CHARACTERIZATION OF C10-C17 CHLORINATED PARAFFINS IN OVEN-BAKED PASTRY PRODUCTS AND UNPROCESSED PASTRY DOUGH BY HPLC-ESI-Q-TOF-MS

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Introduction

Chlorinated paraffins (CPs) are structurally complex mixtures of polychlorinated n-alkanes with varying chain lengths and degrees of chlorination. CPs are classified according to their carbon chain lengths: short-chained CPs (C_{10}–C_{13}, SCCPs), medium-chained CPs (C_{14}–C_{17}, MCCPs), and long-chained CPs (C_{18+}, LCCPs)[1]. CPs have a broad range of industrial uses, predominantly in the metalworking field and polyvinyl chloride processing, as well as plasticizers and flame retardants[2]. Despite classification of SCCPs as POPs, the annual worldwide production of CPs remains high (~ one million tons per year). CPs are chemically stable and highly lipophilic compounds and over the past decades CPs have been reported with alarming frequency in almost all environmental matrices[1]. CPs can biomagnify through food webs and display endocrine-disrupting characteristics[3].

Exposure to CPs poses a potentially major threat to human health while several studies have assessed the presence of CPs in domestic items (e.g., household baking oven doors[4] and kitchen hoods[5]), showing that significant emission sources of CPs are present in the kitchen environment. These observations suggest that migration of CPs might occur during food preparation and contribute to an additional dietary exposure to CPs.

Despite the instrumental advances, analysis of CPs remains a challenging task due to thousands of possible congeners. Gas chromatography (GC) in combination with electron capture negative ion (ECNI) low-resolution mass spectrometry (LRMS) has been the commonly applied analytical technique for over two decades. However, an obvious drawback for applications of LRMS is its lack of selectivity because other organochlorine contaminants and CPs themselves can cause interfering MS peaks. In order to eliminate this issue, several studies have relied on high-resolution MS (HRMS). As of now, the majority of recent CP detection methods relay on GC coupled to time-of-flight MS (TOF-MS) or Orbitrap-MS techniques to separate and characterize CPs[6,7]. Moreover, a study by Li et al.,[8] reported that HPLC techniques can be applied to chromatographic separation of CPs, thus making the analysis of CPs even more accessible for the scientific community.

In our study, we present an easily applicable HPLC-Q-TOF method to analyse CPs without any additional use of ionization enhancers (e.g., DCM). The aim of this study was to assess the levels of SCCP contamination in thermally processed foods, in this case oven-baked pastry products. The acquired SCCP and MCCP homologue profiles were compared to unprocessed dough. Data of this type can lead to further insights regarding CP transformations occurring during the food preparation and provide complementary information about possible contamination sources.

Materials and methods

Reagents and materials

Stock standard mixtures of SCCPs (fixed carbon chain length, 10 ng μL^{-1} in cyclohexane), MCCPs (100 ng μL^{-1} in cyclohexane), and 1,2,4,5,6,9,10-heptachlorodecane (CP-7) (10 ng μL^{-1} in cyclohexane) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The chain lengths and chlorine content (wt%) for SCCP and MCCP standards were as follows: C_{10} (50.2%, 55.0%, 60.1%, and 65.0% chlorine content), C_{11} (45.5%, 50.2%, 55.2%, 60.5%, and 65.3% chlorine content), C_{12} (45.3%, 50.2%, 55.0%, 65.1%, and 70.0% chlorine content), C_{13} (60.0% and 65.2% chlorine content), and C_{14}-C_{17} (57.0% chlorine content). All solvents, sorbents and other chemicals were of analytical grade and were obtained from FLUKA and Sigma-Aldrich.

Samples

Thirty eight samples of oven-baked pastry products (e.g., pies, buns, strudels) and fifteen dough samples (e.g., puff pastry, pizza dough) were acquired from local confectioneries in Riga, Latvia. Samples were lyophilized, the dry residue was homogenized and stored in polypropylene containers at room temperature.

Sample extraction and clean-up

Approximately 10 g of freeze-dried sample aliquot was extracted with a mixture of *n*-hexane and dichloromethane (1:1, v/v) at 130°C, using a Soxtec™ 2055 Fat Extraction System (Hillerød, Denmark). One gram of fat aliquot was cleaned up by using two sequentially connected columns containing 8 g of acidic silica gel (44% H_2SO_4, w/w) and 3 g of deactivated Florisil (2% H_2O, w/w). Both columns were conditioned with 30 mL of *n*-hexane/dichloromethane (1:1, v/v), followed by of *n*-hexane (30 mL). The extract was dissolved in *n*-hexane (5 mL) and quantitatively transferred onto acidic silica column, flushed with *n*-hexane (50 mL), and the

n-hexane fraction was discarded. The top column containing acidic silica was disconnected and the Florisil column was eluted with 50 mL of *n*-hexane/dichloromethane (1:1, v/v). This fraction was concentrated to dryness using rotary evaporator and a gentle N_2 stream. If the evaporated sample extract contained any visible fatty residues, it was subjected to additional acidic treatment with a mixture containing concentrated sulfuric acid (100 µL) and *n*-hexane (200 µL). After centrifugation the *n*-hexane layer was evaporated under N_2, and reconstituted in 50 µL of acetonitrile for the following instrumental analysis.

Chromatographic separation
CPs were chromatographically separated using a Dionex UltiMate 3000 HPLC system (Thermo Scientific, Sunnyvale, CA, USA) equipped with analytical column Kinetex C18 (50 mm × 3 mm, 2.6 µm particle size, 100 Å) from Phenomenex (Torrance, CA, USA). Mobile phases consisted of water (A) and acetonitrile (B). The following gradient program was applied: 0–2 min, 40% B; 2–5 min, linear increase to 100% B; 5–20 min, 100% B; 20–25 min, 40%. Initial flow rate was 0.3 mL min^{-1}, which was reduced to 0.1 mL min^{-1} from 11 to 20 min during the gradient elution program. Injection volume was 5 µL and column temperature was set to 40°C.

Mass spectrometry analysis
Mass spectra were acquired on a Bruker Compact Q-TOF system (Bremen, Germany) equipped with an electrospray ionization (ESI) source (Bruker Daltonics, Bremen, Germany) and operated in negative ionization mode. The main instrumental parameters were set as follows: endplate offset, 500 V; capillary voltage, 4500 V; nebulizer pressure, 2.0 bar; dry gas flow, 10 L min^{-1}; dry temperature, 200°C; vaporizer temperature, 260°C. A full MS spectrum was acquired from 100 to 1000 *m/z* at the rate of 1 Hz.

Processing of MS data and quantification of CPs
Three most abundant $[M-H]^-$ traces were extracted from the full MS data for each of the analysed CP congener groups. The acquired peak areas and their respective *m/z* values were used to identify the source of overlapping MS interference and to calculate the true intensity of target signal. Deconvolution procedure was conducted with RStudio™ software (Version 1.1.383), which was used as an integrated development environment for R programming language (Version 3.4.2). Five different SCCP mixtures were prepared using the available standard solutions at different compositions. The corresponding response factors (RFs) for SCCP homologue groups were calculated at five concentration levels (from 0.25 to 5.0 ng μL^{-1}). Each sample measurement was subjected to SCCP pattern reconstruction procedure and quantified in accordance with the most fitting combination between two standard solutions.

Results and discussion
Optimization of electrospray ionization
The initial optimization of the ESI source parameters was carried out by direct injection of CP-7 (0.5 ng μL^{-1}). When acetonitrile (ACN) was used as the elution medium, only $[M-H]^-$ and $[M+Cl]^-$ species were observed, whereas methanol produced additional formic acid adduct $[M+HCOOH-H]^-$, thus only ACN was used further.

The sheath gas flow rate was set to 5 L min^{-1} and equally enhanced the signal intensity for both species, while the vaporizer temperature also affected the relative abundance of $[M-H]^-$ and $[M+Cl]^-$ ions. In-source formation of chlorinated olefins (COs) was not observed. The best conditions for source temperature were found at 260°C where the response factor for $[M-H]^-$ ion accounting for 89±3% of the total CP-7 signal. Experiments with low-energy in-source collision-induced dissociation (IS-CID) showed that both species disintegrated almost simultaneously. The enhancing of the formation of chloride adducts was also evaluated by adding DCM directly to the eluent between the LC column and the ionization chamber through a T-type connector. Although the predominance of deprotonated species was eliminated, the signal was still present and did not drop below 12±3%. (Figure 1A). Moreover, the absolute intensity of the $[M+Cl]^-$ ion signal did not exceed that of the $[M-H]^-$ ion signal, which was obtained without adding DCM. Therefore, DCM was not used as enhancer of chloride-containing ionic species in the final method.

Since it was not possible to completely eliminate the formation of chloride adducts, experiments were carried out to investigate whether the formation of $[M+Cl]^-$ species depended on the concentration. This aspect had to be considered, because fluctuations in the relative abundance between both species could affect the quantification of CPs. A mixture of SCCPs and MCCPs was directly injected at different concentrations and the results showed that the ratio between the $[M-H]^-$ and $[M+Cl]^-$ was independent of analyte concentration and the carbon chain length. However, the degree of chlorination did have an impact on the composition of ionic species (e.g. for CP homologues having 7 and 8 chlorine atoms the abundances of $[M+Cl]^-$ ions were 13±5% and 17±4%, respectively (relative to [M-H]-), whereas in the case of 9 and 10 chlorine atoms the relative abundances were 25±7% and 33±7%, respectively). Nevertheless, our experiments showed that $[M-H]^-$ ion can be used as a quantification trace as its signal intensity is largely proportional to the analyte concentration, while the relative ratio between [M-H]- and $[M+Cl]^-$ does not significantly fluctuate.

Deconvolution of mass spectral interferences and reconstruction of SCCP homologue profiles

Quantification of CPs remains the most challenging aspect of their analysis and the majority of methods cannot completely resolve overlapping MS signals from MCCPs, COs, and other chlorinated non-polar interferences, therefore a deconvolution procedure should be used.

In all studies on the Q-TOF application to the analysis of CPs it was reported that Q-TOF did not have the necessary resolving power (RP) (>20 000 FWHM). In this study, a major issue was caused by self-interferences, because each CP homologue group simultaneously produced two detectable species, which interfered with each other. Moreover, the presence of COs in the samples also should be considered. To overcome the risk of erroneous *m/z* peak selection the three most abundant $[M\text{-}H]^-$ traces per each C_nCl_m homologue were extracted from the full MS data using a broad isolation window (±0.02 Da). This enabled the detection of target peaks, which were affected by both interferences. The selected traces were then subjected to a two-way deconvolution procedure. In order to eliminate the negative effect of *m/z* peak broadening caused by the interference, an additional resolution threshold (at 8000 FWHM units) was applied, therefore highly interfered peaks can be marked as non-compliant and subsequently discarded.

SCCPs were quantified using deconvolution procedure where it was assumed that each homologue group with the same carbon chain length can be expressed as a combination between two reference standards with a known chlorination degree[9]. No standard can provide the perfect match to completely mimic the distribution of homologues in real samples. However, the applied procedure can compare the similarity between reference profiles and the measured sample. Calculations were performed separately for each carbon chain length. Reference profiles were generated by simulating pair-wise combination between two SCCP standards. Combinations were produced for each pair and the relative composition of components was varied from 5% to 95% (with a step of 5%). The similarity was calculated as Euclidean distance between two normalized homologue profiles, with lower value indicating a better fit between them. For instance, if an unknown $C_{11}Cl_m$ homologue pattern matched the combined profile between $C_{11}Cl50.2\%$ and $C_{11}Cl65.3\%$ standards with a ratio of 20:80 (w/w), then 20% from the total $C_{11}Clm$ signal was quantified using RF derived from the $C_{11}Cl50.2\%$ calibration curve, while the remaining 80% were quantified using RF derived from $C_{11}Cl65.3\%$. The final concentration of C_{11} CPs in the sample was expressed as the sum of both fractions.

Levels of SCCPs in dough and baked products

The present study focuses on oven-baked products primarily because various studies have reported that kitchen environment contains significant sources of CPs[4,5]. All concentration values in the following paragraph are expressed relative to dry weight. Traces of SCCPs were detected in all samples. The total Σ_{SCCP} concentrations in products ranged from 0.3 ng g^{-1} to 23.0 ng g^{-1} (mean: 6.3 ng g^{-1}), while for dough samples it ranged from 5.8 ng g^{-1} to 22.8 ng g^{-1} (mean: 12.9 ng g^{-1}). The most abundant homologue group in product samples were chlorinated dodecanes (C_{12} CPs, from 0.1 ng g^{-1} to 8.7 ng g^{-1}, mean: 2.6 ng g^{-1}) followed by chlorinated undecanes (C_{11} CPs, from 0.1 ng g^{-1} to 8.6 ng g^{-1}, mean: 2.0 ng g^{-1}, Figure 1B). On the contrary, dough samples contained considerably higher levels of C_{11} CPs (from 1.8 ng g^{-1} to 16.4 ng g^{-1}, mean: 5.2 ng g^{-1}), while C_{12} CPs were the second most abundant CP homologue group (from 1.1 ng g^{-1} to 4.6 ng g^{-1}, mean: 3.0 ng g^{-1}). Our results show that exposure to 180-220°C temperature (typical baking temperature) shifts the relative CP homologue group distribution towards the higher chain length SCCPs. These changes could be most likely associated with thermal decomposition of higher chain length CPs (MCCPs or LCCPs) which therefore release SCCPs via carbon chain cleavage. Our results revealed that the mean and median values of SCCPs were in all cases higher for products with a filling although data appear to suggest that filling is not the primary SCCP contamination source, since the relative increase of SCCP concentrations in samples with filling does not account for the majority of the total contamination.

Relative distribution, and changes of SCCP and MCCP homologue profiles in dough and baked products

The most frequently found CP homologues in the samples were $C_{11}H_{17}Cl_7$, $C_{12}H_{19}Cl_7$, $C_{14}H_{23}Cl_7$. Higher detection frequencies were observed for hepta- and octa- CPs. No traces of deca- chlorinated SCCPs were found in any of the analysed samples. The occurrence of nona- CPs (in the case of SCCPs and MCCPs) and deca- CPs (only in the case of MCCPs) in baked products was much less frequent then in dough. These data confirm that dehydrochlorination reaction could be one of the major decomposition pathways, thus lower occurrence rates can be expected for higher chlorinated CPs in thermally processed products. In order to examine the effect of baking, the relative distribution between CP homologues was assessed. This was done by calculating the ratio between individual peak areas of each detected CP component ($C_nH_{2n+2-m}Cl_m$) and the total peak area within a single homologue group with the same carbon chain length (C_n). In agreement with the detection frequency, hepta-CPs occupied the largest proportion of the total peak area between SCCPs. The proportion of hepta-CPs in product samples ranged from 70% (C_{10} CPs) to 43% (C_{13} CPs), while in the case of dough the range was from 57% (C_{10} CPs) to 43% (C_{13} CPs). In both sample types, the relative distribution of hepta-CPs decreased along with growing chain length. A similar situation was observed for MCCPs, but octa-CPs then had the highest relative

distribution, which ranged from 36% (C_{14} CPs) to 40% (C_{16} CPs) and from 29% (C_{17} CPs) to 38% (C_{15} CPs) in product and dough samples, respectively (Figure 1C). Namely, the proportion of CPs with lower chlorination degree (hexa- and hepta- CPs) was higher for baked product samples in comparison to dough, while a decrease was observed for CPs with higher chlorination degree (octa-, nona-, and deca-CPs). (Figure 1D). In all cases the relative distribution between CP homologues was rather congeneric and the average relative standard deviation for product and dough samples was 26% and 16%, respectively. This finding largely indicates that there is probably one major source of CP contamination, because the homologue patterns are similar from sample to sample. Considering the best match for SCCP profiles and the fact that CPs with lower chlorination degree migrate more rapidly into the environment, our results indicate that the original CP contamination source has a chlorination degree somewhere around 50% to 55% and there is a high likelihood that commercial CP-52 product in particular might be the main source.

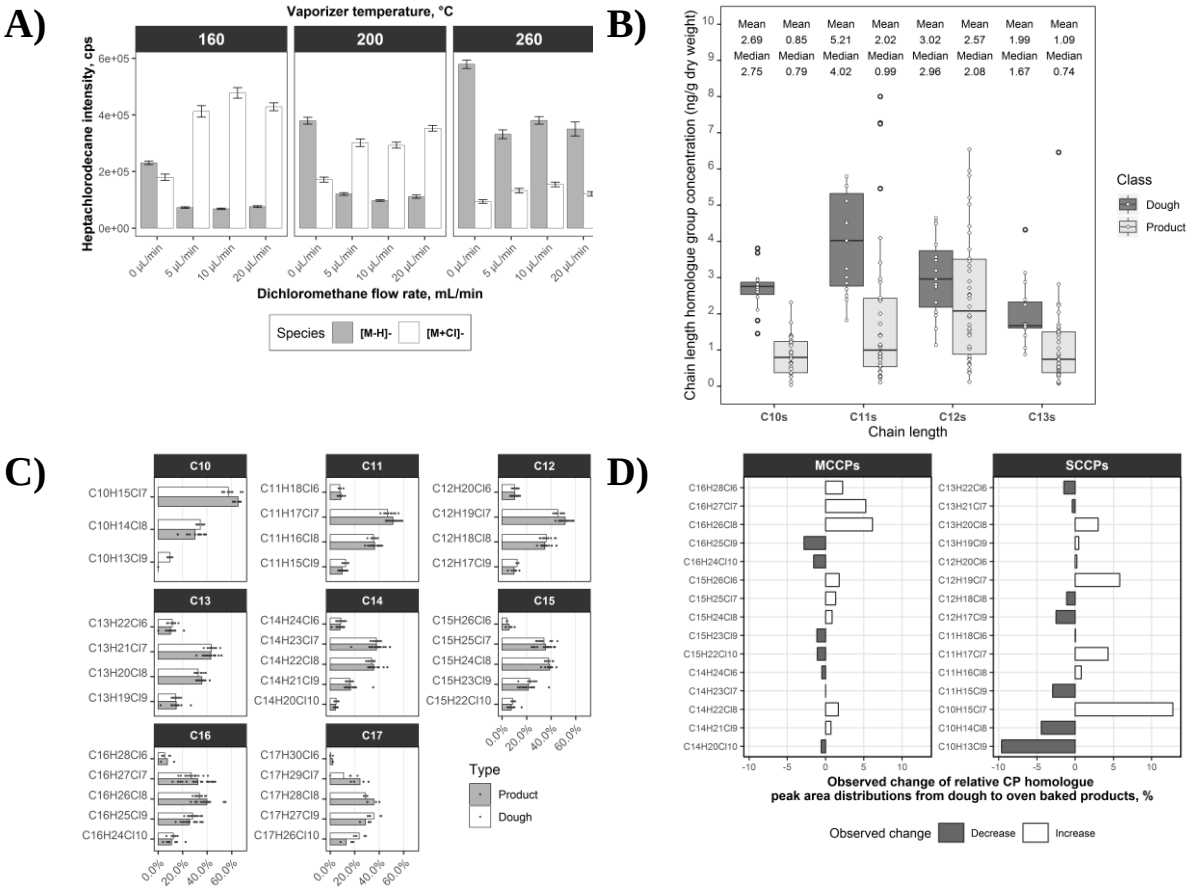

Figure 1. Distribution between $[M-H]^-$ and $[M+Cl]^-$ species at different ESI source parameters and DCM flow rates (1A); levels of SCCP homologue groups in the samples (1B); profiles of CP homologues (1C); the difference in relative distribution of CP homologues (1D).

Acknowledgements:
This work was supported by the European Regional Development Fund under project No. 1.1.1.2/VIAA/I/16/001, Post-doctoral research project No. 1.1.1.2/VIAA/2/18/247.

References:

1. Van Mourik LM, Leonards PEG, Gaus C, et al. (2015). *Chemosphere*. 136: 259–272.
2. Hahladakis JN, Velis CA, Weber R, et al. (2018). *Journal of Hazardous Materials*. 344: 179–199.
3. Zhang Q, Wang J, Zhu J, et al. (2016). *Environment International*. 94: 43–50.
4. Gallistl C, Sprengel J, Vetter W. (2018). *Science of the Total Environment.* 615: 1019–1027.
5. Bendig P, Hägele F, Vetter W. (2013). *Analytical and Bioanalytical Chemistry*. 405: 7485–7496.
6. Gao W, Wu J, Wang Y, et al. (2016). *Journal of Chromatography A.* 1452: 98–106.
7. Krätschmer K, Cojocariu C, Schächtele A, et al. (2018). *Journal of Chromatography A.* 1539: 53–61.
8. Li T, Gao S, Ben Y, et al. (2018). *Environmental Science and Technology*. 52: 1862–1870.
9. Bogdal C, Alsberg T, Diefenbacher PS, et al. (2015). *Analytical Chemistry*. 87: 2852–2860.