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# Determination of 3-MCPD and 2-MCPD esters in edible oils, fish oils and lipid fractions of margarines available on Polish market

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ABSTRACT

3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) are intensively investigated food contaminants, which recently emerged as a serious problem within the discovery of high levels of their fatty acid esters in lipid samples. In order to monitor the concentrations of mentioned toxicants in edible oils and fats, several analytical methods have been developed so far. This paper presents the screening research on bound 3-MCPD and bound 2-MCPD contents in edible oils, fish oils (in the form of dietary supplements) and lipid fractions of margarines available on retail market in Poland. Applied SGS "3-in-1" methodology developed by Kuhlmann (2011) was based on mild alkaline trans-esterification reaction. MCPD esters were not detected in cold-pressed, non-refined edible oils. In all samples of refined oils and products, containing lipid fraction consisting of refined fats, bound 3-MCPD and 2-MCPD were detected. The highest amounts of analytes were present in lipid fractions of margarines and dietary supplements containing refined fish oils (7,3 and 5.5 mg $kg^{-1}$ respectively).



## 1. Introduction

Chlorinated propanols have been known as food toxicants for over three decades. 3-monochloropropane-1,2-diol (3-MCPD), one of their representatives, was discovered by Velišek et al. (1978) in acid-hydrolysed vegetable protein used for soy sauce production. Soon after that toxicological research conducted towards its carcinogenic activity on living organisms proved the risk of chloropropanols presence in heat-processed food products (Lynch, Bryant, Graham, Nestmann, & Munro, 1998). This problem emerged again quite recently within the discovery that chlorinated propanols, especially chloropropanediols, occur in some types of foodstuffs not only in free form but mainly in the esterified one (Svejkovská et al., 2004). 3-MCPD esters were determined so far in edible oils and fats and foods containing high amounts of lipid fraction such as deep fried products, spreads or powdered infant formulas (Ermacora & Hrncirik, 2014; Hamlet et al., 2011; Zelinková, Svejkovská, Velíšek, & Doležal, 2006). The formation of MCPD esters in food lipids from chlorinated compounds and acylglycerols as precursors is affected by the oil composition as well as the oil treatment during refining process (Ermacora & Hrncirik, 2014). The re-investigation in this field is related to the difficulties in reliable risk assessment related to esterified chloropropanediols presence in foods popular in human diet. The hypothesis of free 3-MCPD and related propanols release from esterified form during digestion still needs further investigation, as well as the level of the release since there is still no data obtained from toxicological studies on human beings. So far, the research on carcinogenic activity of chloropropanediols, particularly 3-MCPD, carried out on rodents resulted in the discovery that it may cause the lesions in kidneys and testis (Abraham et al., 2013; Liu et al., 2012). For this reason, for assessing the risk related to chloropropanediols presence in food, it is assumed that free 3-MCPD and related compounds are released in human gastrointenstinal tract with 100% efficiency (Bakhiya, Abraham, Gürtler, Appel, & Lampen, 2011).

In order to determine the level of 3-MCPD esters in lipid samples, two analytical approaches may be applied: direct and indirect. The principle of direct methods relies on each single fatty acid ester determination (including both monoesters and diesters) and final quantification by liquid chromatography-mass spectrometry (e.g. LC-MS/MS, LC-TOFMS) (MacMahon, Begley, & Diachenko, 2013; MacMahon, Mazzola, Begley, & Diachenko, 2013; Part 1 & 2; Haines et al., 2011; Hori et al., 2012; Weisshaar, 2011; Zelinková, Doležal, & Velíšek, 2009). It requires quite easy sample clean-up (based usually on SPE technique) and enables obtaining complete

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information about sample composition however it didn't find application in routine analysis because of the need of plenty analytical standards purchase and challenging separation (Crews et al., 2013). For monitoring the concentrations of 3-MCPD esters in lipid samples the indirect methods are more frequently applied. They are based either on acidic or alkaline transesterification reaction in order to release free chloropropanediol from esterified form followed by purification (liquid–liquid extraction), derivatisation (usually by PBA, HFBI or BSTFA/TMCS) and quantification by gas chromatography-mass spectrometry. This approach, although involving multi-step sample preparation process where possible side reactions may occur, is widely applied in quality control laboratories because it is relatively cheap and simple in case of chromatographic separation (Dubois et al., 2012). The critical step in indirect methods is the transesterification reaction which may result in partial conversion of the original content of 3-MCPD into glycidol (related food toxicant) if the reaction is carried out in alkaline conditions. The solution for this problem may be lowering of pH of the reagent or carrying the transesterification reaction in acidic conditions (Ermacora & Hrncirik, 2013; Hrncirik, Zelinková, & Ermacora et al., 2011).

The scope of this work was to conduct screening research on the concentration of chlorinated propanediols, 3-MCPD and its isomer 2-MCPD in esterified form present in lipid products available on Polish market including rapeseed, sunflower, olive, linseed, corn, sesame oils, lipid fractions of margarines and fish oils in the form of dietary supplements. The last group of samples bring novelty to the field of chloropropanols presence in food and related products, as dietary supplements haven't been monitored so far more widely according to available literature data. Moreover, the presence of bound MCPD in foodstuffs from retail Polish market have not been reported so far in the paper reaching international attention. This screening research will enrich the knowledge regarding the presence of MCPD in products available on European market and the level of MCPD exposure to consumers. Publication of these results may lead to greater call for setting the regulations regarding 3-MCPD and related chloropropanols concentrations in both free and bound form in lipid products because by now only the tolerable daily intake was set for 3-MCPD present in soy sauce (at a level of 2 μg/kg body weight) (EC, 2001).

## 2. Materials and methods

### 2.1. Chemicals and samples

1,2-Bis-palmitoyl-3-chloropropanodiol, 1,2-Bis-palmitoyl-3-chloropropanediol-$d_5$, 1,3-distearoyl-2-chloropropanediol and 1,3-distearoyl-2-chloropropanediol-$d_5$ were purchased from Toronto Research Chemicals (Toronto, Canada). Diethyl ether, methanol, sodium hydroxide, sodium bromide, ortophosphoric acid, ethyl acetate, phenyloboronic acid (PBA) and acetone were purchased from Sigma–Aldrich (Steinheim, Germany). Compressed gases as nitrogen and helium were purchased from Linde Gas. The hexane was purchased from Merck (Darmstadt, Germany) and anhydrous sodium sulphate from Eurochem BGD (Tarnow, Poland). All chemicals used were of analytical grade and were used as received without any further purification.

Sodium hydroxide solution in methanol was prepared by dissolving 250 mg of NaOH in 100 mL of MeOH. The neutralizing solution was prepared by dissolving 50 mg of sodium bromide in 100 mL of deionised water and adding to the solution 3.5 mL of ortophosphoric acid (85%) in order to obtain slightly acidic conditions. The solution for derivatisation was prepared by dissolving 200 mg of phenylboronic acid in 10 mL of diethyl ether. Stock solutions of 1,2-bis-palmitoyl-3-chloropropanodiol and 1,3-distearoyl-2-chloropropanediol (both 5 mg $mL^{-1}$) and 1,2-bis-palmitoyl-3-chloropropanodiol-$d_5$ and 1,3-distearoyl-2-chloropropanediol-$d_5$ (correspondingly 5 mg $mL^{-1}$ and 2 mg $mL^{-1}$) were prepared by dissolving pure solid analytical standards in ethyl acetate. Spiking solutions, were obtained by the dilution of stock solutions to the desired concentrations: 5 μg $mL^{-1}$ and 50 μg $mL^{-1}$ solutions of non-deuterated standards and 50 μg $mL^{-1}$ solutions of deuterated standards.

Examined samples can be divided into three groups:

1. Edible oils (described below) – 27 samples
2. Margarines (different brands) – 5 samples
3. Fish oils (3 types of capsulated fish oil and 2 types in a liquid form) – 5 samples

For comparison of the influence of pressing and refining conditions on the chloropropanols content in edible oils two types of samples were examined: cold-pressed unrefined oils such as corn, sesame, linseed, rapeseed, sunflower and extra virgin olive oil and refined oils - rapeseed, sunflower and olive oil (three samples from different supplier of each oil variety). The selection of margarines and fish oils for the investigation was based on the popularity of different brands and suppliers among Polish consumers.

### 2.2. Sample preparation procedure

The lipid fractions of investigated margarines were obtained by centrifugation of warmed product and then the upper lipid phase was collected and filtered through the anhydrous sodium sulphate.

The fish oils from encapsulated supplements were collected from the capsules by a sterile needle.

The bottled fish oils and edible oils were collected by a pipette directly from the bottle in which they were stored on the market.

The procedure applied in this work was performed according to the paper published by Kuhlmann (2011). Originally the methodology was dedicated for determination of bound 3-MCPD and bound glicydol in refined oils. According to the available literature data this so called SGS “3-in-1” method provides accurate measurement avoiding underestimation of the analytes concentration. The mild alkaline transesterification minimizes the possibility of MCPD transformation into glycidol. In this study, only the concentration of MCPD esters was examined.

The general principles of each step of the procedure are provided by flow chart and reaction presented in Figs. 1 and 2 respectively.

The samples were melted and homogenized if necessary and dissolved in 600 μL of diethyl ether. At this step 100 μL of each internal standard solution of deuterated 3-MCPD and 2-MCPD esters was added to the reaction mixture. Next, the transesterification reaction was carried out by the use of 350 μL of methanolic sodium hydroxide solution in order to release free 3-MCPD and 2-MCPD from bounded form. The samples were incubated for 16 h in freezer at −25 °C with the aim of complete transesterification and then the reaction was stopped by the addition of slightly acidic NaBr solution neutralizing the reaction mixture. The organic phase was evaporated under gentle stream of nitrogen in the room temperature to approximately 100 μL and extracted by 600 μL hexane twice (the organic phase was discarded) in order to remove remained fatty acid methyl esters. Remaining aqueous phase was extracted three times by 600 μL of moderately polar mixture of diethyl ether and ethyl acetate (3:2), organic extracts were collected in new vials and dried with the use of anhydrous sodium sulphate. Derivatisation reaction was carried out by addition of 80 μL of derivatisation agent - phenyloboronic acid (PBA) into the collected extracts. In order to enhance the reaction ultrasound treatment was applied for 5 min.