Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
2015, volume 100, livre 4, pp. 96-99
First National Conference of Biotechnology, Sofia 2014

THE IMPACT OF DIFFERENT SOLVENT RATIOS ON THE RECOVERY OF LINDANE IN EGG

I. STOYKOVA[1,2*], T. YANKOVSKA-STEFANOVA[1],
L. YOTOVA[2], D. DANALEV[2]

1 – Central Laboratory of Veterinary Control and Ecology, Bulgarian Food Safety Agency, Sofia 1528, Blvd. Iskarsko Shousse 5
2 – Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia 1756, Blvd. Kliment Ohridski 8
**Corresponding author: stoykova.iskra@abv.bg*

Keywords: extraction, egg samples, pesticides, lindane

Abstract: Pesticides play a key role in pest management in agriculture, public health and medical applications. Unfortunately, this widespread use means residues can be found in a variety of matrices like animal tissue, milk, honey, eggs, etc. Food safety is an integral part of the EU policy, ensuring protection of consumer's health therefore maximum residue levels of pesticides are defined in specific Regulations.

Analysis of pesticide residues in foods from animal origin includes a wide variety of extraction methods and cleanup steps, followed by the determination generally with chromatographic techniques (Ahn et al., 2006). Extraction of pesticides in egg samples is much more complicated than other samples of animal origin, due to the presence of phospholipids. Therefore the choice of the solvent mixture used for extraction is of great importance for the determination of the fat, which affects the final recovery (Suoza et al., 2008).

Herein, we report the impact of several different solvent mixtures on the recovery of the organochlorine pesticide Lindane, in hen eggs. Various combinations of polar and non-polar solvents were used for extraction by means of Accelerate Solvent Extractor followed by Florisil column as a cleanup step. Gas chromatography with electron-capture detector was used for the quantification of the pesticide content. The obtained results show that a mixture of 20/80 (V/V) polar/non-polar solvent (ethanol/toluene) is the best ratio for both - fat determination and recovery of Lindane.

INTRODUCTION

Egg yolk lipids have a very high nutritional value. Due to the favourable fatty acid profile, fat-soluble vitamin and lecithin content egg yolk can be considered

a good addition to human nutrition (Lewis et al., 2000). Solvents are chosen in order to extract as much lipids as possible from the raw material, keeping in mind that a low boiling temperature is favourable to allow easier and faster evaporation. Favouring less toxic solvents (Boselli et al., 2001).

The difference between extraction efficiency in the raw liquid egg or egg yolk powder is that the water content in raw material makes extraction with non-polar solvents inefficient due to the different polarities between the solvent and the water in the egg yolk (Cizkova et al., 2004). Polar solvents, such as low molecular weight alcohols, cause denaturation of egg yolk proteins by destroying hydrogen bonds or electrostatic interactions causing permanent changes in the tertiary protein structure. This facilitates access to the neutral lipids, thus enabling an extraction with non-polar solvents. Without this protein denaturation, polar solvents will only extract polar membrane-associated lipids from the egg yolk (Ahn et al., 2006). In this case a combination of polar and non-polar solvents can be chosen, yielding better extraction efficiencies from liquid egg yolk.

The aim of this study was to compare the impact of several solvent mixtures (ethanol/toluene in different proportions) on the extraction of lipids and on recoveries of the organochlorine pesticide Lindane, in hen eggs. The choice of the solvents was based on solvent polarities. Volatility and toxicity of the solvents were taken also into account (ISO 5508, 1990).

MATERIALS AND METHODS

1. Reagents and stock solutions

The standard of organochlorine pesticide Lindane (γ-HCH) used in this study was purchased from Supelco (ps71) of which a solution 1mg/ml was prepared in n-hexane. This solution was used to spike egg samples to the required concentrations.

As a drying agent anhydrous Na_2SO_4 backed overnight at 150°C was used. Florisil 60–100 mesh obtained from Sigma-Aldrich backed overnight at 170°C and deactivated with 3% water was used as cleanup sorbent. All of the solvents used (toluene, ethanol, hexane, dichloromethane) were analytical grade, purchased from Sigma-Aldrich.

2. Fat determination

The whole egg commodity was blended after removing and discarding shells. The sample was spiked with 0,02 mg/kg of Lindane.

An appropriate amount of the sample (10 g) was mixed with 5 g of the drying agent in a glass mortar using a glass pestle. This homogenized sample was introduced into a cell from a DIONEX Accelerate Solvent Extractor (ASE), with a filter at the bottom. The samples were extracted with 10 different solvent ratios:

Ethanol/ Toluene in following ratios: 0:100; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80 and 10:90. The extracts were collected into ASE

bottles, and after evaporation of the solvents under vacuum the amount of fat was gravimetrically determined

3. Cleanup

In this step, a Florisil column as described in point 3.1 was used. A glass column with a 1cm inner diameter was filled with 5g of sorbent. Of each sample 100mg of the extracted fat was eluted with dichloromethane/hexane (1:1) and the eluting solvent mixture was initially evaporated with vacuum evaporator up to 2-3ml, and subsequently evaporated till complete dryness under a gentle stream of nitrogen. The residue was resolved with 1ml of n-hexane and 2µl are injected into the gas chromatographic system with Electron Capture Detector (ECD)

4. Apparatus

GC-ECD chromatographic analysis was performed using a Thermo Finnigan gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) at 350°C. Analytes were separated with a DB-5 column (J & W Scientific, Fol-som, CA, USA), 30mm×0.25mm i.d., containing 5% phenyl-methylpolysiloxane with a phase thickness of 0.25µm. The following temperature gradient was used for the analysis: from 100°C (hold 2 min) to 280°C (rate 20°/min) and hold for 3 minutes. The injector was set to 240°C in the splitless mode. Nitrogen was used as a carrier gas at 1.2 ml/min and as make-up gas at 40 ml/min. Identification of peaks was based on the comparison of the retention times of compounds in the standard solutions.

RESULTS AND DISCUSSIONS

Medium values of extracted fats and recoveries of the analyte of interest Lindane, are shown in Table1.

Table 1. Performance of the results for fat content and recovery.

Solvents		**Results**	
Ethanol [%]	**Toluene [%]**	**Fat content [%]**	**Recovery of Lindane [%]**
10	90	7,89	30
20	80	7,65	85
30	70	7,97	25
40	60	7,20	0
50	50	5,99	0
60	40	6,60	0
70	30	5,44	0
80	20	4,20	0
90	10	4,53	0
100	0	5,15	20

The results presented in Table 1 show the parameters of all of the extracted raw egg samples. The fat extracted from eggs with 30:70 ethanol/toluene solvent was higher than the others, but did not differ significantly from the 10:90; 20:80; and 40:60 ratios. According to DG SANCO (Guidance document on analytical quality control and validation procedures for pesticide), recoveries for pesticides in food have to be at least 60%. Therefore, the results obtained with the 30:70 ratio are not satisfactory. The highest recovery of Lindane is reached by 20:80 ethanol/toluene, while simultaneously obtaining a good determination of the fat content.

The use of 5g Florisil in the cleanup was considered appropriate as it has the capacity to process 100 mg of fat and allowing proper fractionation resulting in the separation of the pesticide from the fat. Furthermore, the final extract was considered clean, evidenced by the low noise on the chromatogram's baseline.

CONCLUSION

The combination of polar solvents and non-polar solvents are efficient for both the extraction of lindane in the egg and the determination of the fat content. This study reveals that 20/80 ethanol/toluene is the optimal ratio, allowing a good determination of the fat content and gives the best recovery of Lindane.

Acknowledgements: This work was supported by project BG051PO001-3.3.06-0059. In addition, the authors would like to thank to Bulgarian Food Safety Agency for the opportunity to use their specific equipment.

REFERENCES

1. Ahn D.U., Lee S.H., Singam H., Lee E.J., Kim J.C. (2006) Sequential separation of main components from chicken egg yolk. *Food Science and Biotechnology*, Vol. 15, No. 2, p. 189–195
2. Boselli E., Velazco V., Caboni M.F., Lercker G. (2001) Pressurized liquid extraction of lipids for the determination of oxysterols in egg-contained food. *Journal of Chromatography* A, No .917, p. 239–244
3. Cizkova V., Prokoratova V., Voldrich M., Kvasnicka F., Soukupova V. (2004): Determination of egg content in pasta. *Czech J. Food Sci.*, No. 22, p. 197–203.
4. ISO 5508 (1990) Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids.
5. Lewis N.M., Seburg S, Flanagan N.L. (2000) Enriched eggs as a source of N-3 polyunsaturated fatty acids for humans, *Poultry Science*, No. 79, p. 971–974
6. Souza J.G., Costa F.G.P., Queiroga R.C.R.E., Silva J.H.V., Schuler A.R.P., Goulart C.C. (2008) Fatty Acid Profile of Eggs of Semi-Heavy Layers Fed Feeds containing Linseed Oil, Brazilian Journal of Poultry Science, No.10 (1), p. 37-44.[accessed on 12.02.2014.]. Available: http://www.scielo.br/pdf/rbca/v10n1/a06v10n1.pdf