

Analysis of the Fumigant Phosphine Applying Headspace-GC-MSD

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Due to its volatility, the residue levels of phosphine in food are expected to be very low. To face this analytical methods are needed, that are able to achieve determination limits at about 0.1 µg/kg. Amstutz et al. published a method in 2003 with sufficient sensitivity using a Headspace-GC-FPD. Since a flame-photometric detector is not available in every lab, our aim was to use a universal MSD instead.

Chemicals and standards

sulfuric acid (concentrated) analysis grade (Merck Darmstadt, Germany)
phosphine (Purity ≥ 99.9 %) in nitrogen (Linde AG München, Germany).

Apparatus

Grindomix GM 200 knife mill (Retsch Haan, Germany)
Tedlar gas sampling bags (Nr. 24633 Supelco #, Germany)
gas tight syringes (PN 1710 100 µL and PN 1001 1000 µL from Hamilton #, Germany)
analytical balances capable of weighing to 0.1 mg or to 0.01 g (Mettler-Toledo Greifensee, Switzerland).
GC-MSD: Agilent 6890 GC coupled to 5973 MSD (Waldbronn, Germany) manually tuned to achieve high sensitivity in the low mass area,
Autosampler: MPS2 sampler (Gerstel Mülheim/Ruhr, Germany) with a head space agitator unit and a 2.5 mL syringe
Injection system: KAS 4 PTV with a cryo system at -80°C (liquid nitrogen)
Liners: filled with Tenax (Gerstel Mülheim/Ruhr, Germany).
Column: Rt-Q-Bond PLOT column (30 m × 0.32 mm × 10 µm) (Restek Bad Homburg, Germany) connected to a restriction capillary (5 m × 0.25 mm) (Agilent Waldbronn, Germany), to prevent single loose particles of the stationary phase from entering the ion source of the MSD

Sample Preparation

Lumpy samples, such as nut kernels or legumes, were ground with a knife mill.

Powdery samples or milled samples:

1 g of sample was weighed into a head space vial and 7 mL of water were added. Then the vial was closed with a rubber plug and vigorously shaken. Subsequently, it was filled up to a 15 mL level with sulfuric acid 10 % by pouring from a small beaker before it was immediately closed. The use of pipettes is not appropriate because of the slower liquid handling which leads to analyte loss.

From the moment on when sulfuric acid is added, 4 hours of waiting time before injection should not be exceeded because of analyte loss.

For the analysis of granular samples like whole grains, up to 3 g were weighed in and sulfuric acid 5 % was speedily added up to a level of 15 mL into the vial.

Headspace GC MS analysis

Headspace conditions:
agitator temperature: 80°C,
incubation time: 10 min,
shaking speed: 500 rotations per min
shaking interval: 5 s followed by a 2 s break
syringe temperature: 85°C,
injection volume: 2000 µL,
draw speed: 200 µL/s,
injection speed: 500 µL/s

PTV conditions:
-80°C initial temperature with 1.0 min initial time,
heating ramp to 150°C with a rate of 12.0°C min⁻¹,
hold time 2 min

Oven:
carrier gas flow (helium) was set to 2.2 mL/min in constant flow mode,
split ratio was 5:1
oven temperature: started at 35°C for 3 min,
heating ramps: 10°C/min to 100°C, then 35°C/min to 200°C
final time: 4 min
total run time: 16.4 min.

MSD:
transfer line temperature: 240°C
solvent delay 4 min
SIM mode recording the ions m/z 31, 33 and 34
dwell time: 100 ms for each ion
For sufficient sensitivity, the use of a manually tune for very low masses is essential.

Calibration:
Calibrations are linear up to 50 ppb at least. Due to quenching effects dependent on the matrix present external calibrations without matrix are only recommended for screening purposes. For exact quantification either by matrix matched calibration or better by standard addition is necessary.

The method was tested and validated by participating in an interlaboratory comparison test organized by Coop Central Laboratory and State Laboratory Basel-City (Determination of phosphine in plant materials: method optimization and validation in inter-laboratory comparison tests, publication under review).

Analysis of samples

To get an overview of the residue situation, 115 samples of cereals, spices, oilseeds, legumes of conventional production and, to a smaller extent, of organic production were analyzed. In 38 of the tested samples residues of phosphine were detectable. However, as expected, concentrations were well below official maximum residue limits. In fact only 14 samples out of 115 contained residues above 1 µg/kg.

In EU legislation for phosphine and phosphides, maximum residue limits are set from 0.01 to 0.1 mg/kg foodstuff, depending on the commodity (Reg. (EC) No 149/2008).

All conventional samples had residue levels well below the defined MLR, see also Table 1.

Table 1: Results for phosphine in conventional food samples:

Commodity	No. of samples	No. of positive samples	Maximum (µg/kg)	Minimum (µg/kg)	Mean value (µg/kg)
Cereals	19	2	0.45	0.09	0.27
Cereal products	3	2	0.4	0.2	0.30
Spices	1				
Legumes	15	6	1.69	0.14	0.92
Oil seeds	1	0			
Nuts	12	5	0.91	0.08	0.37
Tee	1	0			
Pasta	13	9	30	0.09	4.51
Sum	65	24	30	0.08	2.04

For organic foods, the general rules including use of pesticides and products for cleaning and disinfection (Article 16) are laid down in Council regulation (EC) No 834/2007. Detailed rules with regard to organic production, labeling and control are described in Commission Regulation (EC) 889/2008. In Annex 2 a restricted list names the pesticides which are allowed in organic production. Phosphine and phosphides are not listed; therefore, residues resulting from an application should not be found in organic products. Furthermore, in Article 26 of Commission Regulation (EC) 889/2008 is regulated, that every measure has to be taken to avoid cross contamination of organically grown products with conventional products including separate storage and suitable cleaning of the production equipment. Following that phosphine residues should not to be expected in organic food. Table 2 shows the results for phosphine in organic food samples, with 14 out of 50 samples having residues of phosphine.

Table 2: Results for phosphine in organic food samples:

Commodity	No. of samples	No. of positive samples	Maximum (µg/kg)	Minimum (µg/kg)	Mean value (µg/kg)
Cereals	13	3	5.3	0.16	2.12
Cereal products	3	2	10.6	0.1	5.35
Legumes	17	8	18.7	0.5	5.67
Oil seeds	5	0			
Nuts	11	1	0.9		
Pasta	1	0			
Sum	50	14	18.7	0.1	4.52

Seven of the 14 positive organic samples had residue levels above 1 µg/kg, see table 3.

Table 3: samples with phosphine residue > 1 µg/kg

Commodity	Country of origin	Phosphine (µg/kg)
Chickpea	Turkey	5.7
Lentil	Turkey	2.7
Bulgur	Turkey	10.6
Lentil	Turkey	13.5
Lentil	Turkey	2.5
Lentil	Unspecified	18.7
Millet	China	5.3

The phosphine amounts detected in conventional and organic products were in the same range. This indicates either cross-contamination during handling and transportation, mingling of organic and conventional products or illegal application of phosphine. Further investigations are needed to identify the entrance paths and to ensure that the rules of Commission Regulation (EC) 889/2008 are applied accordingly, also in Third Countries.

References:

Amstutz, Richard und Anton Knecht, Daniel Andrey. Detection of phosphine residues in (organic) cereals. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*. Bd. 94, 6/2003, S. 603-608.