

## Simple Analysis Method to Measure Phosphine Residue in Grains, Herbs, and Spices Using Headspace Gas Chromatography

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**Abstract:** Trials were conducted to determine whether an analysis method for measuring phosphine residue using a headspace autosampler, which provides rapid analysis and easier handling, is equally applicable to herbs and spices and other kinds of grains as it is to wheat and small beans. For measuring residue levels, the optimal equilibrium time, best sample amount per vial, and impact of cracking a sample before fumigation were investigated using 17 kinds of grains, herbs, and spices: popcorn, dent corn, soybeans, black pepper, cumin seed, celery seed, fennel seed, white peony, huang qi, rosemary, turmeric, coriander seed, hemp seed, oregano, basil, mustard seed, and horseradish. Optimal sample amounts varied from 1 g to 5 g depending on the kind of sample. Large and dense samples required a long holding time (120 minutes), and small and low-density samples did not require a long holding time. The analysis method concerned could be applied to grains, herbs, and spices as a simple analysis method of measuring phosphine residue instead of absorption spectrophotometry at a 1 ppb level quantitative limit. However, grains, herbs, and spices with a larger grain size would often provide dispersive data, although this seems to depend on the kind of grain, herb, or spice. Homogenization of samples or increasing sample size by using a larger vial is appropriate to conduct accurate analysis. Fumigation of a sample in fractions or chip shapes which has been artificially cracked is inadequate to quantify phosphine residue because in turmeric, residual rates increased according to fraction size, and smaller laurel chips indicated a higher residue than larger ones.

**Key words:** residue analysis, fumigation, phosphine, headspace, gas chromatography

### Introduction

The use of methyl bromide for quarantine and preshipment is exempted despite MB being listed as an ozone-depleting substance. The development of alternative techniques to MB fumigation is expected, however (UNEP, 2006). Phosphine generated from tablets of aluminum phosphide is widely used to control infestation of stored product insects, including many kinds of quarantine pests. Recently, phosphine stored in cylinders has been used, and this technique could be applied to a wide range of commodities because no residue of aluminum hydroxide remains after fumigation, unlike with the use of aluminum phosphide tablets. Although the High Pressure Gas Safety Law (Law No. 204 of 1951) restricts the use of phosphine gas in cylinders in Japan, a phosphine gas generator has been developed instead (SOMA *et al.*, 2002). Consequently, the expansion of phosphine fumigation holds promise not only for general purposes but also for quarantine.

The Food Sanitation Law was amended in 2003 in order to introduce the so-called positive list system, which prohibits the sale of foods that contain agricultural chemicals above maximum residue limits (MRLs). Simultaneously, MRLs were set for all kinds of foods in the amendment (MHLW, 2006). Therefore, in the interest of safety and the appropriate use of phosphine to comply with the positive list system, it should be determined whether phosphine residue levels in food commodities is exceeding MRLs or not. Various kinds of grains, herbs, and spices are imported to Japan, and herbs and spices especially are often imported in small quantities. Absorption spectrophotometry for phosphine is officially specified (MHLW, 2005; MoE, 1971) in an analysis method that oxidizes phosphine into phosphate using bromine water. The phosphate generated is then quantified by ultraviolet spectrophotometer. This method requires a large

sample amount (500 g per analysis) and specialized experimental apparatus; moreover, it takes rather a long time and a lot of work (SOMA, 2004). A rapid and simple analysis method which does not require a large sample is necessary to determine phosphine residue for herbs and spices fumigated in the plant quarantine process.

Headspace gas chromatography for phosphine residue has been used, and sample extraction into a solvent is usually required in the analysis process (NORMAN and LEONARD, 2000). A method for accelerating solvent extraction has also been researched (REN and ALLEN, 2001). Sample homogenization using a blender is utilized for the analysis of fruit (KING *et al.*, 1981), but grains, herbs, and spices are usually harder than fruits, and some of them (e.g. turmeric) are quite a lot harder, requiring special tools and devices to break them. Consequently, even headspace gas chromatography with an autosampler requires a certain amount of sample conditioning to prepare vials for analysis.

An analysis method of detecting phosphine residue using a headspace autosampler has been reported (JFTA, 2003). This method allowed rapid analysis and provided easier handling in the analysis of wheat and small beans, and the residue level detected compares evenly with residue data from absorption spectrophotometry. Furthermore, higher sensitivity would be expected than with absorption spectrophotometry.

We conducted this study as to whether the reported headspace gas chromatography method (JFTA, 2003) is applicable to herbs and spices and other kinds of grains, and we also tried to establish applicable analysis conditions to measure a lot of samples rapidly in sequences of analysis.

## Materials and Methods

### 1. Determination of the best sample amounts per vial and optimal equilibrium time

Seventeen kinds of grains, herbs, and spices (Table 1) were fumigated with  $\text{PH}_3$  3 g/m<sup>3</sup>, at 10°C or 15°C for 72 hours. After fumigation was completed, fumigated samples were stored 1–3 days at room temperature, and then phosphine residue levels were measured. Each of the samples weighing a certain amount were put into a 20 ml vial, then 10 ml of distilled water was added, and the vial was immediately sealed with a PTFE (polytetrafluoroethylene) lid liner and aluminum cap. Sample vials were analyzed with a gas chromatograph (FPD) equipped with a headspace autosampler to determine both optimal equilibrium time (holding time in the oven) and the optimal amount in grams of sample per vial. Analysis conditions of the gas chromatograph and the headspace autosampler were as follows. These conditions were based on the method described in the project report (JFTA, 2003).

**Best sample amount per vial:** Sample amount per vial was set at 0.5 g, 1 g, and 1.5 g for oregano and basil, 1 g, 2 g, and 3 g for rosemary and coriander seed, and 1 g, 2 g, and 5 g for the other herbs and spices. Sample vials containing these amounts were heated for 75 minutes at 90°C in the oven of the headspace autosampler, then analyzed, and the detection response was compared among the different sample amounts.

**Optimal holding time:** Each of the vials containing 2 g samples was heated at 90°C in the oven of the headspace autosampler with a holding time of 45, 60, 75, 90, 105, and 120 minutes, and then the detection response of each holding time was analyzed.

The reported phosphine analysis method achieves a level of 1 ppb (0.001 mg/kg) as a quantitative limit (NORMAN and LEONARD, 2000). It is important to attain a quantitative limit of 1 ppb for grains, herbs, and spices to measure fixed MRLs in the positive list system in Japan. The quantitative limit for each grain, herb, and spice was also investigated using the absolute calibration method.

**Table 1.** List of grain, herb, and spice samples fumigated with  $\text{PH}_3$   $3 \text{ g/m}^3$  for 72 hours at  $10^\circ\text{C}$  or  $15^\circ\text{C}$  and analyzed by headspace gas chromatography.

1. Popcorn ( <i>Zea mays</i> var. <i>everta</i> ), Indonesia	10. Rosemary ( <i>Rosmarinus officinalis</i> ), Morocco
2. Dent corn ( <i>Zea mays</i> var. <i>indentata</i> ), Japan	11. Turmeric <sup>2</sup> ( <i>Curcuma longa</i> ), China
3. Soybean ( <i>Glycine max</i> ), Canada	12. Coriander seed ( <i>Corianderum sativum</i> ), Canada
4. Black pepper ( <i>Piper nigrum</i> ), Malaysia	13. Hemp seed ( <i>Cannabis sativa</i> ), China
5. Cumin seed ( <i>Cuminum cyminum</i> ), India	14. Oregano <sup>3</sup> ( <i>Origanum vulgare</i> ), Albania
6. Celery seed ( <i>Apium graveolens</i> ), India	15. Basil <sup>3</sup> ( <i>Ocimum basilicum</i> ), Egypt
7. Fennel seed ( <i>Foeniculum vulgare</i> ), India	16. Mustard seed ( <i>Sinapis alba</i> ), Canada
8. White peony <sup>1</sup> ( <i>Paeonia lactiflora</i> ), Japan	17. Horseradish <sup>3</sup> ( <i>Armoracia rusticana</i> ), China
9. Huang qi <sup>1</sup> ( <i>Astragalus membranaceus</i> ), Japan	

## Notes:

<sup>1</sup> White peony and huang qi had been chopped into 10–20 mm lengths were used.

<sup>2</sup> Turmeric was manually crushed into 2–5 mm diameter pieces with a grinder before fumigation.

<sup>3</sup> Oregano, basil, and horseradish had been shredded into 1–2 mm pieces were used.

## Analysis conditions

- Headspace sampler (Perkin-Elmer TurboMatrix HS-40 or HS-110)
  - Equilibrium temperature:  $90^\circ\text{C}$
  - Transfer line and needle temperature:  $120^\circ\text{C}$
  - Equilibrium time: 45, 60, 75, 90, 105, and 120 minutes
- Gas chromatograph (Shimadzu GC-2014 with Flame Photometric Detector)
  - Column: Chromosorb 101, 2.6 mm i.d., 60/80 mesh, length 3.1 m
  - Column temperature\*<sup>1</sup>:  $70^\circ\text{C}$  initial; after 6 minutes holding at initial temperature, temperature raised gradually to  $200^\circ\text{C}$  (rising rate:  $40^\circ\text{C}/\text{min.}$ ), then held 2–10 minutes
  - Detector (FPD) temperature:  $230^\circ\text{C}$
  - Injection temperature:  $120^\circ\text{C}$
  - Injection: 2.0 ml (needle loop; pressuring: 3 min.; venting: 0.5 min.; injecting: 0.05 min.)
  - Gas flow: Carrier (He): 40 ml/min., hydrogen 130 kPa, air 50 kPa

## 2. The effect on residue levels of cracking a sample before fumigation

The size of some of the herbs and spices is larger than the diameter of the vials. It is inevitable that large samples must be cracked so as to put them into vials. However, if samples are cracked after fumigation, phosphine gas is probably emitted from the sample at the moment they are cracked. Samples should be cracked before fumigation and the effect of cracking on residue levels should also be investigated.

Three sizes of turmeric (*Curcuma longa* from China) pieces (fine:  $< 710 \mu\text{m}$ ; medium:  $710 \mu\text{m}$ –2 mm; coarse: 2–5 mm), and two sizes of laurel (*Laurus nobilis* from Morocco) pieces (fine: 2 mm  $\times$  2–5 mm; coarse: 5–10 mm  $\times$  10–20 mm), which had been manually cracked or shredded, were fumigated with phosphine  $3 \text{ g/m}^3$  for 72 hours at  $10^\circ\text{C}$ . After fumigation was completed, each of the samples was stored for one day at room temperature and then analyzed for phosphine residue in the above-mentioned headspace autosampler and GC conditions. Sample amounts per vial of turmeric were 2 g, whereas vials for laurel are loaded with only 1 g because it is difficult to stuff 2 g of laurel leaves into a vial due to laurel's low density.

## Results and Discussion

### 1. Determination of the best sample amount per vial and optimal equilibrium time

**Best sample amount per vial:** Figures 1–1, 1–2, and 1–3 show detection responses for each of the samples for the 17 kinds of grains, herbs, and spices. From these results, 5 g per vial is considered to be adequate for popcorn, dent corn, black pepper, and white peony because detection responses were higher and increased proportionally with the sample amount. Inflation of the sample due to both heat and water absorption occurred, and parts of the sample rose above the

\*<sup>1</sup> On preliminary analysis of horseradish at column temperature  $70^\circ\text{C}$  constant, large peaks were detected at retention time of 70–120 minutes. That situation could arise in the analysis of other commodities because most spices and herbs have a characteristic flavor. Accordingly, to prevent collision of peaks of phosphine that would present an obstacle in subsequent analysis of samples, the rising temperature program in analysis conditions is set to purge interfering components from the column after the detection of phosphine.

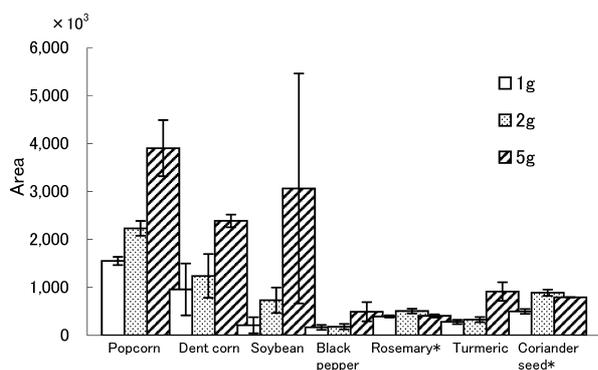


Fig. 1-1. Detection responses of differing sample amounts per vial.

Note: \*Sample amounts for rosemary and coriander seed were 1 g, 2 g, and 3 g.

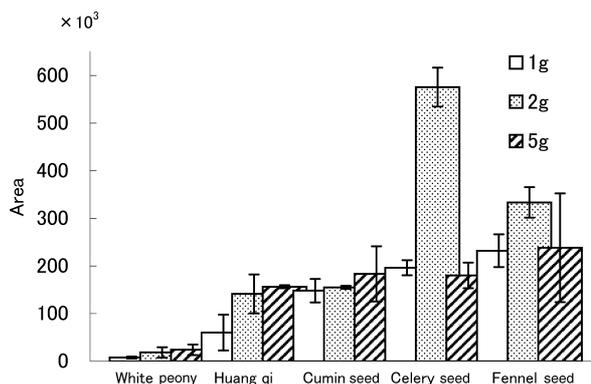


Fig. 1-2. Detection responses of differing sample amounts per vial.

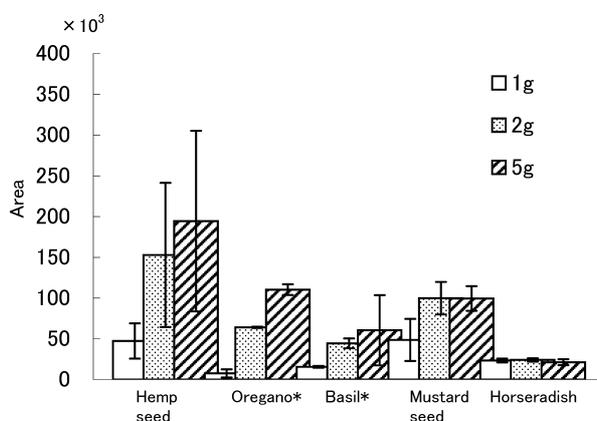


Fig. 1-3. Detection responses of differing sample amounts per vial.

Note: \*Sample amounts for oregano and basil are 0.5 g, 1 g, and 1.5 g.

liquid level in the liquid phase in vials that contained 5 g or 3 g of soybean, rosemary, turmeric, coriander seed, cumin seed, celery seed, fennel seed, oregano, basil, and horseradish. Most of the detection responses did not increase proportionally to the sample amount, and the coefficient of variance (%) was relatively larger for soybean, cumin seed, fennel seed, and basil. In particular, the detection responses of the 5 g sample for coriander seed, celery seed, and fennel seed were less than for 2 g samples of the same material. The part of the sample above the surface of the liquid phase might influence equilibrium in the vial in the headspace, and that is likely to provide unstable detection responses. Therefore, a 2 g sample per vial is preferable. For oregano and basil, an amount of 1 g is relevant because of steady detection responses. Although all parts of a 5 g sample in the vial settled in the liquid phase, detection responses of 5 g sample vials for white peony, Huang qi, hemp seed, mustard seed, and horseradish did not show proportional responses according to an increase in the sample amount.

A trial of small beans indicated that the data from a larger sample amount per vial, which showed a portion of the small beans existed in a gaseous phase in the vial, provided better detection response and accuracy than the smaller sample amount, and it can be concluded that a 5 g sample per vial is suitable for obtaining maximum accurate detection response (JFTA, 2003). However, the types of herbs and spices vary in wide categories, such as seeds, stems, leaves, etc. Size and density may also differ among sources. Particularly, herbs possess the property of low density. A 5 g sample per vial seems to be considered too large an amount for most kinds of herbs and spices.

**Optimum holding time:** Figures 2-1 and 2-2 indicate detection responses and proximate quadratic curves of 15 kinds of samples for holding times from 45 to 120 minutes. Holding times of

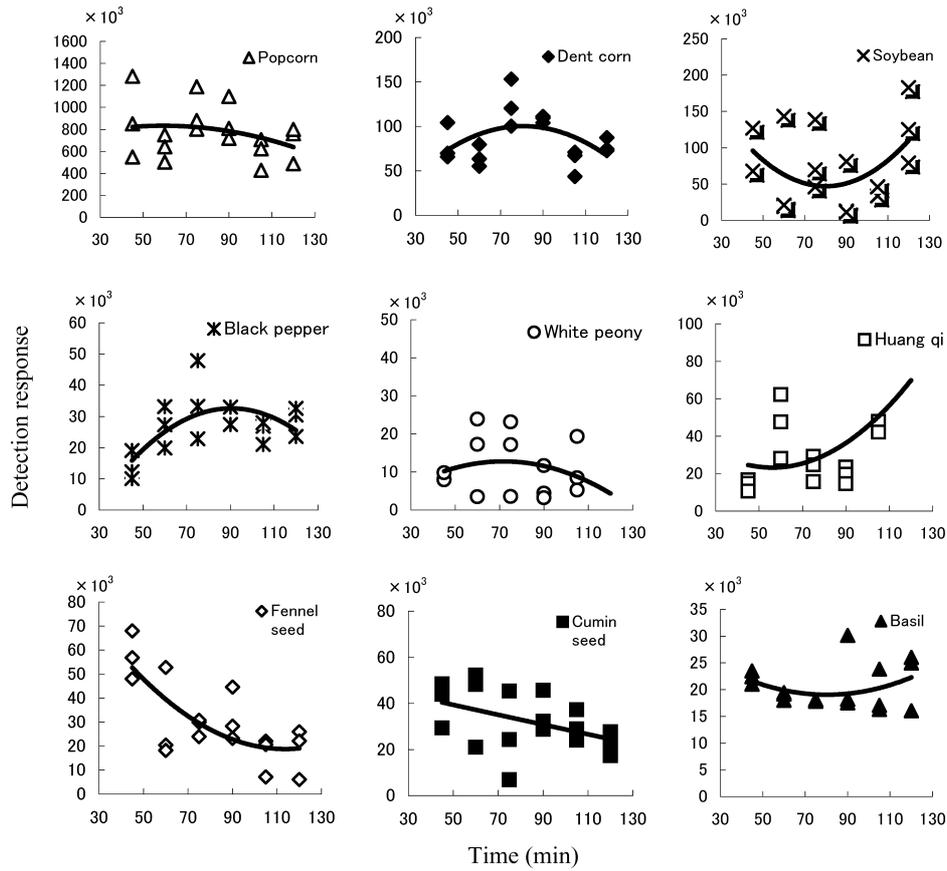


Fig. 2-1. Detection responses to holding times from 45 to 120 minutes, and their quadratic curves.

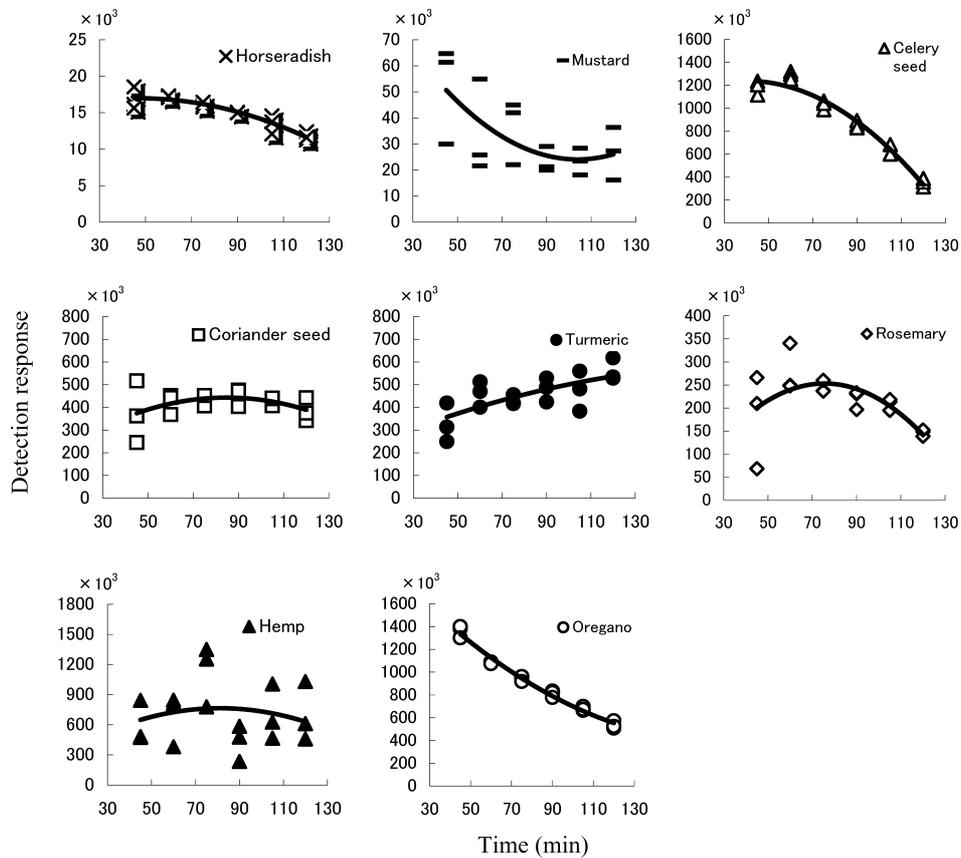


Fig. 2-2. Detection responses to holding times from 45 to 120 minutes, and their quadratic curves.

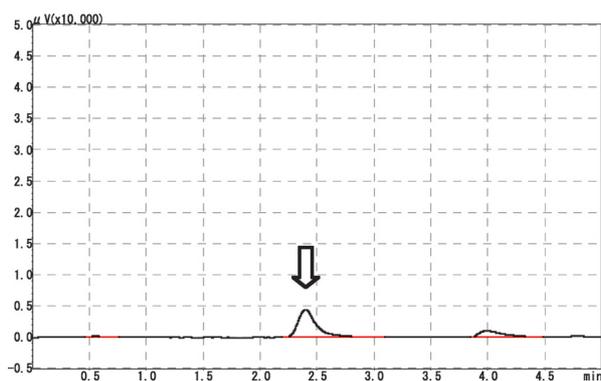


Fig. 3. Chromatogram of white peony with 0.93 ppb phosphine.

75 minutes for grains except soybeans, as well as black pepper, white peony, coriander seed, hemp seed, and rosemary were considered suitable. Fennel seed, cumin seed, basil, oregano, horseradish, mustard seed, and celery seed provided maximum detection response at 45 minutes, and huang qi and turmeric showed 120 minutes to be effective. It is inadequate to determine the optimum holding time for soybeans due to large dispersion, which means that more than 110 CV% was found for detection responses of some holding times. For cumin seed, white peony and hemp seed also showed relatively dispersed data; however, the highest values of CV% for data of some holding time in hemp was under 40% level, and these in both cumin seed and white peony were fewer than 70% level. The project report (JFTA, 2003) described the optimal equilibrium time at 90°C as 75 minutes for both wheat and small beans. The same equilibrium time can be adopted for grains that have similar properties to wheat and small beans. However, the properties of spices and herbs are obviously different, and the best equilibrium times might differ among commodities. The results of this analysis roughly suggested that a sample that has the properties of large size and high density require a longer holding time, while small and low density samples do not require long holding times to acquire maximum detection responses.

Figure 3 shows a chromatogram for white peony with 0.93 ppb phosphine (standard gas, Takachiho Chemical Industrial Co., Ltd.) acquired from a 2.0 g sample per vial at 75 minutes equilibrium time; this commodity indicates the lowest detection response among the 17 kinds of samples. It appears that a signal-to-noise ratio of 10 was fulfilled with sufficient levels even though analysis conditions were not optimal. Optimal equilibrium time for Japanese pear is also reported at 75 minutes (SOMA *et al.*, 2004). Therefore, although it is not optimal, a 75-minute holding time and a 2 g sample amount per vial could be adapted to analyze grains, spices, and herbs efficiently at a 1 ppb level quantitative limit.

Consequently, the analysis method concerned would be applied to grains, spices, and herbs as a simple phosphine residue analysis method instead of absorption spectrophotometry at a 1 ppb quantitative limit. However, grains, spices, and herbs in which there are some larger grain sizes would be regarded as occasionally providing dispersive data. Figures 2-1 and 2-2 reveal that some kinds of herbs and spices are also dispersive. Homogenization of samples or increasing sample amount per vial by using a larger-volume vial are appropriate measures in conducting accurate analyses.

## 2. The effect on residue levels of cracking samples before fumigation

Each detection response and its coefficient of variances (CV %) for three sizes of turmeric pieces and two sizes of laurel pieces are shown in Fig. 4. Residual rates of turmeric increased in relation to larger fraction sizes. Turmeric is firm and has the property of relatively high density compared to other spices. It is thought that adsorbed phosphine gas on turmeric might be released more easily from smaller fractions than larger ones after fumigation. In the case of laurel, a contrary result was obtained. Pieces of fine size indicated higher residue levels than those of coarser pieces. It appears that higher surface area of fine pieces could contribute to increased sorption, especially adsorption of phosphine in laurel leaf pieces, and it might result in

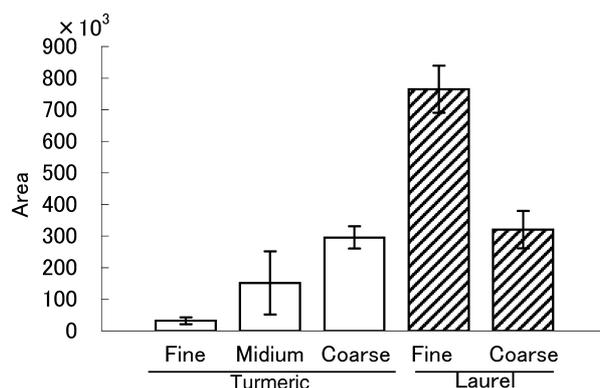


Fig. 4. Differences in phosphine residual rates by piece size in fumigation for turmeric and laurel.

high residual rates for fine-sized pieces. There is the possibility that differences in density and surface structure between turmeric and laurel might influence residual levels of fractions and pieces. Fumigation of samples as fractions or piece shapes that have been cracked or shredded artificially are inadequate to quantify phosphine residue. A phosphine residual level in maize of half was indicated if the grains of maize had been cracked after fumigation by analysis with absorption spectrophotometry (AKIYAMA *et al.*, 1977), which means that samples should be cracked under sealed conditions and/or dozens of degrees below the freezing point to prevent phosphine emission from samples as much as possible. Samples which are disproportionately large compared to vial size should be cracked just before analysis while preventing phosphine emission from the sample. Absorption spectrophotometry should also be applied.

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## 和 文 摘 要

ヘッドスペースガスクロマトグラフィーによるリン化水素  
くん蒸された穀類・香辛料の簡易残留分析法

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小麦と小豆で開発されたヘッドスペースガスクロマトグラフィーによるリン化水素残留分析法が17種類の穀類及びハーブ・香辛料（ポップコーン、デントコーン、大豆、黒コショウ、クミン、セロリシード、フェネルシード、シャクヤク、オウギ、ローズマリー、ウコン、コリアンダーシード、ヘンプシード、オレガノ、バジル、マスタードシード及びホースラディッシュ）にも適用できるかどうか調査し、最適保持時間及びサンプル量、並びにくん蒸前にサンプルを破碎することの影響も調査した。それらハーブ・香辛料等について、リン化水素 3 g/m<sup>3</sup>、10℃又は15℃で72時間くん蒸し、ヘッドスペースオートサンプラー付きガスクロマトグラフ（FPD）で残留レベルを調査した結果、ポップコーン、デントコーン及び黒コショウは20 mlバイアルあたり5.0 gのサンプル量が適当と考えられ、大豆、クミン、セロリシード、フェネルシード、シャクヤク、オウギ、ローズマリー、ウコン、コリアンダーシード、ヘンプシード、マスタードシード及びホースラディッシュについては2.0 gが妥当であり、オレガノとバジルは1.0 gが適当であり、ハーブ・香辛料にとって5.0 gのサンプル量は多すぎると考えられた。最適保持時間については、大豆を除く穀類、黒コショウ、シャクヤク、ローズマリー、コリアンダーシードは75分の保持時間が最も適当であったが、クミン、セロリシード、フェネル

シード、バジル、マスタードシード及びホースラディッシュは45分が適当であり、オウギとウコンは120分が適当であった。大豆については残留レベルがばらつき、最適保持時間を決定することができなかった。よって、サンプルサイズが大きく、密度が高い品目は長い保持時間を必要とするが、細かいサイズで密度が低いサンプルは長い保持時間を必要としない傾向が示唆された。17種類のうち、最も応答面積が低いシャクヤクに0.93 ppbのリン化水素を添加した場合のクロマトグラムのS/N比は10以上を十分に満たしていた。以上から、当該分析方法は公定分析法に代わり、穀類及びハーブ・香辛料の分析法として定量限界1 ppbレベルでの分析が可能であると判断された。しかしながら、サンプル粒の大きさが大きい品目は種類によるが、データにばらつきが認められた。分析サンプルのホモジナイズやバイアルサイズを大きくしてサンプル量を多くすることが精度の高い分析のため適切と考えられた。くん蒸前にサンプルを破碎することは、ウコンでサイズを大きめに破碎したほうが小さいものより残留レベルが高くなり、ローレルでは逆の関係が見られたため、適当ではないと考えられた。したがって、分析バイアルに入らない大きさのサンプルの分析は、その破碎を分析直前にリン化水素ガスの放出を防ぎながら実施するか、あるいは、公定分析法で実施すべきと考えられた。