Extraction procedure optimization of the method for detecting ethylene oxide and 2-chloroethanol in sesame seed

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Abstract: This study describes modifications of the extraction procedure within the European Union Reference method for determination of ethylene oxide and 2-chloroethanol in sesame seed. The method suggests utilisation of uniform stainless steel balls in order to facilitate extraction in small seed samples. Experiment was conducted with combination of balls of different sizes and the extraction efficacy was assessed by measurement of 2-chloroethanol in sesame seed samples from the local market. Increased efficacy of 18.3% for ethylene oxide and 16.2% for 2-chloroethanol was observed when the combination of two diameters of balls was used compared to samples extracted by the guidelines in original method and alternative approach with uniform balls of the lower diameter.

1. Introduction

Chemical contamination of food is an important aspect of food safety [1]. It is also a very complex field, consisting (among the other) of prevention and control of thousands of compounds from entering the food chain, whether those compounds are intended to be used in food manufacturing/processing, or abused, due to their proven beneficial properties in food, however at the high cost of their harmful effects on humans. Such is the case with ethylene oxide (EO), the compound with one of the highest production rates worldwide. It is a precursor or intermediate in production of many chemicals e.g. polyethylene glycols, PET, cosmetics additives, construction materials, etc.

However, due to the volatility and high reactivity of the EO, one of its uses is also in fumigation of objects, warehouses, sterilization of medical equipment and (before 1980s) food commodities, especially spices and herbs. Being a small molecule, EO can easily penetrate cellular membranes of bacteria, their spores and viruses thus reacting with DNA/RNA and complex molecules resulting in their inactivation.

Although fumigation can be classified as “niche” use of EO accounting for only 0.05% of the global production [2], having in mind its overall production, this is still a significant proportion of the chemical that is being used for this purposes. However, the same properties for which EO represents an excellent fumigant (interactions with genetic material of the microorganisms), are also responsible for its genotoxic, mutagenic and carcinogenic potential. European Chemicals Agency (ECHA) has classified EO in category 1B in respect to carcinogenic, mutagenic and genotoxic properties and category 3A in respect to acute toxicity [3].

After fumigation of food commodities, EO quickly produces several reaction products, of which 2-chloroethanol (2-CE) is the most significant, and legally relevant as well, since it is included in the residue definition scope in the European Regulation 868/2015 [4] (sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide).
Although EO is not an authorized substance for fumigation in the EU from 1991, it is still used in countries that are large producers of certain commodities such as sesame seed (e.g. India, China, Nigeria), in order to reduce contamination with Salmonella and other faecal bacteria. This was apparent in mid-2020 after numerous Rapid Alert System for Food and Feed (RASFF) notifications concerning EO residues in sesame seed from India. Number of notifications amounted to about 140 by November 2020 [2]. Certain quantities of contaminated sesame seed were imported to Serbia as well resulting in prompt withdrawal from the market.

European Union Reference Laboratory for Single Residue Methods (EURL-SRM) published a comprehensive analytical observations report [2] in December 2020 concerning EO and 2-CE, covering historical, toxicological and analytical aspects of this subject. Detailed analytical method based on QuEChERS method described in ISO EN 15662 [5] and quantitative determination by gas chromatography-tandem mass spectrometry (GC-MS/MS) is provided. One of the important steps in analysis of EO/2-CE, especially in the case of samples containing of small seeds (poppy, sesame) is extraction of as much residue as possible from the matrix consisting of very small (1-3 mm in diameter) units. In the case of more persistent chemicals, this could be accomplished by grinding and homogenization in laboratory mills with or without freezing. However, in the case of EO which is highly volatile this could result in significant analyte loss.

Therefore, EURL-SRM analytical method stipulates utilization of extraction aids (4-5 stainless steel balls, 9.5 mm in diameter) in order to facilitate maceration of small sesame seed and increase availability of EO and 2-CE and speed up the extraction process. Polypropylene tube (50 mL) is to be loaded with 2 g of sesame seed, 10 mL of acetonitrile with 5% of water and stainless steel balls. The tube is to be shaken for 15-30 minutes, centrifuged and an amount of the crude extract is to be subjected to further clean-up. However, during the extraction procedure, we noted that steel balls can be stuck in the tube not performing the seeds maceration efficiently, especially if the shaker speed is low.

The aim of this study is to assess modifications of the EURL-SRM method for determination of EO and 2-CE related to sample extraction and determine whether these modifications can contribute to extraction efficacy.

2. Materials and methods

Analytical standards of EO and 2-CE were purchased from Sigma-Aldrich (St. Louis, MO, USA), as well as HPLC purity acetonitrile and water. QuEChERS mixture for oily samples clean-up (150 mg C\textsubscript{18}; 150 mg PSA; 900 mg MgSO\textsubscript{4}) was obtained from Phenomenex (Torrance, Canada). Stainless steel balls (5 mm and 10 mm in diameter) were obtained from the local hardware store, thoroughly washed with detergent, rinsed and submerged in hexane and acetonitrile in ultrasonic bath for 20 minutes. Balls were dried and stored in sealed container until the analysis.

GC-MS/MS system consisted of Shimadzu (Kyoto, Japan) Nexis GC 2030 with split/splitless injector, AOC-20i plus auto sampler and AOC 20s plus auto-injector, coupled to Shimadzu GCMS-TQ 8050NX triple quadrupole mass spectrometer operating in EI mode.

Sesame seed was obtained from the local retail and was subjected to the analysis according to the original analytical method in order to determine absence of residues of EO and 2-CE. Then, 100 g of seeds was weighted in the polypropylene jar and spiked with solutions of EO and 2-CE in order to achieve concentration of 0.05 mg/kg of each compound. Although current MRL for sesame seed is lower (0.02 mg/kg for sum of EO and 2-CE), our intention is to get clear signals in order to interpret the results correctly. The jar was sealed and shaken on the overhead shaker for 1 hour. Tightly sealed jar was left on room temperature for 2 days before the analysis. Six samples were taken and analysed again in order to determine homogeneity of the residues. Established values were satisfactory (cv less than 5%) and this sample was used for further experiments.

Detailed description of the analytical method for GC-MS/MS determination of EO and 2-CE in sesame seed has been presented in the EURL-SRM document [2]. Briefly, 2 g of sesame seed is weighted with accuracy of ± 0.02 g in polypropylene tube, 10 mL of 5% water in acetonitrile was added
as well as stainless steel balls. The tubes were closed and shaken on the Neuation Technologies (Gujarat, India) shaker at 40 revolutions per minute for 25 minutes.

The modifications of the extraction procedure were conducted as follows:

Instead of extracting the sesame seed with 5 stainless steel balls 10 mm in diameter, three batches each consisting of 6 samples of the same sesame seeds were extracted with various size balls. Batch 1 was control batch and was extracted according to the method (four 10 mm balls). Batch 2 used ten 5 mm balls and Batch 3 used mixture of three 10 mm balls and three 5 mm balls. One millimetre balls were available as well, however, initial experiments confirmed that their weight if applied alone in any number is not sufficient to macerate sesame seeds entirely leaving intact seeds in the tube. Therefore, the smallest balls were excluded from the experiment.

After the extraction, samples were centrifuged at 3000g for 5 minutes and 6 mL of the extract was transferred to the 15 mL polypropylene tube with QuEChERS mixture for oily samples clean-up (C18/PSA/MgSO4). The prescribed mass fraction of constituents of the mixture is 25/25/150 mg per mL of extract, therefore, for 6 mL the 150/150/900 mg of each clean-up agent was used. The tubes were manually vigorously shaken for one minute, centrifuged for 5 minutes at 3000g and 1 mL aliquot was transferred into the GC vial.

GC column was Shimadzu SH-Rxi-5MS (30m x 0.25 mm id x 0.25 mm df). Injector temperature was 260°C, splitless injection was performed. Oven program was as follows: start at 50°C, hold 2 min, ramp to 150°C at 40°C/min, ramp to 280°C at 12°C/min, hold till 20 min.

Mass spectrometer was operating in EI mode, source temperature was 270°C, transfer-line temperature was 250°C.

Transitions for the EO were 44>14 (CE 20), 44>28 (CE 5) and 44>29 (CE 5) while for 2-CE transitions were 80>31 (CE 5), 80>43 (CE 5) and 82>31 (CE 5).

Calibration was not performed in these experiments since the aim of the study was to assess extraction efficacy only and for that purpose, quantification was not relevant. Therefore, obtained peaks were integrated and resulting peak areas were compared. One sesame sample was analysed before the analysis of three batches according to the original method (five 10 mm stainless steel balls as an extracting aid). Since batch 1 consists of six samples of sesame seed extracted in the same manner as initial sample, the results for this batch are expected (92-106% for EO and 94-110% for 2-CE) and can account for the variability of the analytical method. However, batch 2 (ten 5 mm balls) shows significant decrease (76-87% for EO and 76-96% for 2-CE) of the original intensity and batch 3 (combination of three 10 mm balls and 3 5 mm balls) shows significant increase of extraction efficacy (109-125% for EO, in average 18.3% increase and 109-122% for 2-CE, in average 16.2% increase).

### Table 1.
Standardized peak areas in three batches of sesame seed

| Sample  | Batch 1 (EO) | Batch 2 (EO) | Batch 3 (EO) | Batch 1 (2-CE) | Batch 2 (2-CE) | Batch 3 (2-CE) |
|---------|--------------|--------------|--------------|----------------|----------------|----------------|
| Sample 1 | 1.06         | 0.87         | 1.13         | 0.99           | 0.88           | 1.22           |
| Sample 2 | 0.96         | 0.78         | 1.21         | 0.94           | 0.93           | 1.17           |
| Sample 3 | 0.92         | 0.82         | 1.19         | 1.1            | 0.96           | 1.15           |
| Sample 4 | 0.96         | 0.81         | 1.09         | 0.97           | 0.89           | 1.19           |
| Sample 5 | 1.05         | 0.76         | 1.23         | 1.06           | 0.76           | 1.07           |
| Sample 6 | 1.03         | 0.86         | 1.25         | 1.08           | 0.82           | 1.17           |
These findings can be explained by the variations in mechanics of stainless steel balls depending on their size and weight. Diameter of 50 mL polypropylene tube is 29 mm and although 4-5 balls (10 mm in diameter) fit into the tube, they can stick at slower shaker speeds, reducing crushing and maceration of sesame seeds. From the opposite side, large diameter balls provide sufficient weight to perform the maceration very well. Combination of three heavy 10 mm balls and three lighter 5 mm ones reduce the possibility of getting stuck, provide adequate weight and therefore crushing of seeds and at the same time the mixing of larger and smaller balls during shaking process is continuous and more efficient. This results in increased efficacy of the extraction process.

4. Conclusion
Presented modification of analytical method for determination of EO and 2-CE in sesame seed in respect to extraction mechanism, increases transfer of these compounds from matrix to the solvent and therefore increase efficacy of the whole process. Further investigation can be performed in order to investigate this modification on other matrices that require utilization of mechanical extraction aids.

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References
[1] Nastasijevic I, Veskovic S and Milijasevic M 2020 Meat Tech. 61 97
[2] EURL-SRM Analytical Observation Report https://eurl-pesticides.eu
[3] European Chemicals Agency https://echa.europa.eu/en/regulations/clp/classification
[4] Commission Regulation (EU) 2015/868 of 26 May 2015 Official Journal of the European Union 145 1–71
[5] International Organization for Standardization CSN EN 15662 Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method