution (440 mg/L) was prepared by adding 1.0 g of formaldehyde to 1 L of distilled water. A NaBH4- isopropanol solution (5 g/L of NaBH4, 100 mg/L of isopropanol) was prepared by adding 0.5 g of sodium borohydride and 0.1 mL of an isopropanol stock solution (100

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g/L) to 100 mL of a salt solution containing 600 g/L of K_2CO_3. A isopropanol solution (100 mg/L of isopropanol) was prepared by adding 0.1 mL of the above isopropanol stock solution to100 mL of a solution containing 600 g/L of K_2CO_3.

❖ Apparatus and operations

HS-GC measurements were carried out with an automated headspace sampler (DANI HS 86.50, Italy) and a GC system (Agilent GC 7890A, US) equipped with a flame ionization detector and a DB-5 capillary column (with an i.d. of 0.53 mm and a length of 30 m) (J&W Scientific, US), operating at a temperature of 35°C with nitrogen carrier gas (flow rate = 3.8 mL/min). Headspace operating conditions were as the follows: strong shaking of the tested sample solution at 105°C for 45 min; pressurization pressure = 2.00 bar; carrier gas pressure = 1.5 bar; vial pressurization time = 15 s; sample loop fill time = 10 s; and transfer time = 20 s.

❖ Preparations and procedures

A 25-mLof K_2CO_3 solution (600 g/L) was added to a proximately 5.00-gram samples in a 50-mL Erlenmeyer flask that was placed in a water bath at 40°C for 60 min. Then, 5 mL of the solution and 0.1 mL of the $NaBH_4$- isopropanol solution were placed in a 20-mL headspace sample vial, which was sealed immediately with a PTFE/butyl septum and aluminum cap. For a separate blank test, 5 mL of the solution and 0.1 mL of K_2CO_3 solution (containing 100 mg/L of isopropanol) was placed in a 20-mL headspace sample vial. The vials were placed in the headspace sampler and allowed to equilibrate at 105°C for 45 min with a strong shaking and then the methanol in the vapor phase of the vials was measured by HS-GC.

Results and Discussion

❖ Conditions for the sample extraction

The addition of salt in the solution can effectively limit many organic species (less hydrophilic compounds) be extracted from canned food samples. Since the conversion of formaldehyde to methanol by borohydride must be performed at an alkaline medium, we chose a K_2CO_3 solution (instead of Na_2SO_4) as the extraction medium in the present study.

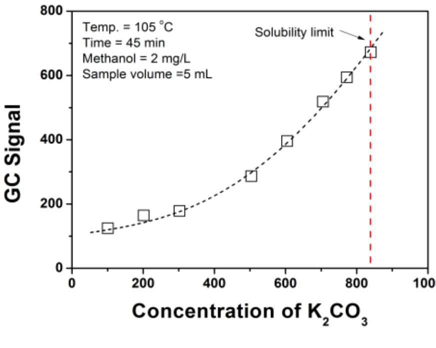

Fig. 1. Effect of K_2CO_3 concentration on methanol detection in HS-GC.

Due to the salting-out effect on the Henry's law constant of methanol [33], the detection sensitivity of methanol in headspace analysis can be improved by adding more salt in the testing solution (cleansing water), as shown in Fig. 1. However, high salt concentration can affect the extraction efficiency of the hydrophilic compounds (i.e., methanol and formaldehyde) from aqueous environmental samples. As a compromise, we chose a K_2CO_3 solution (600 g/L, pH≈12.4) as the extraction medium in this study.

❖ Method establishment

The present method is based on measuring GC signals for the original methanol before the reaction, and the total methanol after the reaction (including the part from formaldehyde conversion). Since the GC signal in HS-GC measurement is linearly proportional to the content of methanol in the vial, the methanol contents in the above two separate measurements can be written as.

$$C_m = KA_1 \tag{2}$$

and

$$C_m + C_f = KA_2 \tag{3}$$

or

$$C_f = K(A_2 - A_1) \tag{3-1}$$

where C_m and C_f represent the content of methanol and formaldehyde; A_1 and A_2 represent the GC signals of methanol at HS measurements before and after the reaction; and K is a coefficient.

Based on Eqs. (2) and (3-1), the contents of methanol and formaldehyde can be determined by HS-GC method.

Since Eq. (3-1) requires the GC signals from two separate HS measurements, any uncertainty caused by the sampling and measurement could be led to a significant error in the formaldehyde quantitative analysis, especially for the case when its content is very low. In order to minimize the error, an internal standard addition calibration method is introduced. In this work, we spiked isopropanol into the solution from sample extraction. Then, the same volumes of the solution are taken and add into two separate sample vials with and without the reduction reaction by $NaBH_4$. Since the spiked contents of isopropanol in these solutions are identical. Any differences in the signals for isopropanol from these two separate tests can indicate the uncertainty in sampling and HS-GC measurement. Thus, the analysis error in the experiment can be minimized by taking the isopropanol GC signals as the references, i.e., the signals of methanol obtained from HS-GC measurements are di-vided by the corresponding isopropanol signals, i.e.,

$$R_1 = \frac{A_1}{A_{s1}} \tag{4-1}$$

and

$$\mathrm{R}_2 = \frac{A_2}{A_{s2}} \tag{4-2}$$

where As1 and As2 represent the GC signals of isopropanol before and after the reaction. R1 and R2 represent the corrected GC signals of methanol (i.e., the ratio of methanol to isopropanol signals) for the sample solutions before and after the reaction.

Thus, Eq. (3-1) for formaldehyde quantification can be revised as

$$C_f = K^{'}(\mathrm{R}_2 - \mathrm{R}_1) \tag{5}$$

i.e., an equation based on the internal standard calibration.

where K' is the coefficient.

Error analysis for formaldehyde quantification

Based on Eqs. (3-1) and (5), the error equation using the conventional (external) and internal standard calibration method can be written as

$$s_{C_f}^2 = \left(\frac{\partial C_f}{\partial K}\right)^2 s_K^2 + \left(\frac{\partial C_f}{\partial A_1}\right)^2 s_{A_1}^2 + \left(\frac{\partial C_f}{\partial A_2}\right)^2 s_{A_2}^2 \tag{6}$$

and

$$s_{C_f}^{'2} = \left(\frac{\partial C_f}{\partial \mathrm{K}^{'}}\right)^2 s_{\mathrm{k}^{'}}^2 + \left(\frac{\partial C_f}{\partial \mathrm{R}_1}\right)^2 s_{\mathrm{R}_1}^2 + \left(\frac{\partial C_f}{\partial \mathrm{R}_2}\right)^2 s_{\mathrm{R}_2}^2 \tag{7}$$

Where S_{C_f} and S'_{C_f} are the standard errors of formaldehyde content by the conventional and the internal standard calibration method, respectively. S_{A1} and S_{A2}, S_{R1} and S_{R2} are the standard errors of GC and the corrected GC signals of methanol before and after the reaction, respectively. SK and SK' are the standard errors of coefficient.

Because the error is mainly caused in the HS-GC measurement, the errors of GC signals in these two separate measurements are basically in the same level, i.e., $S_{A1}=S_{A2} = S_A$ and $S_{R1} =S_{R2} = S_R$. Thus, Eqs. (6) and (7) can be simplified as

$$s_{c_f} = \sqrt{2K^2 s_A^2} = \sqrt{2}Ks_A \tag{8}$$

and

$$s_{c_f}^{'} = \sqrt{2K^{'2} s_{\mathrm{R}}^2} = \sqrt{2}K^{'} s_{\mathrm{R}} \tag{9}$$

According to Eqs. (8), (9) and propagation of uncertainty (or propagation of error), the uncertainty, as the relative standard deviation (RSD), in the formaldehyde quantification using the conventional and internal standard calibration method can be expressed respectively as

$$RSD = \frac{s_{c_f}}{C_f} = \frac{\sqrt{2}Ks_A}{C_f} \tag{10}$$

and

$$RSD = \frac{s_{C_f}^{'}}{C_f} = \frac{\sqrt{2}K^{'}s_{\mathrm{R}}}{C_f} \tag{11}$$

In Table 1, it shows the comparison of the measurement precisions (from quadruplicate measurements) obtained by these two calibration methods on a given aqueous environmental sample (formaldehyde content = 45 mg/kg). It is clear from the table that the measurement error (RSD) is very significant (~27 %) in the conventional calibration method. With the internal standard calibration method, such an error can be significantly reduced (3.6 %). Therefore, it is important to introduce the internal standard calibration for the formaldehyde analysis in canned food samples.

In Fig. 2, it shows the relationships between the measurement errors and the content of formaldehyde in a sample containing a given amount of methanol (3.2 mmol/L), using the conventional and internal standard calibration methods, respectively. It is clear from the figure that the measurement error increases with the decrease of formaldehyde content in the sample whenever using the conventional or internal standard calibration methods. However, the errors with the internal standard calibration method are much lower than those of the conventional method. For example, to have a measurement error less than 10%, the detectable amount of formaldehyde is ~ 0.25 mmol/L by the internal standard calibration, which is much lower than 1.25 mmol/L by the conventional calibration method. Therefore, the introduction of the internal standard calibration can remarkably increase the sensitivity for formaldehyde analysis in the present method.

Table 1 Comparison of the measurement errors in two calibration methods

Testing no.	**GC signals before the reaction**		**GC signals after the reaction**		**Conventional**	**Internal Standard**
	A_1	A_{s1}	A_2	A_{s2}	A_2-A_1	R_2-R_1
1	365	689	412	647	47	0.107
2	340	650	421	660	81	0.115
3	351	670	444	703	93	0.108
4	361	685	430	678	69	0.107
Average	354	674	427	672	72.5	0.109
RSD, %	3.16	2.62	3.20	3.61	**27.1**	**3.55**

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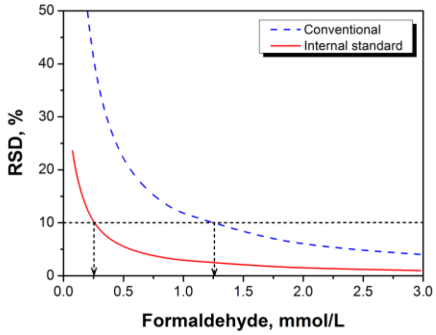

Fig. 2. Measurement errors as a function of formaldehyde content in a aqueous environmental sample containing methanol (3.2 mmol/L).

❖ HS-GC conditions for methanol and isopropanol measurement

To improve the detection sensitivity for methanol measurement, the headspace equilibration was performed at an elevated temperature (105 oC), in which the complete methanol conversion from formaldehyde by Reaction (1) and vapor-liquid equilibrium of methanol can be achieved within 45 min [33]. At the given GC conditions, the methanol and iso-propanol in the sample solution (lotions) can also be well-separated, as shown in Fig. 3.

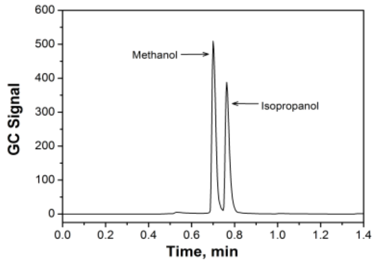

Fig. 3. Chromatogram of a sample solution spiked with isopropanol.

❖ Method calibration and evaluation

In this work, the calibration was performed on the basis of varying the concentrations of methanol (0.15 - 3.2 mmol/L) by spiking a given amount of isopropanol (0.0333 mmol/L) in a set of headspace sample vials, which were then tested by HS-GC. According to the methanol and isopropanol signals from HS-GC measurement on these samples, the corrected signals for methanol can be calculated. By plotting the corrected signals vs. the corresponding content of methanol, a standard calibration curve was obtained, which can be written as

$$R = -0.0209(\pm 0.0330) + 1.85(\pm 0.02)c \quad \text{(n=6, R2=0.999)} \qquad (12)$$

where R and c represent the corrected GC signal and the methanol content (in mmol/L), respectively.

From the information shown in Eq. (12), the limit of quantitation (LOQ) of the present method can be calculated by Eq. (13), which is 0.835 mmoL/kg (26.7 mg/kg of methanol and 25.1 mg/kg of formaldehyde) when a 5-mL of solution with 20 % canned food samples consistency was used in the testing, better than that of 50 mg/kg for methanol and 60 mg/kg for formaldehyde reported by the current methods.

$$LOQ = \frac{a + 10 \times |\Delta a|}{s} \qquad (13)$$

where a,Δa and s represent the intercept, uncertainty of the intercept, and the slope in Eq. (12), respectively.

According to Eq. (12) and sample weight added in the testing, the methanol content and formaldehyde content (in mg/kg) in the aqueous environmental sample can be calculated by the following equation, i.e.,

$$C_M = 800 \times \frac{R_0 - a}{sw} \qquad (14)$$

$$C_F = 750 \times \frac{(R_1 - R_0) - a}{sw} \qquad (15)$$

where w is the weight (in grams) of aqueous environmental sample in the suspension solution. Ro and R1 represent the corrected GC signals before and after the reaction.

To verify the present method, the known amounts of formaldehyde and methanol were spiked into the sample solutions, which contained 5 g aqueous environmental sample in the K_2CO_3 solution. After extraction, the extract solution was spiked with isopropanol. The sample solutions with and without the reaction by borohydride were tested by HS-GC, respectively. According to Eqs. (14) and (15), the amounts of methanol and formaldehyde in these samples can be calculated and the results are shown in Tables 2 and 3.

In these tables, it can be seen that the recoveries in both methanol and formaldehyde testing are in the range of 94-103 %, indicating that the present method is justifiable to be used for the analysis of these species in the canned food products.

Table 2 Method validation for methanol testing.

Sample No.	**Methanol, μg**		**Recovery, %**
	Added	**Measured**	
1	250	239	95.6
2	500	491	98.2
3	750	729	97.2
4	1000	969	96.9
5	1250	1292	103

Table 3 Method validation for formaldehyde testing.

Sample No.	Formaldehyde, μg		Recovery, %
	Added	Measured	
1	250	243	97.2
2	500	470	94.0

3	750	768	102
4	1000	981	98.1
5	1250	1245	99.6

❖ Comparison between the conventional and internal standard calibration

The formaldehyde and methanol contents in 5 canned food samples of various kinds were determined by the present internal standard calibration method. The formaldehyde and methanol contents of these samples were also determined by the conventional calibration method. As shown in Table 4, the present method (with the internal standard calibration) has a good agreement on the methanol testing with the conventional calibration. However, if the sample contains a significant amount of methanol, the conventional calibration can produce a significant error (greater than 100%) in duplicate measurement on the formaldehyde quantification. Such an error will be reduced if the amount of methanol in the sample is lower, and the data obtained by both the conventional and internal standard calibration match well.

Table 4 Results from the conventional and the internal standard calibration on both methanol and formaldehyde testing.

Sample name	Conventional		Internal standard	
	Methanol, mg/kg	Formaldehyde, mg/kg	Methanol, mg/kg	Formaldehyde, mg/kg
Tuna fish	3685	386.7	3710	268.5
	3910	-145.2	3791	275.8
R.D., %	6.10	-137	2.18	2.71
Ground chicken	8275	-205.6	7568	129.5
	7166	150.1	7891	138.1
R.D., %	-13.4	-173	4.26	6.64
Mango pickle	6185	-106.3	6132	325.4
	6695	143.7	6320	315.2
R.D., %	8.24	-235	3.06	-3.13
Industrial food colors	895.2	274.2	861.3	276.5
	846.7	231	875	289.1
R.D., %	-5.41	-15.7	1.59	4.55
Roohafza drink	686.2	158.2	695.6	175.1
	720.9	176.5	713.1	169.5
R.D., %	5.05	11.5	2.51	-3.19

*R.D. – relative difference

Conclusion

A reaction-based HS-GC method for the determination of formaldehyde and methanol content in canned food products has been developed. With the internal standard calibration, the error for formaldehyde analysis in the high methanol containing sample can be significantly reduced, and thus improves the method detection sensitivity. The results from recovery testing indicated that the present method also has a good accuracy in formaldehyde and methanol analysis. The present method is simple and practical, and can be applied for the determination of formaldehyde and methanol in canned food products.

Conflict of Interest: NIL

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Declarations:

Authors' Contribution:

- a-b-c Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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