

Zeitschrift: Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 67 (1976)

Heft: 4

Artikel: Determination and occurrence of ethylene chlorohydrin residues in foodstuffs fumigated with ethylene oxide

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DOI: <https://doi.org/10.5169/seals-982965>

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Determination and Occurrence of Ethylene Chlorohydrin Residues in Foodstuffs Fumigated with Ethylene Oxide

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Introduction

Ethylene oxide (EO) is widely used for sterilization of certain food materials including dehydrated mushrooms, vegetables and spices. The sterilization mechanism of the compound can largely be explained by its reaction with the protein of the cells of the micro-organisms whereby labile hydrogen atoms in OH-, COOH-, NH₂- and SH-groups are replaced by a hydroxyethyl group (1). Ethylene oxide is usually applied as a fumigant, mixed with carbon dioxide, chlorofluorohydrocarbons or methyl formate, to provide non-explosive conditions. The fumigation treatments are normally carried out in specially designed vacuum chambers.

The high reactivity of EO towards all acid hydrogen atoms suggest that the natural constituents of foodstuffs may also react to some extent with the fumigant. However, relatively few data have been published on this subject.

According to the present state of knowledge, possible present residues in a foodstuff after treatment with EO can be listed as follows:

1. EO retained by physical adsorption, which may persist during not more than a few weeks before its disappearance by volatilization or reaction with the natural constituents of the fumigated commodity (2, 3, 4).
2. Ethylene chlorohydrin formed by the interaction of EO with the inorganic chlorides present in a foodstuff (5, 6, 7, 8), and ethylene bromohydrin formed in a similar way by the reaction of EO with inorganic bromide, introduced by previous treatment of the commodity with methyl bromide (9).
3. 1,2-diol-monoesters by reaction of EO with lower fatty acids (10).
4. Small amounts of ethylene glycol and of diethylene glycol produced by the reaction with water under conditions of excessive fumigation (4, 11).
5. Hydroxyethylated derivatives of amino acids, proteins, vitamins, alkaloids and sugars (12, 13, 14, 15, 16, 17).

The food analyst needs a rapid and simple analytical procedure which enables him to identify and quantitate the possible present residues, but it would be most

difficult and unpractical to consider all the possibilities listed above. It is, therefore, necessary to make a selection.

In some countries, e. g. in Holland and Belgium, tolerances have been set for residual ethylene oxide. This is rather unrealistic, because it is most unlikely that foodstuffs reaching the consumer will still contain detectable amounts of the unchanged fumigant (4). Moreover, the determination of free EO is not a simple matter. The classic volumetric procedure of *El Kishen* (2) is not suitable for the detection of small amounts and the recently developed gas chromatographic methods (4, 18) are not specific.

The determination of ethylene glycol and diethylene glycol at ppm levels is feasible with gas chromatographic procedures (4), but it is hardly worthwhile to analyse foodstuffs for these residues as a matter of routine. In fact, detectable amounts of ethylene glycol and its dimer have rarely been found, except in dried fruits (11) and to a lesser extent in flour (4).

So far, only one report exists on the formation of a 1,2-diol-monoester, i. e. ethylene glycol monoacetate, formed in cocoa powder and cocoa beans by interaction of EO with acetic acid (10). The presence of this compound in the said commodities can be considered as an infallible sign that they have received EO treatment. It has not yet been found in other fumigated foodstuffs.

The determination of hydroxyethylated derivatives of amino acids, amines, alkaloids, vitamins and sugars in EO treated foodstuffs is too complicated for routine work. There is no doubt that these food constituents react with EO as has been demonstrated in model studies, but quantitative data on the derivatives actually present in fumigated foodstuffs are not available. It should also be pointed out that the reactivity of amines, amino acids and alkaloids with EO differs considerably even between related compounds. For example, several alkaloids are easily converted to quaternary N-beta hydroxyethylated derivatives as was found by *Terlinden* (16), but this is not the case with the purine bases which are present in coffee and cocoa: model studies performed in the laboratory at La Tour-de-Peilz indicated that caffeine and theobromine hardly react with EO at room temperature, not even when dispersed in water. Moreover, analyses carried out before and after excessive fumigation of cocoa beans and green coffee on a laboratory scale did not give any significant difference in their purine base content. This observation is in contradiction with the analytical results of *Kröllner* who reported a virtually total destruction of «alkaloids» in cocoa powder and a 30 percent reduction in the caffeine content of green coffee after EO treatment (3).

Ethylene chlorohydrin (ECH) is the only fairly stable and easily detectable compound formed when foodstuffs are treated with EO. Although a number of papers have been published on the determination of ECH (4, 5, 6, 7, 8), no validation studies on the different analytical procedures have been undertaken. Moreover, nearly all work on the formation of this compound has been done on a restricted number of raw materials subjected to small scale treatment with EO in the laboratory. This is also the case with ethylene bromohydrin (EBH). No information is available on the ECH and EBH contents of foodstuffs moving into commerce.

In this paper the authors have described and evaluated a simple method for the routine analysis of ECH. Although evidence on the occurrence of EBH residues is scarce, provisions are included for the simultaneous determination of this compound.

The procedure is based on the solvent extraction method for fumigant residues of *Heuser* and *Scudamore* (18), but it also includes guidelines for the determination of both halohydrins without gas chromatographic equipment. Identity of the compounds is confirmed by a selective derivatization procedure.

Furthermore, the authors report the ECH contents found in a number of commercially available raw materials. In addition, the formation of ECH residues in small scale laboratory fumigation experiments and the persistence of the compound in various commodities have also been studied.

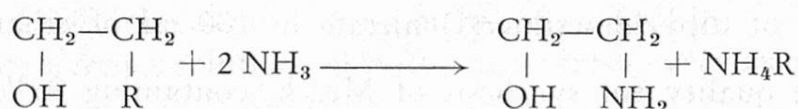
Method

Field of application

The method can be applied to all foodstuffs likely to have received ethylene oxide treatment. These are, among others, spices, dehydrated vegetables, -fruits and -mushrooms, flours and cocoa powder.

Principle of the method

Ethylene chlorohydrin (ECH) and ethylene bromohydrin (EBH) are isolated by cold extraction with a mixture of acetonitrile-water and determined by gas chromatography with a flame ionization detector. Confirmatory analysis is carried out after steam distillation by derivative formation with ammonia whereupon both halohydrins undergo the following reaction:



R = Cl or Br

ethanolamine + ammonium halogenide

The derivatives, ethanolamine, ammonium chloride and ammonium bromide are chromatographed in two suitable thin-layer chromatographic systems.

Laboratories who do not dispose of gas chromatographic equipment may perform a semi-quantitative determination by thin-layer chromatography of the derivatives formed after treatment of the distillate with ammonia.

Reagents

Acetonitrile

Test for absence of interfering impurities by simply injecting 3 microlitres of the solvent into the gas chromatograph under conditions described under section gas chromatography. If impurities are observed, purify by adding 1 g of silver nitrate p. a. to 500 ml and distilling in an all-glass apparatus, collecting the fraction which distills at 81—82°C.

Extraction mixture

Add 1 volume of distilled water to 5 volumes of purified acetonitrile.

Ammonia solution, 25 percent, p. a. quality.

Acetone, p. a.

Acetic acid, purum quality, should contain at least 95 percent of CH_3COOH .

1-Butanol, any purum quality of Fluka or Merck is suitable.

Ethanol 96 % p. a.

Chloroform p. a.

Ninhydrin, triketohydrindene monohydrate, p. a.

Silver nitrate p. a.

Pyridine p. a.

Dipyridinesilver(I) nitrate

Dissolve 8,5 g of dried powdered silver nitrate in 10 ml of pyridine. Allow to cool at about 4°C and gather the crystalline solid on a small buchner funnel. No further purification is necessary.

Chromogenic reagent for the TLC of halogen anions

Dissolve 0,5 g of dipyridinesilver(I) nitrate in 100 ml of ethanol.

Ethanolamine, the quality for synthesis of Merck, containing 99% by titration, is suitable.

Chromogenic reagent for the TLC of ethanolamine

Dissolve 0,3 g of ninhydrin in 100 ml of 1-butanol. Add 3 ml of acetic acid. Store in a brown bottle.

Ethylene chlorohydrin, 2-chloroethanol, ECH, boiling point 127—129°C. Purum quality of Fluka.

Ethylene bromohydrin, 2-bromoethanol, EBH, boiling point 149°C. Purum quality of Fluka.

N. B.: Handle these very toxic compounds with utmost care. Do not inhale the vapour and prevent any contact with skin. Wash off with water immediately even the smallest quantities that, in spite of precautions, have come into contact with the skin.

Reference solutions for gas chromatography

Prepare solutions of ECH and EBH containing 1,00 mg pro ml in acetonitrile-water, 5:1 v/v. From these stock solutions prepare dilutions in the same solvent mixture with concentrations of 0,25, 0,10, 0,05, 0,01 and 0,005 mg/ml. These final reference solutions contain respectively 250, 100, 50, 10 and 5 nanograms of ECH and EBH pro microlitre.

Prepare fresh every two weeks.

Reference solutions for thin-layer chromatography

a) Ethanolamine

Prepare an ethanolic solution of this compound containing 1 mg/ml. Dilute an aliquot of this stock solution 20 times with ethanol. This final reference solution has a concentration of 0,05 mcg pro microlitre.

Prepare fresh every month.

b) Chloride anion

Dissolve 105,1 mg of dried potassium chloride p. a. in 5 ml of distilled water. Use four 5 ml portions of water to transfer the solution quantitatively to a 50 ml volumetric flask. Make up to volume with acetone and shake well to mix. This stock solution contains 1 mg of Cl' pro ml. Prepare the final reference solution containing 10 ng pro microlitre by diluting an aliquot of the stock solution a 100 times with acetone-water 1:1 v/v.

c) Bromide anion

Dissolve 74,5 mg of dried potassium bromide p. a. in 5 ml distilled water. Prepare the stock solution and the final reference solution as described under b). The final dilution contains 10 ng of Br' pro microlitre.

Prepare the reference solutions of both anions fresh every two weeks.

Equipment

Gas chromatograph, equipped with a flame ionization detector.

Column for gas chromatography of ECH and EBH

Use a 5 feet long stainless steel column with an inside diameter of $\frac{1}{8}$ inch filled with 10% Carbowax 1540, coated on solid support Chromosorb W, 60—80 mesh, HMDS-treated.

Weigh 2 g of Carbowax 1540 in a beaker. Dissolve in chloroform and transfer to a 1 litre round bottomed flask. Add 18,0 g of solid support, swirl the flask and allow to stand for 15 minutes. Connect the flask to a rotavapor apparatus and remove the solvent slowly, using a 40°C water-bath and slight vacuum. Avoid foaming of the mixture. When solids appear damp, increase vacuum. Remove the last traces of chloroform by heating the powder in a stove at 100°C. Use only free flowing powder for column preparation.

Prepare the column in the usual way and condition at 150°C with a nitrogen flow of at least 30 ml/min during 36 hours.

Syringe for GLC

Any type suitable to deliver exact volumes from 1—10 microlitres.

Special distillation apparatus for isolation of ECH and EBH

See figure 1. Any skilled glass blower will be able to furnish these apparatus. At least 6 specimens are required for routine analyses.

Waterjet pump, equipped with a mercury manometer.

Glass beads, 5 mm diameter.

Ready made plates for thin-layer chromatography

- For ethanolamine: TLC aluminium sheets, coated with silica gel, 0,25 mm thickness, 20×20 cm, from Merck. Catalogue number 5554/0025
or, alternatively
TLC aluminium sheets, coated with cellulose, 0,1 mm thickness, 20×20 cm, from Merck. Catalogue number 5552/0025.
- For bromide and chloride: aluminium oxide, type E, f 254, alufoil sheets, 20×20 cm, from Merck. Catalogue number 5550/0025.

Ultraviolet lamp for photochemical revelation of bromide and chloride on TLC

Most commercial available lamps have sufficient energy for this purpose, provided that their filter is removed.

Micropipettes for chromatography, graduated from 1—10 microlitres and ditto from 1—50 microlitres.

All glass tanks for developing chromatograms of 20×20 cm.

Various glassware.

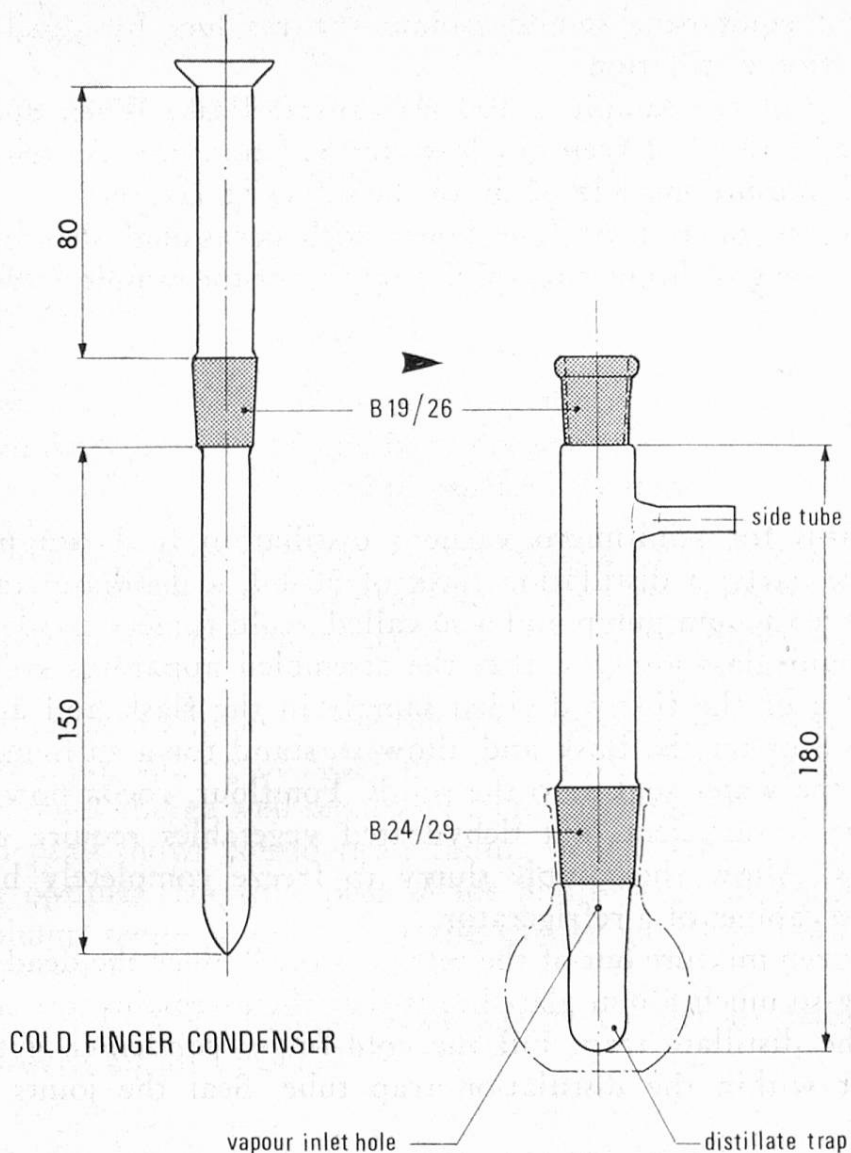


Figure 1. Vacuum distillation apparatus for isolation of ECH

Procedure

Preparation of the sample

Grind whole spices such as pepper, cardamon, turmeric, etc. to obtain a more or less fine powder. Extreme fineness is not essential, but the ground sample should be free of large fragments.

Cut dried fruits, leafy spices (herbs), dehydrated vegetables and mushrooms in very small pieces.

Solvent extraction

This extraction method should only be used by laboratories using gas chromatography for the determination step. Laboratories that do not dispose of gas

chromatographic equipment should isolate the residues by distillation as described under section distillation.

Weigh 5,00 g of the sample a 100 ml conical flask. When analysing flours, spices or nuts, add 30 ml of acetonitrile-water 5 : 1 mixture. To dehydrated fruits, -vegetables and -mushrooms add 60 ml of the solvent mixture.

Allow to stand for at least four hours with occasional shaking and analyse according to section gas chromatographic analysis of the sample extract.

Distillation

The apparatus for semi-micro vacuum distillation is shown in figure 1. It consists of three parts: a distillation flask of 50 ml, a distillate trap with a side tube to connect a vacuum pump and a so called «cold finger» condenser. All three parts have ground glass joints so that the assembled apparatus will be gas-tight.

Weigh 1,00 g of the finely divided sample in the flask and add 3,00 ml of distilled water. Stopper the flask and allow to stand for a sufficiently long time in order to let the water soak into the solids. For flour, cocoa powder or ground spices one hour is sufficient, but dehydrated vegetables require about 4 hours for rehydration. Allow the sample slurry to freeze completely by placing the flask in the cold cabinet of a refrigerator.

Take the frozen mixture out of the refrigerator. Reduce the dead volume in the flask by adding so much 5 mm glass beads that there remains just sufficient room to introduce the distillate trap. Fill the cold-finger condenser with crushed ice and position it within the distillation trap tube. Seal the joints with vacuum grease.

Before the sample in the flask has thawed, connect the assembled apparatus to a waterjet pump and evacuate.

Check the vacuum with a mercury manometer; the final pressure should be about 60 mm. Close the connection to the pump by means of a screw clamp, preferably positioned immediately after the side tube.

Disconnect the evacuated apparatus from the pump, incline it about 45° from the vertical and place it in a beaker of boiling water. Keep the flask half-way up to the neck under the water surface and slowly rotate the apparatus.

Due to the very low pressure in the flask, distillation begins immediately, the vapours pass through the inlet hole and condensate collects in the trap.

When all the ice in the condenser has melted, loosen the screw clamp, thus allowing air to enter. Remove the flask from the water-bath and let the apparatus stand vertically in order to let the droplets of condensate drain into the trap. After 30 minutes, remove the condenser and transfer the distillate by means of a pipet to a small glass bottle.

A good yield of distillation is 1,5—2 ml, although lower volumes may be analysed without difficulty.

Analyse the distillate according to section derivatization procedure.

Gas chromatography

1. Adjustment to optimal operation conditions

Recommended operation conditions for a 5 feet \times $\frac{1}{8}$ inch 10% Carbowax 1540 column are:

— injection temperature	175°C
— column temperature	95°C
— detector	200°C
Carrier gas flow	30 ml of nitrogen/min
Flame ionization detector	30 ml of hydrogen/min
	300 ml of purified air/min

Set range and attenuation at positions providing a sensitivity of about 20 times less than the maximum possible. For example, if the electrometer of the apparatus indicates a maximum possible sensitivity of $0,1 \times 1$, set the range at position 1 and the attenuation at 2.

Inject a 3 microlitre aliquot of the reference solution for gas chromatography (page 407) containing 100 ng of ECH pro microlitre. After about 5 minutes the peak of ECH should emerge well separated from the slope of the solvent peak.

If the ECH peak shows considerable tailing or if it elutes too early, the conditions are not optimal. Improve peak shape and/or time of elution by slightly altering the column temperature and/or the carrier gas flow until the optimum conditions are found.

If necessary, adjust the hydrogen flow again in order to maintain the ratio carrier gas/hydrogen/air an 1 : 1 : 10.

2. Determination of the minimum detectable quantity

Set range and attenuation at a sensitivity at which the vibration of the recorder pen does not exceed 2 percent of full scale deflection. At this high sensitivity inject 3 microlitres of the standard solution of ECH containing 5 ng pro microlitre. If instrument performance and chromatographic conditions are good, those 15 ng will yield a measurable peak which exceeds at least 10 times the noise level. If this is the case, inject lower quantities until the peak height obtained is only four times over noise level. This last quantity represents the minimum detectable amount.

Repeat the procedure for finding the minimum detectable amount of EBH. This compound elutes in approximately 8 minutes.

3. Gas chromatographic analysis of the sample extract

Set range and attenuation at positions necessary for visualizing the minimum detectable quantity (see under 2.). Inject 3 microlitres of the extract obtained under the section solvent extraction.

The following chromatograms may be obtained:

- a) No ECH or EBH peaks are observed, i. e. no peaks exceeding four times the noise level.

In this case the injected amount contains less than the minimum detectable quantity of both compounds. The result is, therefore, negative and the extract can be discarded.

- b) The chromatogram shows a definite ECH and possibly also an EBH peak at or over four times the noise level, but remaining on the scale.

Wait until possible present peaks with longer retention times than that of EBH have emerged and inject a suitable quantity of the appropriate reference solution(s). Compare the size of the residue peak with the size of the peak produced by the standard. Sufficient accuracy is achieved when simply using peak height (expressed in mm) for quantitation.

- c) An ECH and possibly also an EBH peak is eluted which exceeds the 100 percent mark on the recorder chart.

Quickly decrease the sensitivity as much as is necessary to keep the peak within the scale. The final attenuator setting indicates the appropriate sensitivity for quantitative evaluation of the peak. Wait until possible present higher boiling compounds have been eluted and repeat the injection at the appropriate sensitivity.

Subsequently, inject a 3 microlitre aliquot of a suitable reference solution. The degree to which the sensitivity was lowered should be taken into account: if only two times, inject 0,15 mcg, if four times 0,30 mcg, etc.

Determine the ECH and possibly also the EBH content of the injected volume of sample extract by comparing the size of the residue peak to the size of the peak from the standard.

Remarks:

1. Although the extracts obtained from most foodstuffs are relatively pure, those of spices as, for example, pepper and turmeric, generally contain appreciable quantities of essential oils of which the components elute with long retention times. This renders the time of waiting between injections often as long as 40 minutes.
2. It should be pointed out that residues arising from other fumigation treatments may also be present in the sample. If the foodstuff was sterilized with propylene oxide, the corresponding isomers of propylene chlorohydrin (formed by interaction with inorganic chloride) will also be visualized on the chromatogram. In that case, peaks will be observed at (ECH = 1,00) 0,68 and 0,91.

Although sterilization treatments with propylene oxide are seldom practiced in Europe, the possibility of finding residues derived from such a treatment cannot be altogether excluded.

If the gas chromatographic result is positive for ECH and possibly also for EBH, the identities of these compounds should be confirmed. As the acetone-water sample extracts are too impure to be subjected to confirmatory procedures, prepare a distillate of the sample as described under section distillation.

Derivatization procedure

Pipette accurately 1 ml of the distillate obtained under section distillation into a 5 ml glass bottle. Add 0,10 ml of concentrated ammonia and stopper the bottle tightly. Heat during 90 minutes in a stove at 60°C. Let cool and add by means of a calibrated pipette 0,9 ml of acetone. Mix by swirling and keep well closed until thin-layer chromatographic analysis.

Thin-layer chromatographic determination of the derivatives — Confirmation of identity

The derivatization procedure outlined above converts both ECH and EBH to ethanolamine. In addition, ECH yields ammonium chloride and EBH ammonium bromide. All three derivatives are determined by thin-layer chromatography (TLC).

1. TLC of ethanolamine

Laboratories who do not dispose of gas chromatographic equipment should first run a chromatogram for orientation purposes: spot at regular intervals 1, 5, 10, 25 and 50 microlitres of the derivative solution on a ready made plate of silica gel (page 408).

Quantities over 10 microlitres should be spotted slowly with between times drying of the spot with a fön (hair dryer).

For best results, keep size of spotted quantities as small as possible.

For comparison, spot 0,05 and 0,50 mcg aliquots of the reference solution of ethanolamine (page 407).

Laboratories who have already obtained a gas chromatographic result, will have no difficulties in adjusting the aliquot of the derivative solution to give a spot within range of 0,05—0,50 mcg (1 mcg of ECH will yield 0,76 mcg of ethanolamine). For example, a quantity of ECH found close to the minimum detectable amount will require the spotting of approximately 50 microlitres of derivative solution in order to obtain a visible spot. On the other hand, if GLC indicated a quantity of 1000 ppm, an aliquot of 2 microlitres is amply sufficient.

Apply the reference solution of ethanolamine to give spots of 0,05, 0,10, 0,15, 0,20, 0,25, etc. until 0,50 mcg.

Develop the plate by ascending migration in a presaturated tank using ethanol 96% — ammonia solution 25% 4:1, v/v as a mobile phase. The time for migration over a distance of 15 cm will take approximately 3 hours. Remove the plate from the tank and let evaporate completely the adherent solvent.

Spray with the chromogenic reagent (page 406), using lateral motions of the spray bottle perpendicular to the direction of solvent flow. After spraying, heat the plate for 5 minutes at 60°C. Ethanolamine will yield reddish violet spots at a Rf-value of 0,40.

The revelation may be considered as satisfying if 0,05 mcg of the reference compound is clearly visible. If this is not the case, visibility may be improved by spraying the plate lightly with distilled water and subsequent heating at 60°C.

Possible present propylene chlorohydrin isomers will have been converted to their corresponding propanolamines during the derivatization procedure. If present, they will yield a single spot at Rf 0,60.

N.B.: In some exceptional cases the interpretation of the chromatogram may be obscured by a curtain effect, i. e. upon spraying with ninhydrin and heating, the whole lower part of the chromatogram turns reddish. The source of this troublesome phenomenon is not completely understood, but it is probably caused by an interaction between ammonia and impurities in the silica gel layers. In that case, it is recommended to repeat the analyses on cellulose ready made plates (page 408), using 2-propanol-acetic acid-water 5:1:4 v/v as a mobile phase.

The time for development in this system is rather long, but it is sufficient to have a migration distance of only 8 cm. When the chromatogram is treated with ninhydrin reagent as described above, ethanolamine is revealed as a dark violet spot at Rf 0,78.

As the limit of detection on cellulose is about 10 ng, i. e. 5 times better than that observed on SiO₂, the quantities of sample and reference solutions to be spotted may be correspondingly lowered.

2. TLC of chloride and bromide

The application of the sample aliquots is performed as described under 1., but the volume applied should represent a quantity of chloride and/or bromide in the range of 10—200 nanograms. Spot reference solutions chloride anion and bromide anion accordingly.

Adsorbent: Al₂O₃E ready made plates 20×20 (page 408).

Mobile phase: acetone-n-butanol-25 percent ammonia-water 65—20—10—5 v/v.

Before use, allow the mobile phase to saturate the chromatography tank overnight.

When the mobile phase has reached the front line, remove the plate from the tank and let the adherent solvent evaporate. Spray abundantly with the chromogenic reagent for the TLC of halogen anions. Place the chromatogram under the ultra-violet lamp with removed filter and irradiate until 10 nanograms of bromide

are clearly visible. If visibility leaves to be desired, spray the chromatogram lightly with distilled water and irradiate again. Chloride and bromide appear as dark spots with R_f-values of respectively 0,35 and 0,55.

Evaluation of the chromatograms

After having made chromatograms for orientation purposes, inspect carefully the different aliquots for presence of a spot of a derivative.

If even the 50 microlitre aliquots show absence of spots, the result may be considered as negative. On the other hand, if the result is positive for ethanolamine and chloride, and negative for bromide, the sample contains ECH. In that case, repeat only the chromatography for ethanolamine by applying that volume which has yielded a spot between 0,05 and 0,50 mcg. It goes without saying that a suitable range of standards should also be applied.

If the result is also positive for bromide, the sample contains ECH and EBH residues. Repeat chromatography of the appropriate aliquots in both systems.

Estimate the approximate quantity of each of the derivatives by comparison with the spots given by different quantities of the reference compounds.

Calculation

From GLC results

The calculation of the ECH and EBH content of a foodstuff can best be illustrated by the following example:

sample size 5,00 g. Volume of extract 30 ml.

3 microlitres injected into the gas chromatograph yielded a peak corresponding with 0,15 mcg of ECH.

This equals 1500 mcg/30 ml/5 g of sample.

1 ppm of 5 g is 5 mcg

ECH concentration in sample is $\frac{1500}{5} = 300$ ppm

From TLC results

If result was only positive for ECH, calculate the ECH content from the found quantity of ethanolamine using the following equation:

$$1,32 \frac{2 A \frac{1000 V}{b}}{P} = \text{ppm of ECH in sample}$$

$$1,32 = \text{stoichiometric factor} \frac{\text{molecular weight ECH}}{\text{molecular weight ethanolamine}}$$

- A = quantity of ethanolamine estimated on the chromatogram, expressed in mcg.
V = volume of water used for distillation in ml.
b = aliquot of derivative solution applied to the chromatogram expressed in microlitres.
P = weight of 1 ppm of the quantity of sample expressed in mcg (mostly 1 mcg).

If the result was positive for both ECH and EBH, calculate both compounds according to the same formula, using the stoichiometric factors 2,26 for conversion of Cl' to ECH and 1,56 for Br' EBH. It goes without saying that in that case A represents the quantity of Cl', respectively of Br' estimated on the chromatogram.

N. B.: It is most unlikely that samples treated with ethylene oxide should only contain EBH as a residue.

For very accurate determinations it is necessary to apply a correction factor to the yield of the distillation. This factor is obtained in a separate determination using 1 g of a not fumigated sample, plus 3 ml of an aqueous standard solution containing comparable concentrations of the halohydrins.

Ideally, the results of GLC and TLC analyses should be quantitatively the same. However, appreciable differences are to be expected due to the relative inaccuracy of the TLC evaluations. In the range of 100—1000 ppm of ECH, the discrepancy between the results of both methods is usually below 25 percent.

Sensitivity of the method

By GLC: 1—10 ppm, depending on instrument performance.

By TLC: 10—20 ppm, depending on the limit of detection on the chromatogram and the quantity of derivative solution spotted.

Discussion

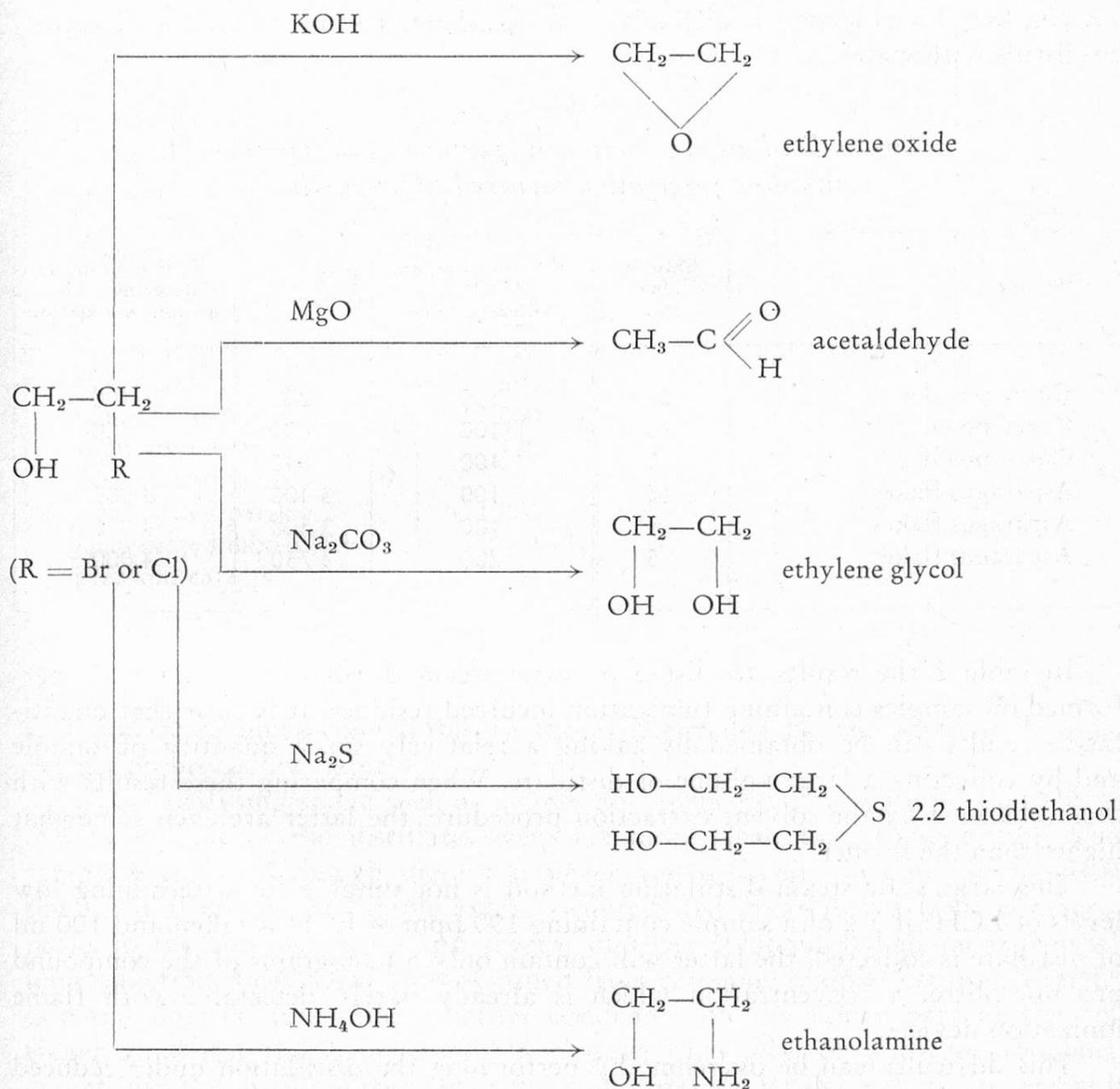
Because of its simplicity and accuracy solvent extraction is undoubtedly the most appropriate method for screening large numbers of samples for presence of halohydrin residues. In case of finding positive results, the identity of the residues present is easily confirmed by carrying out a distillation in the convenient semimicro apparatus and by subjecting the distillate to the derivatization procedure.

Experience has proven that this method is reliable: no spots of ethanolamine have been observed in distillates of untreated fruits, vegetables, mushrooms and spices.

Before we adopted the ethanolamine procedure, we investigated several reactions of the halohydrins which are listed in table 1. Unfortunately, none of

Table 1

Confirmation of identity of the halohydrins:
 Reactions which are suitable for derivatization purposes



these confirmatory procedures could be applied to the crude acetonitrile sample extracts, because of interference by co-extracted impurities or by decomposition of the solvent. We obtained good results for all 5 reagents on sample distillates (the derivatives could be visualized by GLC on suitable columns), but the ethanolamine procedure gave the highest yield, and this derivative, as well as the simultaneously formed halogen anions could be easily determined by thin-layer chromatography.

In their now classic paper *Wesley et al.* (5) reported steam distillation as a suitable means for the isolation of ECH prior to the determinative step. For this purpose, they slurried a quantity between 20 and 100 g of the sample according to the possible ECH content and steamdistilled until 100 ml of distillate was obtained. The distillate was subsequently analysed by gas chromatography.

It has repeatedly been stated that this method gives much too high results (1, 19, 20), but this objection is difficult to accept if one considers that ECH readily co-distills with water.

Table 2
Steam distillation experiments performed on samples containing fumigation incurred ECH residues

Sample	Sample size in g	Volume of steam distillate collected in ml	ECH found in ppm	ECH-content as determined by solvent extraction
Curry powder	25	100	200	700
Curry powder	10	100	600	700
Curry powder	5	100	640	700
Asparagus flakes	10	100	3 400	4 000
Asparagus flakes	5	100	3 500	4 000
Asparagus flakes	5	200	3 750	4 000

In table 2 the results are listed of some steam distillation experiments performed on samples containing fumigation incurred residues. It is clear that quantitative results can be obtained by taking a relatively small quantity of sample and by collecting a large volume of distillate. When comparing these results with those obtained by the solvent extraction procedure, the latter are even somewhat higher than the former.

This large scale steam distillation method is not suitable for determining low levels of ECH: if 5 g of a sample containing 100 ppm of ECH is taken and 100 ml of distillate is collected, the latter will contain only 5 nanograms of the compound per microlitre, a concentration which is already barely detectable with flame ionization devices.

This difficulty can be overcome by performing the distillation under reduced pressure in the semi-micro apparatus as described in this paper. This instrument, which is of the same design as that used by *Wesley* (21) for his routine analyses, permits the quantitative entrainment of the halohydrins with a far more favourable ratio sample/distillate.

Recovery experiments carried out on fortified samples indicate that the yield of the semi-micro distillation procedure is close to a 100 percent, both for ECH and EBH (see table 3).

Comparative analyses performed on a number of different commodities fumigated with ethylene oxide revealed that the results obtained by semi-micro di-

Table 3
Recoveries of ECH and EBH from fortified
Samples using semi-micro vacuum distillation

Sample	Quantity of ECH added in ppm	Quantity of EBH added in ppm	Percentage recovery for	
			ECH	EBH
Distilled water	1 000	1 000	110	92
Wheat flour	100	—	92	—
Cocoa powder	136	200	110	117
Cocoa powder	50	600	120	103
Cocoa powder	—	1 000	—	107
Cocoa powder	—	2 000	—	86
Turmeric powder	500	—	118	—
Turmeric powder	1 000	—	89	—
Black pepper	500	—	116	—
Black pepper	1 000	—	104	—
Dehydrated carrots	120	—	92	—
Dehydrated carrots	17	—	88	—
Dehydrated parsley	1 000	—	99	—
Dehydrated mixed leeks, peas and carrots	34	—	103	—

stillation were quite comparable to those determined by the acetonitrile water extraction procedure (table 4).

Upon analysing spices such as pepper and turmeric, it was found that the gas chromatograms of the distillates were far more clean than those of the crude sample extracts. Although there was never any difficulty in determining ECH, the essential oils in the sample extracts contributed invariably a large number of peaks to the chromatogram and several of these compounds had inconveniently long retention times. Distillates obtained from the same samples contained far less essential oils, because these volatiles condensed on the upper part of the cold finger condenser and, being insoluble in water, they were only leached to a small extent into the distillate trap. In fact, at high residue levels, such as, for example, those in curry powder, the distillates did not produce any aroma peaks at the attenuation setting necessary for visualizing ECH.

ECH residues in commercially available raw materials

During the last two years we performed a total of 250 analyses for ECH and EBH residues in raw materials used for the manufacture of culinary products. In

Table 4

Comparative determinations of ECH in fumigated commodities by semi-micro vacuum distillation and by solvent extraction with acetonitrile-water 5 : 1 v/v

Sample	ECH content in ppm found by distillation	ECH content in ppm found by solvent extraction
Black pepper	1 130	890
Black pepper	680	680
Turmeric	960	880
Turmeric	1 340	1 470
Turmeric	3 400	2 500
Asparagus flakes	4 500	4 020
Dehydrated green beans	380	330
Dehydrated mushrooms	890	560
Dehydrated mushrooms	760	800
Dehydrated mushrooms	260	200
Barley flour	1 710	1 810
Barley flour	1 750	1 780
Rice flour	420	470
Dried prunes	74	80
Whole egg powder	1 220	1 460

116 of these samples we found ECH residues, but EBH was never detected. In table 5 the results are listed for the different ECH concentrations in the various raw materials. Twenty-three of these samples (20 percent) contained less than 100 ppm, whereas fifteen (13 percent) had ECH levels in excess of 1000 ppm. In the majority of the samples (67 percent) the ECH content fluctuated between 100 and 1000 ppm.

Formation of ECH in different substrates during small-scale exposure to ethylene oxide

Small-scale ethylene oxide treatment of various commodities at sterilization levels was carried out under conditions as described by *Scudamore* and *Heuser* (4). After air-washing the fumigation chamber, the samples were transferred to glass bottles which were stoppered immediately and within one hour the samples were subjected to the solvent extraction procedure for the determination of ECH. Prior to the fumigation experiments all commodities were analysed for their inorganic chloride content and the corresponding potential chlorohydrin equivalent was calculated. It was found that in all fumigated samples, the greater part of the ECH had already been formed during exposure to the sterilant (see table 6). In fact, a significant increase with time in ECH content (due to continued reaction

Table 5

ECH levels measured in various commercially available raw materials
250 samples examined, 116 samples found with ECH residues

Raw material	Number of samples containing ECH	Less than 100 ppm	100 to 300	300 to 500	500 to 750	750 to 1000	1000 to 1500	1500 to 2000	Over 2000
Turmeric	7	0	0	1	1	3	1	0	1
Coriandrum	4	3	0	1	0	0	0	0	0
Cardamom	3	2	0	1	0	0	0	0	0
Carum carvi seed	2	1	0	1	0	0	0	0	0
Bay-leaf	2	0	2	0	0	0	0	0	0
Paprika	7	4	3	0	0	0	0	0	0
Majoran	1	0	0	1	0	0	0	0	0
Black pepper	6	2	2	1	1	0	0	0	0
Dehydrated carrots	1	0	1	0	0	0	0	0	0
Pimentoso	8	0	2	4	1	0	1	0	0
Dehydrated parsley	8	0	1	0	2	2	3	0	0
Dill tips	5	0	0	0	0	1	2	0	2
Dehydrated celery	1	1	0	0	0	0	0	0	0
Mushroom powder	16	0	7	5	3	0	1	0	0
Dehydrated sliced mushrooms	36	4	9	6	7	7	3	0	0
Cocoa powder	5	5	0	0	0	0	0	0	0
Chili powder	1	0	0	1	0	0	0	0	0
Oregano	1	0	0	0	1	0	0	0	0
Sesam seed	1	1	0	0	0	0	0	0	0
Asparagus tips	1	0	0	0	0	0	0	0	1

of inorganic chloride with adsorbed EO) was only observed in wheat flour, rice flour and whole egg powder. In the three fumigated flour samples the amount of ECH determined was roughly proportional to the potential chlorohydrin equivalent.

In the other commodities, the ECH formation stopped after only a small fraction of the inorganic chloride had combined with the fumigant. For example, the ECH levels in both Shiitake and dehydrated white mushrooms (*A. bisporus*) represent only about 5 percent of their potential chlorohydrin equivalent and this amount decreased fairly rapidly after fumigation.

All samples were also analysed for ethylene glycol residues, but negative results were obtained. Under the analytical conditions used (which were the same as those for ECH, but gas chromatography was performed at an oven temperature of 120°C) the limit of detection for this compound was approximately 20 ppm. Ethylene glycol is apparently much slower formed than ECH during fumigation.

Table 6
ECH levels formed in different substrates during small-scale fumigation with ethylene oxide

Sample	Inorganic chloride content Cl ⁻ ppm	Potential ECH equivalent ppm	Amount of ECH formed immediately after fumigation ppm	Maximum ECH level attained during storage mostly after a few days ppm	Fumigation conditions concentration-time product: mg EO h/l
Barley flour*	1 300	2 950	2 150	2 160	14 000
Wheat flour*	700	1 590	710	1 140	14 000
Rice flour*	400	910	500	575	14 000
Apple flakes	40	90	40	40	14 000
Raspberry flakes	1 400	3 180	190	190	14 000
Shiitake dehydrated mushrooms	500	1 130	50	60	16 000
Agaricus bisporus dehydrated mushrooms	8 500	19 300	870	decreases immediately	6 400
Whole egg powder	7 100	16 100	1 880	2 540	6 400
Black pepper	2 000	4 540	960	1 000	4 500
Black pepper	2 000	4 540	340	not determined	2 160
Turmeric powder	6 000	13 600	824	not determined	2 160

* Moisture content: approximately 10 percent

It is known that glycol formation is favoured by a low pH and for this reason, we performed ethylene oxide treatment on dried prunes, which contain a considerable amount of organic acids. The following results were obtained:

Fumigation conditions	ECH found in ppm	Ethylene glycol found in ppm
360 mg/l: 6 h	40	< 20
360 mg/l: 12 h	67	120
360 mg/l: 50 h	80	3 350

Appreciable quantities of glycol were only formed after the exposure time of 50 hours which far exceeds that used in practice. We also analysed the fumigated prunes for residues of diethylene glycol, but the results were below the limit of detection, i. e. smaller than 5 ppm.

Influence of ethylene oxide concentration and time of exposure on the rate of formation of ECH

Table 7 lists the ECH levels measured in 5 dehydrated vegetables and 3 flours exposed to ethylene oxide under different conditions of concentration and of time of exposure. It is readily apparent that ECH is more easily formed in the flours than in the dehydrated vegetables. Under normal fumigation conditions, i. e. at 800 mg of EO/l during 6 hours, the ECH concentration in the latter is significantly lower than that measured in the former.

Differences in the inorganic chloride content of the above-mentioned substrates are probably not a limiting factor in the rate of ECH formation: on longer exposure and even with a lower EO concentration the dehydrated vegetables attain an ECH level that is quite comparable to that measured in flours after fumigation under normal conditions.

It is, however, possible that the formation of ECH is facilitated by a higher moisture content, because the dehydrated vegetables contained only 2—5 percent of water, compared to about 10 percent in the flours.

Moreover, ethylene oxide adheres probably more easily to the fine particles of the flours than to the coarse texture of the other substrates.

It is interesting to note that no glycol formation was observed in any of the samples, not even at longer times of exposure.

Table 7
Influence of ethylene oxide concentration and time of exposure on the rate of formation of ECH

Commodity	Fumigation conditions : mg EO/l/h				
	280 mg/2 h	120 mg/6 h	800 mg/6 h	740 mg/19 h	320 mg/90 h
	ECH	ECH	ECH	ECH	ECH
Dehydrated green beans	n. d.	n. d.	144	200	1 150
Carrot flakes	n. d.	n. d.	160	240	850
Celery flakes	n. d.	n. d.	144	230	640
Dehydrated green leeks	n. d.	n. d.	210	290	1 300
Tomato flakes	n. d.	n. d.	138	305	370
Barley flour	125	225	1 100	2 150	—
Wheat flour	22	95	400	710	—
Rice flour	22	63	275	500	—

n. d. = not detectable

Persistence of ECH residues during storage

In order to have an idea about the persistence of ECH residues we re-analysed periodically 14 samples which had been stored at room temperature. In all

Table 8
Decrease of ECH levels in fumigated raw materials on storage

Sample	ECH content in ppm											Conditions of storage at room temperature
	Upon receipt	After 1	2	3	4	6	8	12	15	18	36 months	
Black pepper	1000	—	—	—	1100	1100	—	—	—	570	320	Glass stoppered vessel
Whole turmeric	920	—	—	—	960	—	—	690	—	630	350	Glass stoppered vessel
Ground turmeric	2600	—	—	—	—	—	—	1470	1140	—	—	Glass stoppered vessel
Asparagus flakes	4500	—	—	2200	—	—	—	—	1730	—	—	Sealed in glass
Whole egg powder	2540	1640	1580	—	1660	—	—	—	—	—	—	Glass stoppered vessel
Chili powder	312	—	—	—	—	—	—	n. d.	—	—	—	Sealed plastic bag
Oregano	600	—	—	—	—	—	—	440	—	—	—	Sealed plastic bag
Dehydrated green beans	1150	—	330	—	—	—	—	—	—	—	—	Glass stoppered vessel
Dehydrated mushrooms	895	—	—	—	160	—	—	—	—	—	—	Glass stoppered vessel
Dehydrated mushrooms	870	—	470	—	190	—	—	—	—	—	—	Glass stoppered vessel
Barley flour	2150	1680	—	—	1710	—	—	—	—	—	—	Glass stoppered vessel
Wheat flour	1140	980	960	—	—	—	—	—	—	—	—	Glass stoppered vessel
Rice flour	575	450	470	—	—	—	—	—	—	—	—	Glass stoppered vessel
Dehydrated parsley	1000	—	600	—	—	—	40	—	—	—	—	Sealed plastic bag

— = not measured
n. d. = not detectable

samples we observed a decrease in ECH content with time, but the rate of this decrease varied considerably from one substrate to the other (table 8).

For example, two samples of dehydrated mushrooms lost 70—80 percent of their initial ECH content within 4 months, whereas no decrease in residue was observed after the same period in black pepper and whole turmeric. All four samples had simply been kept in glass stoppered bottles.

Similarly, chili powder lost all of its residue within one year, whereas a sample of oregano only about 30 percent, and both spices had been kept in small plastic bags.

It is, therefore, not likely that the decrease in ECH can be explained by a simple evaporation of the compound.

The fact that the decrease is apparently dependent on the nature of the substrate, suggests that ECH may combine further with certain constituents of the raw materials, probably via intermediary formation of the more reactive ethylene oxide.

This possibility would require intensive investigation.

Conclusion

It is clear from our investigation that sterilization with ethylene oxide is still extensively practiced in the food industry: appreciable concentrations of ethylene chlorohydrin occur in many commercially available raw materials, especially in dehydrated mushrooms and in spices.

Clarification is needed to whether the levels found are of toxicological significance.

Summary

Ethylene oxide (EO) is widely used as a fumigant for cold sterilization of food materials. Among the several possible residues, ethylene chlorohydrin (ECH) formed by the reaction with chloride ions and moisture, is the only compound that occurs in significant and easily quantifiable amounts.

A simple method for the routine analysis of ECH residues is described which is also suitable for the simultaneous determination of ethylene bromohydrin (EBH), a residue formed similarly to ECH by reaction of EO with inorganic bromide, introduced by previous treatment with methyl bromide.

ECH and EBH are isolated by cold extraction with a mixture of acetonitrile-water and determined by GLC using FID. Confirmatory analysis is performed on a steam distillate that is obtained by using a specially designed semi-micro apparatus.

Upon treatment with ammonia both halohydrins are converted to ethanolamine and to the corresponding halogen anion which are chromatographed in two TLC systems. The limit of detection is 1—10 ppm halohydrin by GLC and 10—20 ppm by TLC.

ECH was found in 116 out of 250 samples of raw materials. EBH residues were never detected.

Small scale fumigation experiments indicated that the greater part of ECH was already formed during exposure to the fumigant. The amount of ECH formed in fumi-

gated flours was roughly proportional to the inorganic chloride content of the substrates. In other commodities, notably in mushrooms, ECH formation stopped after only a small fraction of the inorganic chloride had combined with the fumigant.

During these fumigation experiments no ethylene glycol formation was observed, except in dried prunes.

The persistence of ECH residues during storage varied considerably from one food material to the other. Dehydrated mushrooms lost 70—80 percent of their initial ECH content within 4 months, whereas no decrease in residue was observed after the same period in black pepper and whole turmeric.

Zusammenfassung

Aethylenoxid (AO) wird weit verbreitet als Begasungsmittel zur Sterilisation von Lebensmittelrohstoffen angewandt. Aethylenchlorhydrin (ACH), das durch die Reaktion von AO mit Chloridionen in feuchtem Milieu entsteht, ist unter den verschiedenen möglichen Rückständen der einzige, der in bedeutenden und leicht quantitativ bestimmbaren Mengen vorkommt.

Eine einfache Methode für Routineanalysen von ACH-Rückständen wird beschrieben. Sie eignet sich auch gleichzeitig zur Bestimmung von Aethylenbromhydrin (ABH), einem Rückstand, der ähnlich wie ACH durch Reaktion von AO mit Bromidionen entsteht, die in einer vorherigen Behandlung mit Methylbromid hineingebracht wurden. ACH und ABH werden mit einer Mischung aus Acetonitril und Wasser extrahiert und durch GC mit FID bestimmt.

Zur Bestätigung des Befundes wird mit einem speziell dafür entworfenen Halbmikroapparat eine Wasserdampfdestillation durchgeführt. Die dabei überführten Halohydrine werden durch Ammoniakbehandlung in Aethanolamin und in die entsprechenden Halogen-Anionen umgewandelt, die in zwei DC-Systemen bestimmt werden. Die Nachweisgrenzen sind 1—10 ppm Halohydrin mit GC und 10—20 ppm mit DC.

ACH wurde in 116 von 250 Rohstoffmustern gefunden, dagegen ABH nie.

Laborversuche zeigten, daß der größte Teil ACH schon während der Begasung entsteht. Die in begastem Mehl entstandene ACH-Menge war ungefähr proportional zum anorganischen Chloridgehalt des Substrates. In anderen Waren, vor allem in Pilzen, hört die ACH-Bildung schon auf, sobald sich ein kleiner Teil der Chloridionen mit dem Begasungsmittel vereinigt hatte. Eine Aethylenglykolbildung wurde nur in Trockenpflaumen festgestellt.

Die Beständigkeit der ACH-Rückstände während der Lagerung wechselte beträchtlich von einem Lebensmittelrohstoff zum anderen. Getrocknete Pilze verloren in 4 Monaten 70—80 Prozent ihres Anfangs-ACH-Gehaltes; in Schwarzpfeffer und in Kurkumawurzeln wurde hingegen nach derselben Periode keine Rückstandsabnahme beobachtet.

Résumé

La stérilisation à froid des produits alimentaires par gazage à l'oxyde d'éthylène (OE) est une pratique répandue. Parmi les résidus qui peuvent se former, l'éthylène chlorohydrine (ECH), produit de la réaction avec les chlorures en présence d'humidité, est la seule combinaison chimique présente en quantités facilement mesurables.

Une méthode simple est décrite pour l'analyse courante des résidus de ECH. Cette méthode se prête aussi au dosage simultané de l'éthylène bromohydrine (EBH), résidu qui se forme, comme ECH, par réaction de l'OE sur les bromures, introduits par un précédent gazage au bromure de méthyle.

ECH et EBH sont isolés par extraction à froid avec un mélange d'acétonitrile-eau et dosés par CPG avec détecteur à ionisation de flamme (DIF). Les tests de confirmation sont faits sur un distillat obtenu par entraînement à la vapeur d'eau dans un appareil semi-micro spécialement conçu. Un traitement à l'ammoniacal convertit les deux halohydrines en éthanolamine et en anion halogène correspondant qui sont soumis à deux chromatographies sur couche mince (CCM) dont les conditions sont décrites. Les limites de détection sont respectivement de 1 à 10 ppm de halohydrine par CPG et de 10 à 20 ppm par CCM.

250 échantillons de matières premières ont été analysés dont 116 contenaient de l'ECH. EBH par contre ne fut jamais détecté.

Des gazages à petite échelle ont montré que la plus grande partie de l'ECH prenait déjà naissance au cours du gazage lui-même. Dans les farines gazées, la teneur en ECH a été grosso modo proportionnelle à la teneur en chlorure inorganique. Dans les autres produits, notamment les champignons, la formation de ECH s'est arrêtée une fois qu'une petite fraction des chlorures inorganiques a réagi avec EO.

Au cours de ces gazages expérimentaux, la formation d'éthylène glycol n'a jamais été observée, sauf dans des prunes deshydratées.

Pendant l'entreposage, la persistance des résidus de ECH varie considérablement d'un produit alimentaire à l'autre. Des champignons deshydratés ont perdu 70 à 80% de leur teneur initiale en ECH en quatre mois, tandis qu'aucune diminution n'était observée dans le poivre noir et le curcuma entier pendant le même laps de temps.

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