# Survey of 3-Chloropropane-1,2-Diol and its Precursors in Foods in the Czech Republic

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**Abstract:** A survey of the levels of 3-chloropropane-1,2-diol (3-MCPD) and its precursors in a range of selected retail food products in the Czech Republic is reported. The foods were selected according to their content of water, chlorides and lipids and included foods processed at high temperatures and/or stored for a long time. The content of 3-MCPD was determined by the GC/MS method using deuterium-labeled 3-MCPD. Water content and pH value of the analysed foods were determined together with the recognised precursors of 3-MCPD, fat, glycerol and chlorides. An insight into the level of 3-MCPD influenced by these variables has been done by principal components analysis.

**Keywords**: 3-chloropropane-1,2-diol (3-MCPD); GC/MS; 3-MCPD precursors; PCA

## INTRODUCTION

3-Chloropropane-1,2-diol (3-MCPD) is one of a series of chemically related contaminants collectively known as chloropropanols. In view of its toxicity a regulatory limit of 0.02 mg/kg has been adopted for 3-MCPD in soy sauce and acid-HVP in the European Union [1]. 3-MCPD was first identified in acid-hydrolysed vegetable protein (acid-HVP) in 1981 by Davídek *et al*. [2]. Recent studies have shown that elevated levels of 3-MCPD can occur not even in soy sauces and related products [3–5] but also in many foods and food ingredients formulated without acid-HVP [6–9]. Domestic cooking of foods has also been shown to result significantly in elevated levels of 3-MCPD [10].

It has been shown that in the acid-HVP 3-MCPD arises in a reaction of hydrochloric acid with residual lipids contained in the raw material [2]. Glycerolipids and glycerol can also react with chloride ions naturally occurring in foods or with chloride ions from added cooking salt to generate 3-MCPD. In general, the production of 3-MCPD is promoted by a high temperature treatment, high content of chloride ions and low water content [11].

The current study aimed to report the results of analyses of a range of selected foods purchased from retail outlets in Prague in 2004. The foods were selected according to their content of water, chlorides and lipids and included foods processed at high temperatures and/or foods stored for a long time. The aim also was to develop an understanding of the major factors affecting the formation of 3-MCPD.

## EXPERIMENTAL

***Analysed foods***. Malt samples were produced by Obchodní sladovny, a. s. (Prostějov, CZ), all other food samples were purchased from retail outlets in Prague. The samples were stored according to the recommendation of producers.

***Determination of pH, water, chlorides, glycerol and fat***. Approximately 2.5 g of a homogenised sample and 25 ml of distilled water was mixed and pH value of the mixture was measured by the pH meter Radiometer (Copenhagen NV, Denmark) with the electrode THETA 90, type RE 413.

Water was determined by drying 5–10 g of homogenised sample in an oven at 103–105°C, 28 g of dried sea sand was added to the easy sintered samples for better drying [12].

Chlorides were determined in the extract obtained using hot distilled water (50 ml) added to

5 g of sample placed in a beaker. The beaker was covered by a watch glass, its content was boiled for 2 min and cooled to room temperature. The suspension was filtered through a Büchner funnel and the filtrate titrated by the 0.1 M $AgNO_3$ using the above pH meter equipped with a chloride-selective electrode ISE [13].

For the determination of glycerol, 10 g of homogenised sample was mixed with 50 ml of butane-1,3-diol (internal standard) in methanol (0.02 mg/l). The suspension was filtered and solvent evaporated. The residue was dissolved in 2 ml of methanol and 1 µl was analysed by GC. The GC analysis was carried out using an HP 4890A apparatus (Hewlett Packard, USA) equipped with a flame ionisation detector, data processing system (CSW 1.7) and an HP-20M capillary column (20 m, 530 µm I.D., 1.3 µm film thickness). The oven was initially set to 120°C, kept for 2 min, then programmed at a rate of 15°C/min to 180°C and kept at this temperature for 14 min. The injection port (split 1:1) and detector were held at 220°C and 280°C, respectively. Nitrogen at a flow rate of 30 ml/min was used as the carried gas.

The lipid content of cocoa powder, coffee, malts, peanuts, potato crisps, crisp bread, salty sticks, doughnuts, French fries and olive samples was determined by the Soxhlet method using 150 ml of light petroleum ether (8 h). The solvent was evaporated on a rotary vacuum evaporator and the residue was dried in an oven at 103–105°C [14]. The Folche method (extraction with a mixture chloroform:methanol, 2:1, v/v) was used for the determination of lipids in meat products, chicken and fish. The obtained extract was purified by extraction with water, the upper phase was separated and the solvent treated as above. The lipid content of cheeses was determined using the Schmidt-Bondzynski-Ratzlaff method (3 g, hydrolysis with 10 ml of 25% HCl at 100°C for 5 min, extraction with 10 ml ethanol, 25 ml diethyl ether, 25 ml light petroleum ether). The obtained extract was again treated as above.

***Determination of 3-MCPD***. 3-MCPD was determined using capillary gas chromatography with mass spectrometric detection and deuterated 3-chloropropane-1,2-diol as the internal standard [15].

***Statistical methods***. Statistical evaluation of the achieved results was done employing the computer program SPSS for Windows, Release 11.0.0, Standard Version.

## RESULTS AND DISCUSSION

Twenty-four foods were chosen to cover the most common representatives of foods of animal and plant origin (Table 1). The highest amount of 3-MCPD was found in cheese 1 (Parmesan, 82.67 µg/kg). Very high levels exceeding 20 µg/kg were found in dark malt, roasted peanuts, salami 1 and 3, sausage, ham 1 and 2, grilled chicken, pickled herring,

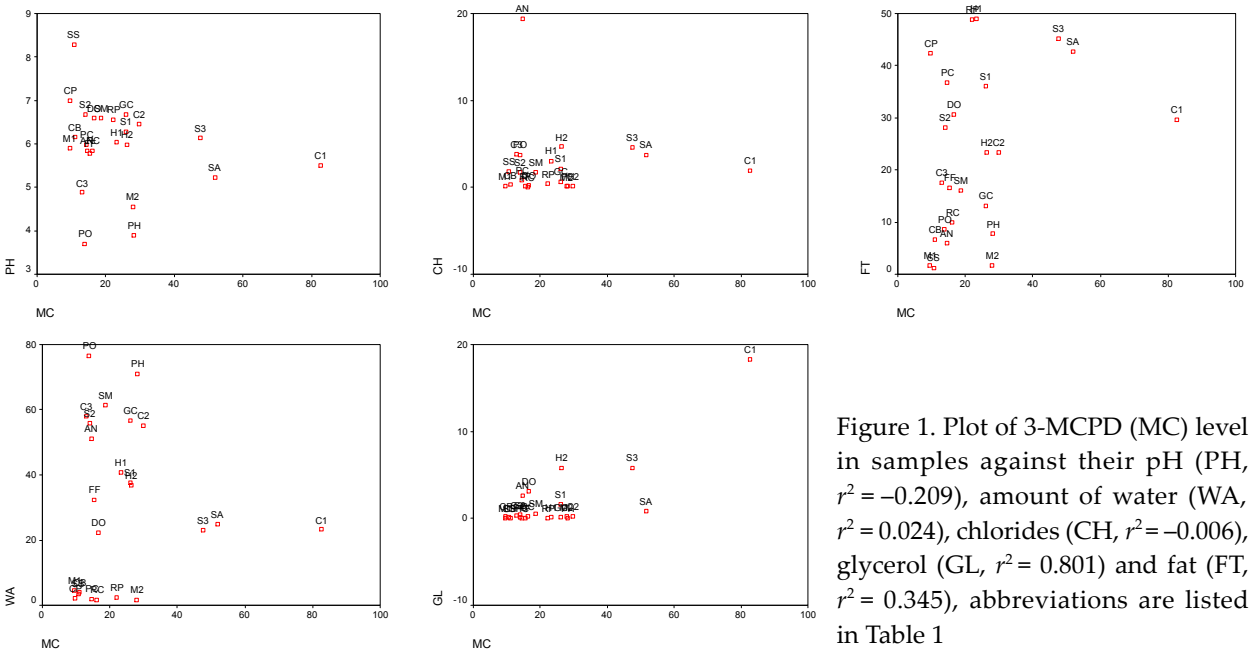



Figure 1. Plot of 3-MCPD (MC) level in samples against their pH (PH, $r^2 = -0.209$), amount of water (WA, $r^2 = 0.024$), chlorides (CH, $r^2 = -0.006$), glycerol (GL, $r^2 = 0.801$) and fat (FT, $r^2 = 0.345$), abbreviations are listed in Table 1

Table 1. Levels of 3-MCPD and its precursors in foods

| Food | pH | 3-MCPD | | 3-MCPDn | Water | | Chlorides | | Glycerol | | Fat | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | µg/kg | RSD | µg/kg | % (w/w) | RSD | % (w/w) | RSD | g/kg | RSD | % (w/w) | RSD |
| Cocoa powder CP | 7.00 | 9.61 | 2.80 | 3.92 | 2.00 | 8.90 | – | – | 0.28 | 3.50 | 42.40 | 0.00 |
| Roasted coffee RC | 5.85 | 16.15 | 4.30 | 6.56 | 1.56 | 0.50 | 0.06 | 12.90 | 0.26 | 0.60 | 10.00 | 0.20 |
| Malt 1 (Pilsener type) M1 | 5.90 | 9.55 | 5.20 | 4.00 | 4.45 | 7.40 | 0.14 | 6.40 | 0.02 | 5.50 | 1.60 | 1.00 |
| Malt 2 (dark) M2 | 4.55 | 27.90 | 2.60 | 11.34 | 1.60 | 0.40 | 0.09 | 8.30 | 0.19 | 0.40 | 1.60 | 0.00 |
| Crisp bread CB | 6.15 | 11.05 | 2.00 | 4.60 | 3.93 | 1.10 | 0.29 | 14.60 | 0.04 | 7.60 | 6.60 | 1.10 |
| Potato crisps PC | 5.98 | 14.48 | 2.40 | 5.91 | 1.93 | 2.60 | 0.80 | 3.50 | tr | – | 36.80 | 0.50 |
| Salty sticks SS | 8.28 | 10.72 | 2.50 | 4.44 | 3.36 | 0.20 | 1.80 | 7.50 | 0.09 | 2.40 | 1.10 | 5.90 |
| Roasted peanuts RP | 6.55 | 22.10 | 2.00 | 9.05 | 2.32 | 3.70 | 0.39 | 1.10 | 0.01 | 12.60 | 48.80 | 0.00 |
| Doughnuts DO | 6.60 | 16.58 | 2.30 | 8.53 | 22.23 | 0.70 | 0.20 | 10.90 | 3.14 | 3.60 | 30.70 | 4.90 |
| French fries FF | 5.78 | 15.41 | 1.30 | 9.10 | 32.24 | 0.40 | 0.13 | 0.00 | 0.05 | 14.10 | 16.60 | 0.90 |
| Salami 1 S1 | 6.28 | 26.01 | 3.10 | 16.68 | 37.61 | 0.00 | 2.12 | 1.70 | 1.63 | 0.40 | 36.10 | 1.40 |
| Salami 2 S2 | 6.68 | 14.03 | 3.80 | 12.73 | 55.92 | 0.20 | 1.76 | 2.40 | 0.39 | 5.40 | 28.10 | 0.20 |
| Salami 3 S3 | 6.13 | 47.63 | 1.00 | 24.75 | 23.03 | 0.20 | 4.62 | 0.80 | 5.76 | 3.70 | 45.20 | 0.90 |
| Sausage SA | 5.23 | 51.80 | 1.40 | 27.59 | 24.89 | 0.30 | 3.69 | 1.20 | 0.85 | 1.30 | 42.70 | 0.30 |
| Ham 1 (smoked) H1 | 6.03 | 23.18 | 1.80 | 15.65 | 40.77 | 0.20 | 3.01 | 0.50 | 0.14 | 7.30 | 49.00 | 1.20 |
| Ham 2 (Bologna) H2 | 5.98 | 26.24 | 0.70 | 16.63 | 36.89 | 0.30 | 4.66 | 1.10 | 5.77 | 4.90 | 23.40 | 1.50 |
| Grilled chicken GC | 6.68 | 26.04 | 2.20 | 24.07 | 56.72 | 0.20 | 0.65 | 6.50 | 0.18 | 5.80 | 13.10 | 4.30 |
| Smoked mackerel SM | 6.60 | 18.62 | 1.40 | 19.27 | 61.34 | 0.50 | 1.73 | 3.30 | 0.56 | 6.70 | 16.10 | 2.20 |
| Pickled herring PH | 3.90 | 28.19 | 7.00 | 38.86 | 70.98 | 0.10 | 0.12 | 1.40 | 0.04 | 16.40 | 7.70 | 0.30 |
| Anchovy AN | 5.85 | 14.64 | 0.90 | 12.00 | 51.20 | 0.10 | 19.39 | 0.30 | 2.58 | 3.60 | 6.00 | 4.70 |
| Cheese 1 (Parmesan) C1 | 5.50 | 82.67 | 0.40 | 43.06 | 23.21 | 0.60 | 1.89 | 0.80 | 18.35 | 1.10 | 29.70 | 0.50 |
| Cheese 2 (processed) C2 | 6.45 | 29.83 | 2.80 | 26.53 | 55.03 | 0.00 | 0.18 | 0.00 | 0.28 | 10.20 | 23.30 | 0.90 |
| Cheese 3 (feta) C3 | 4.88 | 12.98 | 1.20 | 12.39 | 58.09 | 0.00 | 3.84 | 0.20 | 0.35 | 2.10 | 17.60 | 0.80 |
| Pickled olives PO | 3.70 | 13.93 | 1.00 | 23.70 | 76.49 | 0.00 | 3.68 | 3.10 | 0.11 | 8.50 | 8.60 | 0.80 |

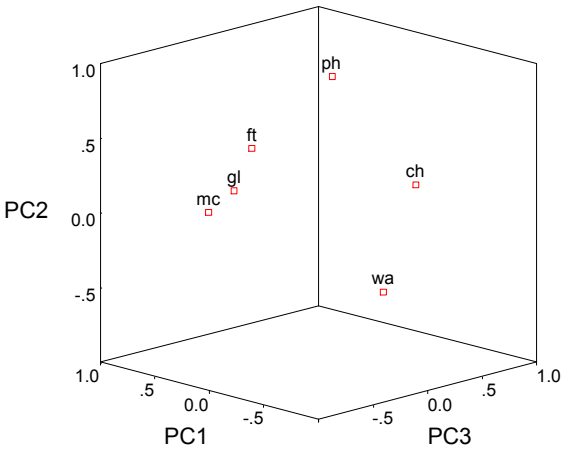
RSD = relative standard deviation; 3-MCPDn = normalized to 40% dry matter content

cheese 1 and 2. The rest of samples had 3-MCPD levels below 20 μg/kg. The lowest amounts of 3-MCPD (< 10 μg/kg) were found in cocoa powder and light malt of Pilsener type. After normalization to 40% dry matter content, 3-MCPD levels in 7 samples (29%) were still above the European Union limit of 20 μg/kg adopted for soy sauces and acid-HVP. Seven of the samples contained 3-MCPD at levels between 10 and 20 μg/kg and in most cases (10 samples) the levels of 3-MCPD were below 10 μg/kg.

Each food was characterised by the following variables: pH value, content of water, chlorides, glycerol and fat (Table 1). It is known that 3-MCPD arises from lipids and chlorides in foods exposed to high temperature or stored for a long time. However, the plot of 3-MCPD concentrations against pH, water, chloride and fat contents (Figure 1) did not reveal any significant relation between these variables. Correlation significant at the 0.01 level (2-tailed) was only found between the 3-MCPD and glycerol concentrations ($r^2 = 0.801$). It is evident that the formation of 3-MCPD is a multivariate problem as it depends not only on water, fat and salt contents but also on pH value of the respective food, temperature during its processing and time of its storage. To visualize the relationships between the individual variables and samples, principle components analysis (PCA) have been done using the data given in Table 1 (pH, 3-MCPD, water, salt, fat and glycerol content). Three extracted PCs (rotation method: Varimax with Kaiser normalization) explained 75.2% of the total variance. The relations between the variables are given in Figure 2. PC1 describes principally the variables 3-MCPD, glycerol and fat. The upper part of PC2 describes the variable pH. The lower part of PC2 describes the variable water and fat. The upper part of PC3 describes the variable chlorides and water. For example, it can be seen that the variable 3-MCPD positively correlates with the fat and glycerol levels and negatively correlates with pH. The relation between the individual samples is given in Figure 3. For example, the sample cheese 1 has the highest content of 3-MCPD and is situated along the positive part of PC1. The sample pickled olives is situated along the positive part of PC2 as it has low pH and relatively high level of 3-MCPD. Nevertheless, some other variables, e.g. the temperature of processing, length of storage and the content of 3-MCPD esters in the respective food, are needed to visualize the relationships between the individual variables and samples more precisely.

## CONCLUSIONS

3-MCPD is present in a number of foods, especially in foods with high content of salt and fat, in sour foods and in foods processed at high temperatures and/or stored for a long time.




ph = pH, mc = 3-MCPD, wa = water, ch = chlorides, gl = glycerol, ft = fat

Figure 2. Saturations of variables plotted in PC1, PC2 and PC3

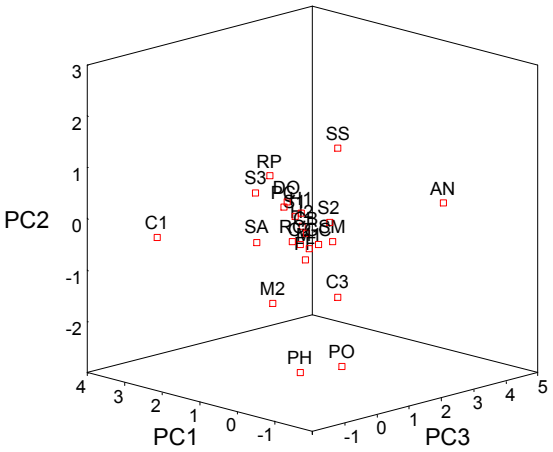



Figure 3. Sample scores plotted in PC1, PC2 and PC3 abbreviations are listed in Table 1

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