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Original Article

Determination of ethylene chlorohydrin as marker of spices fumigation with ethylene oxide

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Abstract

Ethylene oxide (EO) is a gas used to sterilize spices, drugs, packaging materials, medical devices, polyester fibers, plastics and synthetic rubber. When the aeration step foreseen for spices is not properly carried out, residues of EO and its derivative ethylene chlorohydrin (ECH), produced by reaction with chlorine ions always present in the matrix, can be found in these products.

In this way, the reactivity of EO with chlorides in spices provides a suitable marker to confirm the use of EO for fumigation. The ECH derived from spontaneous transformation during the storage and forcibly obtained during the first step of the extraction can be evaluated in spices by a simple GC/MS analytical method, without derivatization.

It has been proven that the EO molecule is carcinogenic for humans; it has been classified as a category 1 carcinogen by the International Agency for Research on Cancer (IARC): ECH, as EO, is a mutagenic substance.

This paper shows the results concerning 25 pepper samples purchased on the Italian market.

The limit of detection for ECH was assumed to be 20 μg/kg (LOD) and was calculated by spiking a matrix pepper that had not been treated with EO. The limit of quantitation was assumed to be 100 μg/kg (LOQ), i.e. 5 times LOD. The reliability of the method was verified by recovery and repeatability tests. Recovery average values are 60–70% (CV% = 9.6–5.5) for the concentration range 100–500 μg/kg.

Only 56% of pepper samples analyzed did not contain ECH at detectable levels, and only 24% of pepper samples contained ECH at levels lower than LOQ. Three samples had a content ranging between 0.2 and 3.3 mg/kg and two samples showed a content of ECH higher than 5 mg/kg.

Keywords: Ethylene Oxide; Ethylene Chlorohydrin; Spices; Fumigation

1. Introduction

The method adopted in this work is useful for detecting ethylene chlorohydrin (ECH) as a marker of fumigation of spices with ethylene oxide (EO). The criterion to detect the inclusive sum of ECH by GC/MS was adopted for the first time; it was derived from spontaneous transformation during storage and forcibly obtained during the first step of the extraction, without derivatization.

It has been proved that the EO molecule is carcinogenic to humans, and it has been classified as a category 1 carcinogen by the International Agency for Research on Cancer (IARC): ECH and ethylene glycol (EG) are also mutagenic substances. The Scientific Committee on Food on 6 May 2002 concluded that the presence of EO should be brought below the detection limit, and the E.C. fixed purity criteria for defoaming additives E 431, E 432, E 433, E 434, E 435, E 436, and E 459, limiting the EO residues to "not more than 200 μg/kg" (Directive 95/EC, 2003).

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Therefore, as treatment of herbs and spices with EO is not considered a positive quality index, it was opportune to develop a rapid method which was easily applicable and specific to detect the ECH as a marker of fumigation. Therefore, this paper does not aim to suggest an analytical method to detect the residues of EO, as for medical devices sterilized with EO gas; nor is it directed to evaluate the potential toxicity of residues of EO and ECH in foods.

When the matrix pepper is fumigated, but the aeration step is not properly carried out, the residue of the EO gas reacts with chlorine ions always present in the matrix to form 2-chloro-ethanol (ECH), which can be found in the treated matrix at levels higher than 20 μg/kg.

The results reported in this paper show that, in the case of pepper, a method with LOD 20 μg/kg and LOQ 100 μg/kg is very easy to execute, and is useful for fast quality control.

As pointed out before, the method described in this paper permits the use of a GC/MS detector and not a GC or GC/ECD as previously proposed.

The headspace analysis, distillation technique or SPME using the direct-immersion method are considered as suitable methods to determine the mentioned gas if EO is the sole analyte (Ayoub et al., 2002): however EO is both highly reactive and volatile, and very small amounts of the gas are detectable a few days after its use in the fumigation of pepper.

In conclusion, the analytical detection of EO and ECH for spices is unnecessary since the total effective exposure is shown by all the ECH.

Various authors have published papers about this analytical theme: among our references we believe that the most interesting are the following:

- Jensen (1988) describes a method for the determination of residues of EO and ECH: sodium hydroxide is added to the sample where ECH is transformed into EO, and the gas is converted into ethylene iodohydrin (EIH) by distillation on dilute sulphuric acid containing sodium iodide. The recoveries of ECH are reported to be 50–60%. The ECH content is determined by gas chromatography using an ECD detector.
- Aitkenhead and Vidnes (1988) propose an acetonitrile–methanol mixture as an extraction solvent for ECH, and capillary GC with FID detector for identification and quantitation of ECH. The authors report that the extraction recovery is approximately 75% for the extraction procedure.
- Gilsbach and Weeren (1999) refer to an interlaboratory study using the GC method after derivatization with sodium iodide, with recoveries of 66–70%. Similar procedures are suggested by NIOSH Manual of Analytical Methods (NMAM) (1994) adopting an ECD or MS detector.

This paper provides information about EO treatment of pepper found on Italian markets in family-size packets with EO. Twenty-five pepper samples, representative of most known trademarks and purchased from various Italian supermarkets, have been analysed using the method described below.

2. Materials and methods

2.1. Materials

Samples of pepper of different varieties and form—black pepper, white pepper, green pepper, ground pepper and peppercorn—were chosen in this study.

All the 25 samples examined, as family-size packets and representatives of packaging commonly used by consumers, were purchased from retailers in Italian markets.

Standard ECH (99%) from Sigma-Aldrich (Milan, Italy) has been used to prepare standard solutions for calibration; the extraction solvent was ethyl acetate (>99.5%) from Merck (Milan, Italy).

Standard EO (50 mg/mL in methanol) from Sigma-Aldrich (Milan, Italy) has been used to prepare the solutions to verify the conversion yield of EO to ECH.

2.2. Apparatus and operating conditions

A gas chromatograph mass spectrometer QP 2010 system (Shimadzu, Milan, Italy) in EI mode (Electronic Impact), equipped with a polar column SupelcoWax-10 (30 m × 0.25 mm ID, 0.25 μm film thickness; Supelco, Bellefonte, PA) was used.

A GC oven was programmed: 60 °C, 1 min, 3 °C/min to 240 °C, 10 min at 240 °C.

The injector temperature was kept on 220 °C (splitless mode 1 min). The flow rate of the carrier gas (helium) was 1 mL/min. The MS detector was operated at 240 °C, ionization energy was 70 eV. The detector voltage was set at 0.9 kV, and the interface temperature at 200 °C. The solvent delay was 10 min.

The acquisition mode was multiple ion detection (MID) monitoring ions at $m/z = 31$ and 80 (rel. abund. 100 and 2 in the ECH standard MS spectrum). The volume injected was 2 μL.

2.3. Extraction procedure

In order to convert completely the residual EO into ECH, an amount of about 2 g of ground sample (pepper), exactly weighed, was charged with 2 mL of 0.02 N H_2SO_4 and with 200 μL of a saturated solution of NaCl. The sample was extracted twice with 80 mL of ethyl acetate for 2 h at 60 °C by stirring in an ultrasonic bath for 30 min. The ethyl acetate phase was filtered

Table 1
Conversion yield of standard EO solutions to ECH

St.sol ECH (teor μg/L)	AC measured (mean of 3 rep)	ECH (recovered μg/L)	% Conversion
91	63552485	76	84
182	130133351	157	86
364	272374299	328	90

each time on anhydrous sodium sulphate (3 g), washing the solid residue three times with 10 mL ethyl acetate and collecting all the resulting liquid phase in a sealed flask. The extract was concentrated in the vacuum of a rotary evaporator to about 1 mL and then transferred to a 2 or 4 mL graduated vial, diluting the volume with ethyl acetate.

To verify the conversion yield of EO to ECH, 200 μL of standard solutions of EO at conc. of 50, 100 and 200 μg/L are treated as described for pepper, obtaining from each of them 2 mL of ECH solution theoretically at concentrations of 91, 182 and 364 μg/L (Table 1).

The results of the GC/MS analysis are reported below (MID mode, m/z 31 and m/z 80).

3. Results and discussions

For the identification of the ECH peak in the extracts, relative intensities for ions $m/z = 31$ and 80 were monitored. All peaks of ECH were confirmed also by spiking and by retention time (13.3 min in the described GC/MS conditions).

The reliability of the method was verified by recovery and repeatability tests.

Recoveries of added ECH were evaluated by analysing samples, proved to be ECH free, spiked with standard ECH in the concentration range between 100 and 500 μg/kg. Recovery experiments were performed in triplicate and the average values were 65.5% for pepper spiked at 100–200 μg/kg (CV% = 8.4) and 70.6% for pepper spiked at 400–500 μg/kg (CV% = 5.5).

Repeatability evaluation tests were carried out on two pepper samples containing ECH at levels of 260 and 3290 μg/kg (mean of five repetitions, corrected by recovery). The CV% values were 5.3 for the first and 7.2% for the second.

The limit of detection for ECH was in the order of 20 μg/kg (LOD) and the limit of quantitation was assumed to be 100 μg/kg (LOQ), i.e. five times that for LOD. The LOD value was estimated not only from the noise value but by considering the minimum peak which produced an MID identifiable as a characteristic of ECH.

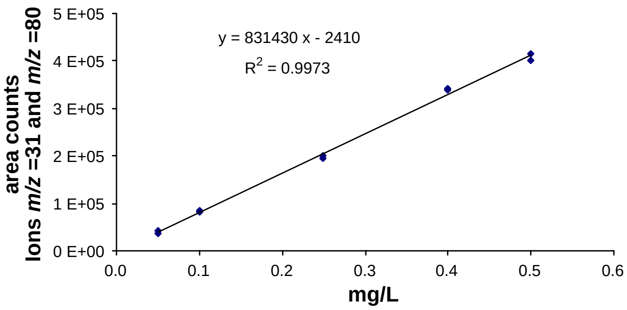

Fig. 1. Calibration curve of standard ECH solution in ethyl acetate in the concentration range 0.050–0.500 mg/L ($R^2 = 0.9973$).

Linearity was tested ($R^2 = 0.9973$) using a standard solution of ECH in ethyl acetate in a concentration range between 0.050 and 0.500 mg/L, monitoring the calibration curve (Fig. 1) and the area count of the ion at $m/z = 31$. No interference with this peak was found after analyzing a large number of pepper samples which were certainly not fumigated.

The calibration curve enables the detection of ECH levels in the sample not exceeding 4 mg/kg, considering that is possible to use not less then 0.5 g of sample for the extraction and to dilute the extract to 4 mL. For levels of ECH > 4 mg/kg in the sample it is necessary to dilute the extract before injection, to avoid detector saturation.

Considering the aim of the application of this method, it is not necessary to do the quantitation of ECH at levels more than 4 mg/kg in pepper samples (i.e. 5.7 mg/kg, corrected by recovery 70%), not reaching the level of 100 μg/kg and not exceeded by involuntary or cross-shaped pollution events. When the ECH level is higher than the value mentioned earlier a quantitative evaluation can be made using a larger dilution of the extract. The results of the research carried out in this work, for the reasons explained before, were expressed as > 5.0 mg/kg when the level of ECH was higher than 5 mg/kg.

To estimate the level of ECH in the sample the quantitative calculation was processed as follows:

$$C_c = (C_e \times V_e \times 100)/(P_c \times R),$$

where C_c is the concentration of ECH resulting in the sample (mg/kg), C_e the concentration of ECH calculated from the calibration curve (mg/L), V_e the volume of the reconstituted extract (2–4 mL), P_c the weight of the sample (g) and R the recovery %.

The MS spectrum in scan mode corresponding to a standard solution of ECH is reported in Fig. 2.

Fig. 3 shows the GC/MS trace of an extract, produced with the method described before, from a pepper sample proved to be ECH free, and spiked with ECH at 0.020 mg/kg (LOD). Fig. 4 reports, as a further example, the GC/MS trace of an extract produced from a pepper

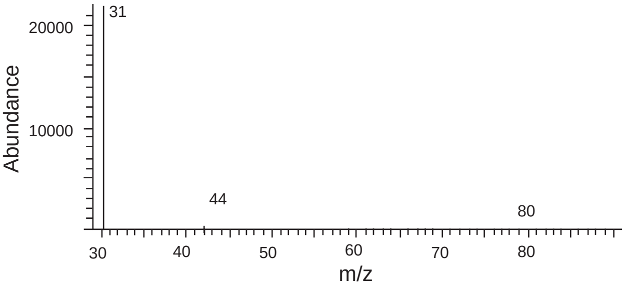

Fig. 2. MS spectrum produced by standard ECH in scan mode.

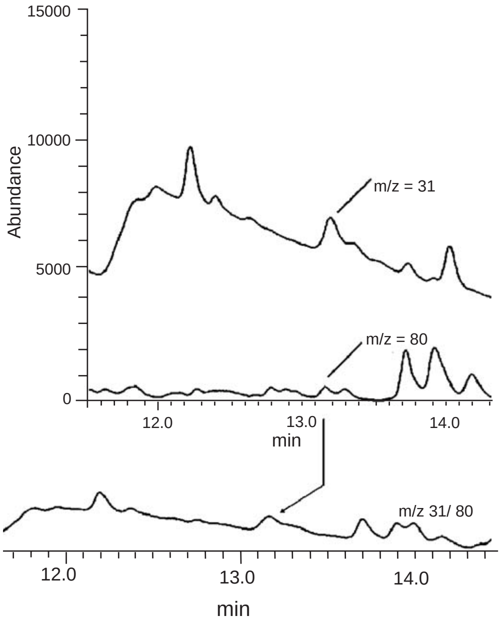

Fig. 3. GC/MS trace ($m/z = 31$ and 80) of an extract obtained from a pepper sample spiked with ECH at 0.020 mg/kg (LOD).

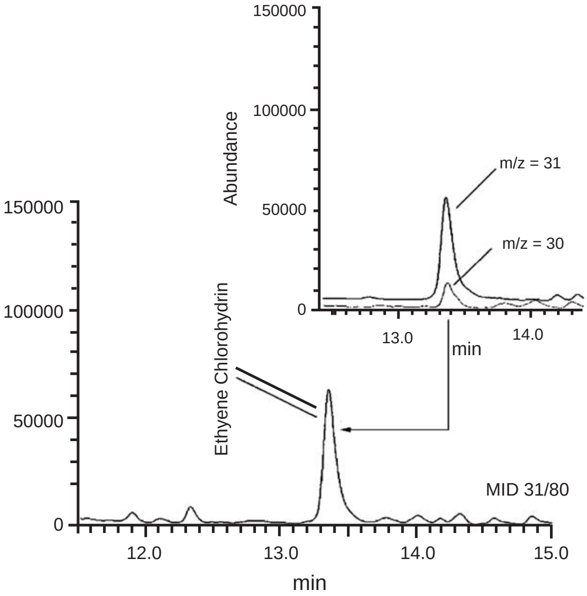

Fig. 4. GC/MS trace ($m/z = 31$ and 80) of an extract obtained from a pepper sample containing 0.634 mg/kg of ECH.

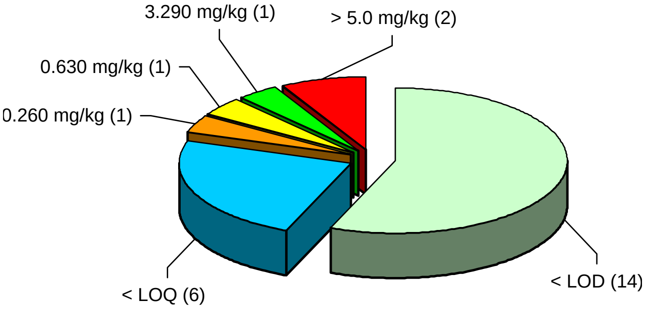

Fig. 5. Distribution of ECH level in 25 pepper samples mentioned in Table 1; the number of samples is reported in round brackets.

sample containing ECH at 0.630 mg/kg, mentioned among the quantitative results shown in Fig. 5. The two figures show the traces of the ions at $m/z = 31$ and 80 for the peak eluted at 13.3 min: the ion ratios and the retention time clearly identify the peak as ECH.

25 samples of commercial pepper from the Italian market were analysed, as described in "Materials and methods". Table 2 reports the label information concerning the examined products, and quantitative results are summarized in Fig. 5, and corrected with recovery data.

The review carried out in this study led to these conclusions: fourteen samples were found to have an ECH level < LOD, six samples produced a content between LOD and LOQ and the remaining five samples contained ECH at levels between 0.260 and > 5.0 mg/kg.

The results obtained show that 11 of the 25 examined samples present ECH at a level higher than 0.020 mg/kg (LOD) and in two samples the content is higher than 5 mg/kg. In conclusion, 44% of the Italian market's most representative samples are found to be fumigated with EO.

The research emphasizes the current problem concerning industrial chemical residues in food. The simple analytical procedure, and the short time required for extraction and GC/MS analysis, allow the consideration of the method adopted in this paper as being useful for routine analysis of pepper or other spices fumigated with EO.

Table 2
Label information for 25 variuos peppers sold in Italian market

	Sample description	Lot	Expiry date	Net weight (g)
1	Black pepper "Ducros"	L3094C	04/2006	48
2	White pepper "Ducros"	L3070C	03/2006	48
3	Black pepper "Drogherie alimentari"	L22073	08/09/2006	40
4	White pepper "Drogherie alimentari"	L06033	13/06/2006	40
5	White pepper "Mondo natura"	L120328	16/07/2005	45
6	Black pepper "Cannamela" bionatura	—	28/08/2006	50
7	Black pepper "Cannamela"	—	29/11/2004	28
8	White pepper "Cannamela"	—	09/04/2006	28
9	Black peppercorn "Cannamela"	—	23/09/2005	28
10	White peppercorn "Cannamela"	—	14/04/2006	28
11	Black pepper "Alpes"	—	20/03/2006	9
12	White pepper "Alpes"	—	20/03/2006	8
13	Black pepper "Kania"	3014BB	2007	50
14	White pepper "Kania"	3281AC	2007	50
15	Black pepper "Tesori dell'arca"	L3020	20/01/2006	50
16	White pepper "Tesori dell'arca"	L3017	17/01/2006	50
17	Black peppercorn "Fertitecnica Colfiorito"	L3182	30/07/04	300
18	White pepper "Fertitecnica Colfiorito"	L3143	30/05/04	300
19	Black pepper "Il Gigante"	L220736	08/09/2006	40
20	White pepper "Il Gigante"	L120236	19/05/2006	40
21	Black peppercorn "Il Gigante"	L25102N	16/04/2006	45
22	Green peppercorn "Il Gigante"	L200238	18/03/2006	15
23	Black peppercorn "Tropic all"	L3203	07/2006	50
24	Black pepper "Shapur"	130 × 1833	31/12/2007	30
25	White pepper "Shapur"	W5121833	31/12/2007	30

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