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METHYL BROMIDE RESIDUES IN FUZZY COTTONSEED

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ABSTRACT

Methyl bromide (MB) is used in quarantine fumigation of fuzzy cottonseed exported from Australia, and we investigated whether MB residues are detectable in the oily commodity after fumigation and airing. Three analytical methods for determining MB residues in cottonseed were trialed: (i) the microwave method of Ren and Desmarchelier (1998), (ii) the modified extraction method of Desmarchelier *et al.* (1998) and (iii), the headspace method of Daft (1992; 1993). The microwave and extraction methods were found to be unsuitable for MB analysis in cottonseed. The headspace method was modified in the present study to improve the recovery of MB in the headspace and reduce the variability of the analysis. This was achieved by increasing the volume of extraction solution and amount of commodity, and by increasing the temperature of extraction. At a fortification level of 0.5 mg kg^{-1}, the recovery of MB from cottonseed solution increased from 22 to 32% and the variability was low (coefficient of variation 9%, n = 5) using the modified technique compared with the Daft headspace method. The residue in cottonseed fumigated in the laboratory with 32 g m^{-3} MB for 24 h was determined using the modified headspace technique after the sample was aired for 72 h. A mean MB residue of 3.4 mg kg^{-1} was detected in the fumigated cottonseed suggesting that MB residues may persist in bulk fumigated cottonseed after routine ventilation. The modified headspace method was found to be adequate for measurement of MB residues in commodities with high oil content.

INTRODUCTION

In a recent report by Norman (2000) persistent methyl bromide (MB) residues were detected in dried fruit, nuts and seeds many weeks after fumigation at 10°C using a recommended concentration time product (200 mg.h L^{-1}). This finding suggests that measurable MB residues may be present in unprocessed nuts, seeds and dry fruit at the point of consumption, especially where overdosing or multiple fumigation of the commodity has occurred. MB is used in quarantine fumigation of cottonseed exported from Australia. Hulled cottonseed contains around 33% fat (Copeland, 1976) and due to the high lipid content, persistent MB residues may occur following fumigation.

There are a number of published methods for fumigant residue analysis. Desmarchelier *et al.* (1998) modified the method of Daft (1987), a multi-residue

method based on extraction of the sample followed by back-extraction of leachate into *iso*octane. Modifications to the method involved performing the analysis in sealed conditions at all times and taking into consideration the partitioning of the fumigant between headspace and extraction solution. Desmarchelier *et al.* (1998) reported a mean recovery of 68% (13% coefficient of variation C.V.) for MB in wheat at a fortification level of 0.5 mg kg^{-1} compared with Daft (1987) who reported a mean recovery of 28% of fortified MB in corn and wheat. A significant improvement in MB recovery was made using the modified method compared with the original Daft (1987) method but the methods were concerned with grain analysis only. A method reported by Norman *et al.* (1995) used the same principle as the methods above by extracting the commodity into aqueous acetone for 24 h, derivatising a sub-sample of the leachate with sodium iodide, followed by automated headspace analysis by gas chromatography.

Ren and Desmarchelier (1998) used microwave treatment to release four fumigants, including MB, from grain. The method involved irradiation of the sample in a sealed flask using a domestic microwave oven at different power settings and time intervals. The power regime giving maximum release of aged residue was determined by measurement of released fumigant in the headspace using gas chromatography (GC). MB residues in wheat determined by the microwave method were virtually the same as the modified extraction method discussed above with a low coefficient of variation. The method is straightforward but has been evaluated for residues in wheat only and the suitability of the method for oil rich commodities, such as cottonseed, is not known.

Another approach to fumigant residue determination is the analysis of the headspace above blended samples (Daft, 1992; 1993). The headspace method for MB involves extraction of the sample blended in a sealed cup, followed by analysis of the headspace by gas chromatography with electron capture detection (GC-ECD). Daft (1993) reported MB recoveries from fortified samples of 28% for oilseeds (C.V. 39%) and 40% (C.V. 29%) for assorted nuts. No residues of MB were detected in the 18 samples analysed by Daft (1992) nor the 200 samples analysed by Daft (1993), at a quantitation limit of about 0.1 mg kg^{-1}. Although the headspace method is one of the few methods to have addressed MB residue analysis in oil-rich samples, it generally gives low recoveries and high variability in the analyses.

The methods described above were applied to the analysis of MB residues in cottonseed, utilising both freshly fortified samples and laboratory fumigated samples. In particular, the headspace method was modified to improve recovery and reduce variability of MB residue analysis in fuzzy cottonseeds.

MATERIALS AND METHODS

Materials

Untreated and unhulled cottonseed was supplied by Cottonseed Distributors Ltd, Wee Waa, Australia. In 'fuzzy' cottonseed, the layer of cotton remains on the hull of

the seeds after processing. MB of 99.5% purity was obtained from Matheson Gas Products Inc, USA.

Desmarchelier *et al.* (1998) modified extraction method

Cottonseed (50 g) was added to extraction solution (100 mL) consisting of acetone and 25% phosphoric acid, in proportions of 8:2, in a 250 mL Erlenmeyer flask equipped with glass sampling port and septum. MB was added by injection of the gas into flasks containing cottonseed and extraction solution to achieve concentrations of 3, 6 and 12 mg kg^{-1} cottonseed. Samples were extracted for 24, 48 and 72 h. Acidified salt solution (10 mL) and *iso*octane (1 mL) were added to a glass vial and sealed with a screw cap and septum. Air (1 mL) was removed from the vial and replaced by an equal volume of cottonseed extraction solution. The vial was shaken for one minute and left on ice until phase separation was complete. The upper *iso*octane layer of the solution was withdrawn and analysed by gas chromatography.

The headspace method - Daft (1992; 1993)

The headspace method of Daft (1992) was applied to the analysis of MB residue in cottonseed. The method was altered from the original by reducing all proportions of sample and extraction solution by one-half and blending in 0.25 L stainless steel blender cups in place of 1 L cups. Cottonseed (25 g) was added to the blender cup containing 0.5 M sodium sulfate solution (125 mL) and homogenised for 3 min in a sealed Waring laboratory blender cup fitted with a gas sampling port. All analyses were carried out at 25°C. For the recovery experiments, cottonseed was fortified with MB added at concentrations of 0.05 and 0.5 mg kg^{-1} cottonseed to the sealed blender cup before blending.

The modified headspace method - Daft (1992)

To a stainless steel 1.25 L blender cup was added 0.5 M sodium sulfate extraction solution (625 mL) and fuzzy cottonseed (125 g). All equipment and the extraction solution but not cottonseed was heated to 65°C prior to analysis. Badawy (1992) selected 60°C as an optimal parameter to improve MB headspace recovery from water samples. Before being used for residue analysis, sealed blender cups had been checked for leakage of air by immersion in hot water. Immediately after closing the lid or after addition of MB in the case of spiked samples, the solution was blended for three min on low speed using a Waring laboratory blender. In trial experiments it was observed that blending for more than 3 min and up to 6 min did not result in a substantially finer homogenate. Therefore, a blending time of 3 min was chosen as providing a fine homogenate but minimising the potential breakdown of MB residues due to prolonged homogenisation. Following blending, samples were stabilised for 10 min at 65°C and then the headspace was sampled for analysis by

GC - ECD. Injections were preformed using a gas-tight syringe with a pressure valve to prevent loss of gas due to the heated headspace gas in the blender cup.

For recovery experiments, samples were fortified by adding MB by gas-tight syringe via the sampling port before blending to achieve concentrations of 0.5 mg kg^{-1} and 5.0 mg kg^{-1} cottonseed. Five replicates at each fortification level were analysed. The partitioning and stability of MB in headspace above extraction solution, or in headspace above blended cottonseed was examined at a single fortification level (0.63 mg MB), injected into the headspace before blending. Five replicates of the partition experiment were carried out in each medium.

Laboratory fumigation of cottonseed

A laboratory-scale fumigation of cottonseed was conducted for the analysis of MB residue. Untreated fuzzy cottonseed (538 g) was added to a 2.7 L desiccator equipped with magnetic stirrer. The volume of the uncompressed cottonseeds gave a 54% filling ratio. The desiccator was sealed and the equivalent of 32 g m^{-3} of MB was added to the headspace, calculated on the basis of the empty volume. Stirring was continued for 2 min after fumigant was added and prior to sampling for GC analysis which was carried out during the fumigation. After 24 h fumigation the lid was removed from the desiccator and the cottonseeds were aired in a fume cabinet for 72 h. Cottonseed was then placed in sealed stainless steel containers and stored at -20°C to prevent loss of fumigant prior to analysis. Residue analysis was performed 48 h after storage.

GC and data analysis

Samples were analysed using a Varian 3300 gas chromatograph equipped with a 30 m x 0.53 mm i.d. GS-Q column (J&W Scientific, USA). For analysis of the *iso*octane layer, the initial column temperature was 125°C for 2 min, increasing to 150°C at 30°C/min, then held at 150°C for 1 min. One to two microlitre injections were carried out. The injection port was held at 160°C and the detector at 300°C. In the case of headspace injections, 40 □L of gas was injected into the instrument held at an oven temperature of 125°C. Under these conditions the retention time for methyl bromide was 2.0 min. Calibration standards of MB were prepared in sealed flasks over the concentration range of 0.02 to 1.00 g m^{-3} (5 points). The response to MB was linear over the range 0.05 to 1.00 g m^{-3} with typical R^2 values of 0.9992 to 1.000. At 0.02 g m^{-3} the signal-to-noise ratio exceeded 50. One aspect that affected the analysis of headspace MB was the accidental aspiration of blended solution into the syringe that may occur when the foam generated by blending fills the headspace area. When injected into the gas chromatograph, the contaminants tended to dampen the response of the ECD.

Methyl chloride is a potential reaction product of MB and blended cottonseed however the column and conditions used for the analysis separated the two compounds completely.

The MB residue in laboratory fumigated cottonseed was determined by applying a correction factor to the measured headspace concentration of a blended sample. The correction factor was derived from the recovery of MB in fortified standards added at a similar concentration to that of the natural residue level. The recovery calculation (%) for fortified standards was made as follows:

$$\text{recovery (\%)} = \frac{\text{amount of MB in headspace above slurry x 100}}{\text{amount of MB added}}$$

The residue level in laboratory fumigated cottonseed was calculated by:

Amount of MB in headspace overslurry x correction factor = total residue present

RESULTS

Modified Daft (1987) extraction method

Two difficulties arose with the use of this method for MB residues in fuzzy cottonseed. At first, it was impossible to immerse the cottonseed in the extraction solution, as they floated on the surface. The seeds did soak in over time but then absorbed most of the solution such that half of the seeds had expanded above the extraction solution level. More importantly, no detectable residue of MB from the fortified samples was partitioned into the *iso*octane layer and hence detected by GC-ECD, even at the maximum fortification level of 12 mg kg^{-1}. Extraneous compounds were also extracted from the cottonseed leachate and these caused interference in the analysis of MB. However, small quantities of MB were detected in the headspace of the fortified samples although the responses for the lowest fortification concentration (3 mg kg^{-1}) were small. The concentrations detected in headspace for the fortified samples were consistent over the 72 h extraction period.

The headspace method – Daft (1992; 1993)

Cottonseeds were fortified with MB at 0.5 and 5.0 mg kg^{-1} and then analysed using the headspace method. The mean percentage recoveries of MB were 22 and 23% for the 0.5 and 5.0 mg kg^{-1} fortification levels, respectively. That is, only 22% of total MB added to the blender cup was present in the headspace above blended cottonseed. No MB peaks or interfering substances were found in the chromatograms of the non-fortified cottonseed samples.

The modified headspace method of Daft (1992)

Partitioning of MB in the headspace above extraction solution, and in the headspace over blended cottonseeds was studied in the blender cup after processing of the solution. The concentration of MB in the headspace decreased with time over 60 min as shown in Fig. 1. In the presence of extraction solution only, half of the added MB was partitioned into the headspace after 10 min at 65°C. With blended cottonseed,

32% of the total added MB was present in the headspace, substantially lower than extraction solution alone. For blended sunflower seeds or oilseed rape, the amount of MB in the headspace was even lower, 13 and 14% respectively, of the added amount.

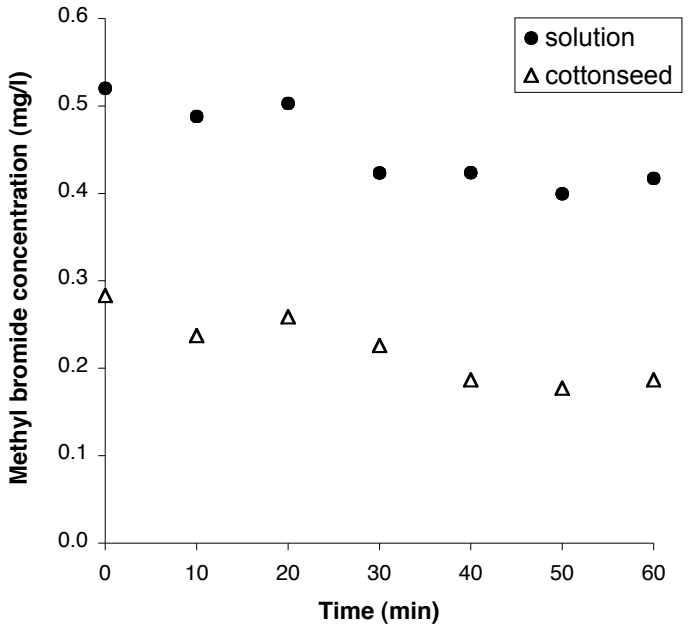

Fig. 1. Change in headspace methyl bromide concentration over sodium sulfate solution (solid circles) or blended cottonseed solution (open triangles) with time at 65°C.

The recoveries of MB at two fortification levels (0.5 and 5.0 mg kg^{-1}) from blended cottonseed solution shown in Table 1. Both fortification levels gave similar recovery values although the variability of analysis was higher at 0.5 mg kg^{-1}. The effect of temperature on recovery of MB added to cottonseed solution (at 5.0 mg kg^{-1}) using the modified headspace method was also examined. When the processing was conducted at 25°C, the recovery of MB in the headspace above cottonseed mixture was 8.8% one minute after processing and fell to 6.4% after 60 min.

TABLE 1
Percentage recovery and of methyl bromide from fortified samples of blended cottonseed using the modified headspace method

	Fortification level in cottonseed	
	0.5 mg kg^{-1}	5.0 mg kg^{-1}
Mean recovery (%)*	31.5	32.3
Coefficient of variation (%)*	9.2	4.9

* n = 5 determinations at each fortification level

Laboratory fumigation of fuzzy cottonseeds with methyl bromide

During the laboratory scale fumigation of cottonseed with MB the headspace was analysed for fumigant. The MB concentration fell rapidly from above 58 to 27 g m^{-3} within one hour of fumigant application, as shown in Fig. 2. After 24 h fumigation, the headspace MB concentration was approximately 8 g m^{-3}. Almost three-quarters of the applied MB was sorbed by the cottonseed during fumigation. Four samples of the fumigated and aired cottonseed were analysed for MB residue using the modified headspace method. A mean residue concentration of 3.4±0.2 mg kg^{-1} was obtained for the fumigated samples.

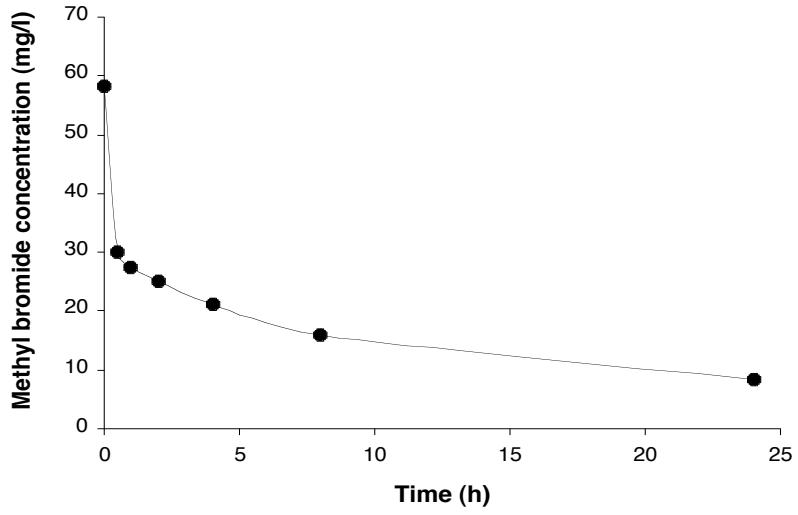

Fig. 2. Loss of methyl bromide from the headspace during a laboratory-scale fumigation of fuzzy cottonseed at 32 g m^{-3} for 24 h at 25°C.the fumigated and aired cottonseed were analysed for MB residue using the modified headspace method. A mean residue concentration of 3.4±0.2 mg kg^{-1} was obtained for the fumigated samples.

DISCUSSION

It is evident from Norman (2000) that MB residues may persist in oil-rich commodities for at least 10 weeks when stored in open conditions. Daft (1992; 1993) described the analysis of a large variety of foods but did not detect any MB residues. To some extent this result may be due to the low recoveries and high variability of the headspace method when applied to oily foods. The detection of substantial MB residue in laboratory fumigated and aired cottonseed (3.4 mg kg^{-1}) in this study indicates that MB residues may be present in fumigated bulks of cottonseed after airing and processing. Cottonseed rapidly sorbed MB in the laboratory scale fumigation (Fig. 2).

The measurement of MB residue in oil-rich commodities is difficult due to the highly lipophilic character and high volatility of MB. Fuzzy cottonseeds have an oily inner seed, a dry fibre covered cellulose outer-shell, which increase the difficulty of residue analysis and a pocket between seed and coat, which aids buoyancy of the seed. Three different methods were applied to the analysis of MB residues in cottonseed.

The modified Daft (1987) method for fumigant determination utilising extraction in aqueous acetone was found to be unsuitable for determination of MB residues in cottonseed. In fortified samples, no MB was detected in the *iso*octane layer although a small quantity of MB was present in the headspace above the leachate. MB was expected to partition into the leachate and undergo back-extraction into *iso*octane. Extraneous material that was extracted into *iso*octane, caused some interference in the GC-ECD analysis.

The microwave method of Ren and Desmarchelier (1998) was attempted for analysis of residues in cottonseed from fortified and fumigated samples but was not investigated in detail. As the cottonseeds were sequentially irradiated with microwave energy MB was liberated into the headspace as indicated by the increasing GC peak. This increase was followed by a rapid falling-off of MB peak size with increasing microwave irradiation time indicating the breakdown of MB residues in the flask. The power regime required to liberate maximal MB residues from the cottonseed differed widely among samples of fumigated commodity (from 50 to 130 sec). This result may have been related to MB residue concentration or the oil content of the individual sample (data not shown). While the microwave method is rapid for analysis of MB residues, in our hands it was difficult to find a standard irradiation time for cottonseed, as each sample appears to require a different level of irradiation to achieve maximal release of the fumigant.

The modified headspace method gave the highest recoveries of MB and low variability (Table 1), and was superior to the modified extraction and headspace methods. The modifications to the headspace method included decreasing the headspace volume and increasing the analysis temperature to improve partitioning into the headspace. Badawy (1992) has used temperature of 60°C as a one of parameters to improve MB headspace recovery from water samples. The use of

temperatures above 65°C may result in higher headspace recoveries of MB but the maximum temperature that could be safely handled with our equipment was 65ºC.

MB partitions to a greater extent into whole or blended cottonseeds than into the headspace. MB was not detected in cottonseed leachate in samples fortified with MB although it was expected to be present. Desmarchelier *et al.* (1998) examined the partitioning of MB between aqueous acetone extraction solution and headspace in a sealed flask and found the ratio to be 0.75:0.25. However, the partitioning of MB is clearly different in the presence of cottonseed and this may preclude the use of analytical methods based on MB extraction into aqueous acetone. In the modified headspace method, the portion of MB present in headspace over blended cottonseed was one-third of the original amount added at 65°C (Fig. 1), demonstrating the high solubility of MB in homogenised cottonseed.

Although the detection limit for MB residue in cottonseed by the modified headspace method was not examined in this study, it can be estimated from the partitioning of MB in the headspace and the detection limit for MB by GC-ECD. Using these parameters, MB residues of 0.1 mg kg^{-1} and above could be easily detected by the modified headspace method. The method is not rapid nor is it the easiest method developed for MB residue analysis. It produces a large volume of waste and where there is large amount of fibre still present on cottonseed, blending of the sample can be difficult. The modified headspace method was adequate for the analysis of MB residues in cottonseed. However future analytical development should focus on improving the recovery of MB from oil-rich commodities.

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