Journal of International Scientific Publications: Agriculture and Food

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

MULTIRESIDUE SCREENING OF PESTICIDES AND THEIR RECOVERY FROM FATTY FOOD MATRICES USING THE QUECHERS METHOD

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Abstract

Although the use of organochlorine pesticides was banned in European Union states, these pesticide residues continue to be present in the environment. These residues enter the human body via diet, therefore it is necessary to monitor their levels in food. This study investigates the presence of selected organochlorine pesticides in representative high-fat dairy products using the QuEChERS method. All of the tested dairy products had lower levels of the pesticides than the currently permitted limits. Effect of the fat content in high-fat dairy products on the recovery of organochlorine pesticides using the QuEChERS method was also investigated. High-fat food matrices had a negative impact on the recovery of most of the tested organochlorine pesticides.

Key words*: QuEChERS, dairy products, GC-ECD, multiresidue screening*

1. INTRODUCTION

Dairy products form an indispensable component of human diet in many countries (Kushi et al., 1995). As dairy products are an excellent source of both macro- and micro-nutrients, both children and adults consume them in one form or another. However, as mammalian milk tends to accumulate pesticide residues (Klinčić et al., 2014; Mannetje et al., 2013; Waliszewski et al., 2001), pesticide use has directly contributed over the years to the negative effect on the human health, apart from proven negative effects on the environment (Köck-Schulmeyer et al., 2013). The European Union MRL Regulation (EC) No 396/2005 database reports a Maximum Residue Limit of 0.04 $mg.kg^{-1}$ for DDT (expressed as a sum of p,p´-DDT, o,p´-DDT, p-p´-DDE and p,p´-DDD), 6 $mg.kg^{-1}$for Aldrin and Dieldrin (expressed as Dieldrin) 8 $mg.kg^{-1}$for Endrin and 0.05 $mg.kg^{-1}$for Endosulfan in milk from cattle (EU Pesticides Database). The United States Department of Agriculture sets a limit of 0.3 ppm for Lindane (γ-BHC) (United States Department of Agriculture: Pesticide Data Program) and the United States Environmental Protection Agency sets a limit of 2 ppm for Endosulfan (Electronic code of Federal Regulations). However, to our best knowledge, other than milk, there have been no reports on the level of such pesticides in dairy products such as yoghurt, cream and cheese.

In many countries, an inventory of pesticide use is maintained, including the Czech Republic (Neumeister & Šarapatka, 2003). All professional users of pesticides are required to record their pesticide use in detail. DDT along with its metabolites, dieldrin, endrin, heptachlor, hexachlorocyclohexanes (HCHs) and chlordane were extensively used before they were banned (Holoubek, et al., 2003). Though such pesticides have been banned for many years (Downie, 2003), at present they can still be detected in the environment due to their persistent nature. Therefore it is worthy to investigate the pesticide levels not only in milk, but also its dairy products.

In general, methods of screening such persistent pesticide residues have largely employed liquid-liquid extraction methods that use large quantities of hazardous solvents and require hours of preparatory steps. Recent methods employ a dispersive-solid phase extraction method which uses a fraction of the solvents, saves time and provide reliable results, QuEChERS being probably the most outstanding approach (Prestes, et al., 2009; Walorczyk, et al., 2014). Even though the QuEChERS method has been primarily developed for non-fatty food matrices, some initial investigations are available for milk (Lehotay, et al., 2005; Selvi, et al., 2012). However, to the best of our knowledge, no study is available for high-fat food matrices, such as cream, yoghurt and cheese.

Therefore, the objectives of this paper are two-fold. Firstly, we want to investigate the effectiveness of the QuEChERS method in recovering these pesticides from milk and cream products with varying fat-content. Secondly, we wanted to perform an initial screening of organochlorine pesticides (OCPs) using the QuEChERS method to provide a comprehensive analytical comparison of pesticide residues in Czech dairy products.

The range of dairy products consumed in Czech Republic ranges from the cow's milk itself with varying fat content, cream ("smetana"), sour milk products such as curd cheese ("tvaroh"), yogurt, butter and an aged cheese ("Olomoucké tvarůžky,") with a strong odour unique to the Czech dairy section.

2. MATERIALS AND METHODS

The Pesticide 8081 Standard mix containing OCPs at a concentration of 200 µg.mL^{-1} purchased from Sigma-Aldrich was used for calibration purposes and recovery studies. This is a mixture of α-BHC, β-BHC, γ-BHC, δ-BHC, Heptachlor, Aldrin, Heptachlor epoxide, γ-chlordane, p,p'-DDE, Dieldrin, α-chlordane, α-endosulfan, Endrin, β-endosulfan, p,p'-DDD, Endrin aldehyde, Endosulfan sulfate, p,p'-DDT, Endrin ketone and Methoxychlor. In addition to the OCPs listed above, the standard mixture also contained 2,4,5,6-tetrachloro-m-xylene and decachlorobihpenyl. HPLC grade acetonitrile was purchased from Sigma-Aldrich. HPLC-grade hexane, anhydrous magnesium sulfate and sodium chloride were purchased from Lach-Ner s.r.o., Czech Republic. Primary secondary amine (Bondesil PSA, 40 µm particle size) was purchased from Agilent Technologies. Calibration solutions with concentrations of 0.002, 0.02, 0.2, 2 and 20 µg.mL^{-1} were prepared by diluting the standard mix with hexane.

Commercially available dairy products listed in Table 1 were obtained from different resources. Most of the products come from the local supermarket, but in addition we also used: (a) the cow milk from vending machine, (b) the cow milk from the local farmer and (c) the cream with 35.5% fat-content which was also purchased from a local farmer's shop.

Exactly 10 g of the dairy product was weighed in a 50 mL screw-capped centrifuge tube and 10 mL of acetonitrile was added, screw cap was closed and the tube vigorously shaken by hand for 1 min. To this, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl was added, and again tube was shaken by hand for 1 min. Then, it was centrifuged at 3000 rpm for 5 min. Two mL aliquot of the upper layer was transferred to a 15 mL centrifugation tube containing 100 mg primary secondary amine (PSA) and 600 mg anhydrous magnesium sulfate. Tube was closed and shaken vigorously for 1 min and centrifuged for 5 min at 3,000 rpm. The sample was evaporated to near dryness under a gentle stream of nitrogen. After which 1mL of hexane was added. This was transferred immediately to an autosampler vial and analyzed using a Gas Chromatograph fitted with an electron capture detector (GC-ECD). Quantification of OCPs was performed using GC (Agilent 7890A) equipped with an EC detector, an autosampler (G4513A).

Recovery rates were determined by spiking the standard mixture at a concentration of 50 µg.mL^{-1} into milk and cream. This level was chosen as an appropriate level using experiments using different concentration. Milk with three levels of fat content was used, namely: 0.5%, 1.5% and 3.5%. The cream with 35.5% fat content was diluted with distilled water to arrive at cream with three levels of fat: 4.44%, 8.88% and 17.75%. The spiked samples were permitted to equilibrate for 30 min before extraction, to allow the spiked solution to penetrate the matrix. The samples were processed by adopting the above extraction procedure. All measurements were performed over five different levels ranging between 0.002 and 20 µg. mL^{-1}. Three replicates for each level were analyzed. Repeatability of the method was evaluated by calculating the relative standard deviation (RSD %). A Signal-to-Noise ratio of 10x the peak height was used for accessing the limit of quantification (LOQ) and a 3x the peak height was used for the limit of detection (LOD). The LOD was determined to be 0.002 ng.mL^{-1} and the LOQ was 0.02 ng.mL^{-1}.

The OCPs were separated using HP-5 (30 m x 0.32 mm internal diameter 0.25 µm film thickness) fused silica capillary column (J & W Scientific Co., Agilent Technologies, USA). The injection was performed at split (1:10) mode with 1 µl injection volume. High purity (99.999 %) nitrogen gas was used as carrier gas and makeup gas flow was set at 2 mL min^{-1} and 30 mL min^{-1}, respectively. The detector temperature was 275°C. The oven temperature ramp was programmed as follow: 150°C, for 30 s, increased to 275°C (4°C.min^{-1}) for a total of 33.75 min. Identification was based on comparison of retention times with spectra of standard pesticide mixture using the software OpenLAB CDS ChemStation edition for GC systems version Rev. C.01.04 [35].

3. RESULTS AND DISCUSSION

All the organochlorine pesticides of interest were clearly resolved in the chromatogram except for α-Chlordane and α-Endosulfan. This phenomenon is known to occur in chromatograms obtained from HP5 columns which was the column used for this study.

Calibration curves were obtained by plotting concentration versus average peak area and the results are summarized in Table 2. As can be observed from Table 2, the calibration curves were found to have a good linearity by with correlation coefficients (R^2) of more than 0.9992 in all cases indicating that the linear

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

regression method can be used to quantify the levels of OCPs in unspiked samples within the concentration range investigated.

The cleanup of the samples was performed by using dispersive solid phase extraction (d-SPE), which involved mixing the extract in a mixer of PSA and $MgSO_4$. The d-SPE with PSA effectively removes many polar matrix components, such as organic acids and sugars from the food extracts (Anastassiades, et al., 2003). All of the tested dairy products had lower than detectable levels of pesticides. This indicates that these products complied well with the current production norms and had not used ingredients with pesticide residues in them.

In order to assess the extraction efficiency of the method, the recovery studies were carried out by spiking the dairy products with the standard mixture of OCPs listed in Table 2, at a concentration levels of 50 $\mu g.mL^{-1}$ and repeated three times to determine repeatability. Unspiked samples of milk and cream were analyzed and the results indicated the blank extracts did not contribute any interference with the target compounds. These results indicated that the QuEChERS method is indeed applicable for the determination of all the analytes of interest in the present study for milk and cream. The percentage of recovery of each pesticide was calculated by comparing the peak area ratio of the spiked standards with those of the pure standards. Average recovery data and relative standard deviations (RSD) obtained for selected matrix spiked with OCPs compounds are shown in Table 3. The recoveries ranged from 47.29–112.16 % with all relative standard deviation (RSD) values less than 20 % indicating a generally acceptable value. It is worthwhile to mention that endrin aldehyde was consistently absent from all the recovery studies using cream. It was also absent in the low-fat milk. This could be partially explained by the fact that this pesticide residue degrades during extraction step (Chung & Chen, 2011). Fat content seem to have a significant effect on the recovery of certain pesticides. This negative effect of recovery of the organochlorine pesticides could also be the effect of other matrix components present in cream and milk.

4. CONCLUSIONS

We have investigated the effects of fat content on the recovery of pesticide residues from the high-fat dairy products such as cream, yoghurt and cheese. Moreover, a comprehensive investigation of the pesticide residues was performed for representative Czech dairy products that represent the range of favorite products available to the Czech consumers. The tested products contained less than the acceptable limits of the pesticide residues, which is important for the consumer safety. This work opens up new opportunities to evaluate matrix components that could influence the use of QuEChERS method.

ACKNOWLEDGEMENTS

The authors would like to acknowledge funding support by the Ministry of Education, Youth and Sport under the project titled, “Development of human resources in food analysis using gas chromatography”, Reg. Nr. CZ.1.07/2.3.00/30.0008.

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

Table 1

Summary of the dairy products used for the detection of OCPs.

Name	Fat Content (%)
Cow milk:	
Low-fat	0.5
Skimmed	1.5
Full-fat	3.5
Organic	3.5
From vending machine	3.5
From local farmer	3.5
Long shelf-life	1.5
Goat milk	3.3
Cream:	
Brand A	12, 16, 33
Brand B	12, 19, 31
From local farmer	35.5
Yogurt:	
Brand A	2.4
Brand B	3.0
Organic	3.5
Other:	
Olomoucké tvarůžky (aged cheese)	0.6

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

Table 2

Retention time, correlation coefficient along with the linear equation obtained from calibration curves.

Pesticide Residue	**Retention Time (min)**	**Correlation Coefficient R^2**	**Linear equation**
α-BHC	15.06	0.9997	y = 25600.1x + 5456.0
β-BHC	16.07	0.9993	y = 8428.1x + 2755.6
γ-BHC	16.32	0.9996	y = 21850.2x + 5371.8
δ-BHC	17.26	0.9997	y = 22633.9x + 4285.8
Heptachlor	19.19	0.9996	y = 21641.1x + 5441.9
Aldrin	20.62	0.9995	y = 23153.4x + 6773.8
Heptachlor epoxide	22.31	0.9995	y = 19851.8x + 6196.9
γ-Chlordane	23.31	0.9995	y = 22304.5x + 6066.7
p,p'-DDE	23.82	0.9996	y = 20037.3x + 5033.9
Dieldrin	23.96	0.9995	y = 21623.2x + 6025.6
α-Chlordane + α-Endosulfan*	24.93	0.9998	y = 42008.8x + 8406.6
Endrin	25.83	0.9994	y = 17612.8x + 5435.4
β-Endosulfan	26.24	0.9997	y = 19460.4x + 4531.7
p,p'-DDD	26.71	0.9992	y = 14705.8x + 5363.3
Endrin aldehyde	27.06	0.9997	y = 15853.8x + 3325.6
Endosulfan sulfate	28.07	0.9999	y = 18572.8x + 2679.6
p,p'-DDT	28.28	0.9996	y = 15225.6x + 3177.8
Endrin ketone	29.98	0.9995	y = 16125.2x + 4764.6
Methoxychlor	30.80	0.9995	y = 7305.2x + 1970.6

* co-eluting peaks

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

Table 3.

Relative recoveries (expressed as mean ± RSD %) from cow's milk and cream with varying fat content spiked with 50 μg.mL^{-1} OCPs.

Pesticide residue	**Milk (fat content)**			**Cream (fat content)**		
	0.5%	**1.5%**	**3.5%**	**4.44%**	**8.88%**	**17.75%**
α-BHC	95.05 ± 4.18	76.29 ± 8.40	76.85 ± 7.76	81.97 ± 1.71	83.29 ± 13.99	77.10 ± 1.54
β-BHC	109.27 ± 3.23	92.28 ± 7.20	93.28 ± 8.59	101.54 ± 0.91	109.26 ± 14.09	106.42 ± 2.44
γ-BHC	98.15 ± 3.80	81.59 ± 8.33	82.63 ± 7.55	89.73 ± 1.33	93.49 ± 14.15	88.65 ± 1.77
δ-BHC	77.01 ± 1.10	57.31 ± 7.78	55.52 ± 1.77	74.72 ± 0.76	76.67 ± 11.87	75.79 ± 0.52
Heptachlor	101.43 ± 4.49	78.89 ± 9.49	74.05 ± 9.77	80.34 ± 1.86	77.87 ± 14.86	66.14 ± 3.21
Aldrin	102.79 ± 3.93	76.73 ± 8.37	66.70 ± 10.22	71.76 ± 1.58	62.32 ± 14.03	47.29 ± 4.90
Heptachlor epoxide	112.16 ± 2.25	93.43 ± 8.58	92.02 ± 9.56	98.66 ± 0.46	101.21 ± 14.73	92.83 ± 1.07
γ-Chlordane	101.91 ± 2.10	80.72 ± 7.67	74.91 ± 10.15	82.15 ± 0.32	79.29 ± 14.81	67.68 ± 2.46
p,p'-DDE	105.08 ± 1.75	84.10 ± 7.77	81.22 ± 9.28	88.28 ± 0.62	87.47 ± 14.49	76.68 ± 1.44
Dieldrin	99.97 ± 2.05	78.47 ± 7.20	72.55 ± 9.85	80.50 ± 0.47	77.58 ± 14.83	66.78 ± 3.17
α-Chlordane + α-Endosulfan*	106.50 ± 2.28	85.34 ± 8.19	79.70 ± 10.01	84.63 ± 0.11	79.85 ± 14.36	66.14 ± 1.08
Endrin	113.27 ± 2.84	93.24 ± 7.73	90.18 ± 9.30	95.85 ± 0.11	95.48 ± 15.08	85.38 ± 1.40
β-Endosulfan	103.58 ± 2.23	86.17 ± 7.60	86.02 ± 8.81	92.45 ± 0.17	95.28 ± 14.18	88.78 ± 0.38
p,p'-DDD	109.33 ± 3.08	90.28 ± 5.83	85.57 ± 9.11	93.10 ± 0.19	93.34 ± 14.56	84.86 ± 1.75
Endrin aldehyde	ND	26.61 ± 2.29	30.96 ± 12.17	ND	ND	ND
Endosulfan sulfate	82.57 ± 0.90	65.29 ± 7.66	67.18 ± 4.40	82.18 ± 0.28	87.28 ± 11.54	88.42 ± 1.74
p,p'-DDT	83.26 ± 3.93	61.33 ± 7.76	56.26 ± 8.20	63.79 ± 1.01	59.21 ± 12.88	51.35 ± 5.05
Endrin ketone	101.91 ± 4.38	84.76 ± 6.42	86.47 ± 6.78	96.01 ± 0.52	100.04 ± 13.14	98.27 ± 0.00
Methoxychlor	84.13 ± 1.87	74.42 ± 7.75	74.71 ± 7.45	78.99 ± 1.75	83.86 ± 11.80	87.31 ± 1.24

* co-eluting peaks

ND = peak not detected

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