Influence of Dough Ingredients on 3-MCPD Formation in Toast

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Abstract: The influences of both traditional dough ingredients like fat and salt and a commercially used baking agent on the formation of 3-chloropropane-1,2-diol (3-MCPD) during toasting of bread were investigated. Whereas varying the fat or salt contents within technologically practicable limits showed negligible effects on 3-MCPD formation, the baking agent turned out to have a crucial impact on generating 3-MCPD in toasted bread slices. We found considerable evidence that the baking agent's main component saccharose was the major cause for its boosting the 3-MCPD formation. Emulsifiers like mono- and diacylglycerols or lecithin did not have any significant influence. 3-MCPD formation showed good correlation with the degree of browning of the bread slices; their 3-MCPD content increased exponentially towards dark brown coloured toasts. The relative proportions between 3-MCPD and 2-MCPD were an average of 3:1 in all samples. Dichloropropanols like e.g. 1,3-dichloropropan-2-ol could not be detected.

Keywords: 3-MCPD; toast; monochloropropanediol; dough; browning

INTRODUCTION

Like acrylamide, 3-chloropropane-1,2-diol (3-MCPD) belongs to a group of contaminants which are formed during processing of food (so-called food-borne contaminants). Several toxicological studies have shown that 3-MCPD produces infertility in rats [1] and causes tumours in rats when administered in high doses. Its *in vitro* genotoxic activity, however, could not be confirmed *in vivo* [2–5]. Based on these evaluations, a maximum tolerable daily intake (TDI) of 2 μg/kg body weight (bw) has been established by the Scientific Committee on Food (SCF). In the European Community (EC), a maximum level of 20 μg/kg 3-MCPD has been set for soy sauce and hydrolyzed vegetable proteins, maximum levels for other foodstuffs are currently under discussion [6].

Several recent studies have shown that 3-MCPD is not only present in foodstuffs which are consummed in comparatively low levels like soy sauce or seasonings, but also in other food ingredients like malts or bread crumbs and moreover in staple foods like bread, cereals, or meat [7–9]. Furthermore, 3-MCPD levels rise significantly when bread is toasted under domestic cooking conditions [8, 10]. Hamlet *et al.* [11] proved that yeast-derived glycerol accounts for a major part of 3-MCPD generated in model dough systems. Since we observed in previous studies [8] that different kinds of toasts produced varying amounts of 3-MCPD, we now concentrated on the investigation whether other dough ingredients than yeast, like e.g. fat or salt, also influence 3-MCPD formation during domestic toast preparation.

EXPERIMENTAL

Chemicals and reagents. Dough ingredients were purchased from commercial suppliers. 3-chloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropan-2-ol (1,3-DCP) were obtained from Aldrich (Taufkirchen, Germany). Phenylboronic acid (PBA) and Extrelut NT 3 solid phase extraction (SPE)

cartridges were purchased from E. Merck (Darmstadt, Germany), heptafluorobutyrylimidazole from Fluka (Taufkirchen, Germany). 3-MCPD-d_5 used as internal standard was obtained from Promochem (Wesel, Germany). All other reagents and solvents were at minimum of analytical quality; deionized water was used for all procedures.

Samples. The basic dough recipe comprised wheat flour (type 550), fresh baker's yeast (5%), and water (54–55%). All given percentages are related to the flour content. After baking (220°C, 35 min, 3 breads from each recipe) and cooling, the breads were cut in slices; slices from each bread were toasted in a customary toaster adjusted to different levels of intensity (four slices per toasting level). The toasting levels ranged from zero (not toasted) to seven (very dark). The toasted slices were kept frozen until analysis.

Determination of 3-MCPD by means of PBA-derivatization. Analyses were performed according to Breitling-Utzmann *et al.* [8], MS/MS conditions were adapted from [12]. GC-MS/MS analysis was performed on a Varian CP-3800 GC coupled to a Varian 1200 MS/MS system (Varian, Darmstadt, Germany). The chromatographic conditions were as follows: capillary column: VF-5ms (Varian, Darmstadt, Germany), 30 m, ID 0.25 mm, film 0.25 μm; injection volume: 2 μl; SSL-injector: 180°C, splitless mode, splitless time: 1.5 min; flow: 1.2 ml/min helium, constant flow mode; oven heating rate: 60°C (1 min) – 6°C/min – 190°C – 20°C/min – 280°C (30 min); interface: 250°C. MS/MS conditions: EI^+, selected ion monitoring (SIM); source: 250°C; detector 1300 V; emission current 150 μA; scan time 0.2 s; span 0.7 amu; SIM masses were m/z 201 (Q1) and 150 (Q3) for the internal standard 3-MCPD-d_5, 196 (Q1) and 147 (Q3) for 3-MCPD, and 196 (Q1) and 104 (Q3) for 2-MCPD, respectively.

Determination of 3-MCPD and 1,3-DCP by means of HFBI-derivatization. An official method for filter papers was adapted for food stuffs [13]. In brief, 2.5 ml of sample solution (sample preparation according to Breitling-Utzmann *et al.* [8]) were applied onto an extraction cartridge. After 20 min of incubation, the analytes were eluted with *t*-butylmethylether/isooctane 95/5 (v/v). 1.75 ml isooctane and 100 μl internal standard (13 μg/ml 3-MCPD-d_5 in isooctane/hexane 95/5 (v/v)) were added, and the *t*-butylmethylether removed *in vacuo*. To the remaining isooctane phase, 25 μl HFBI were added and the reaction mixture kept tightly sealed at room temperature for 15 min. After addition of 1 ml distilled water and vigorous shaking, the organic layer was separated and again washed with water (1 ml). The remaining organic phase was subjected to GC/MS analysis.

GC-MS analysis was performed on a Finnigan TraceGC coupled to a Finnigan TraceMS (Thermo Finnigan, Dreieich, Germany), equipped with a programmable temperature vaporizing injector (PTV). The chromatographic conditions were as follows: capillary column: RTX-5MS (Restek, Bad Homburg, Germany), 30 m, ID 0.25 mm, film 0.25 μm; injection volume: 2 μl; PTV-injector heating rate: 60°C – 12°C/s – 180°C (3 min), splitless mode, splitless time: 0.7 min; flow: 1.0 ml/min helium, constant flow mode; oven heating rate: 50°C (1 min) – 2°C/min – 90°C – 25°C/min – 300°C (10 min); interface: 250°C. MS conditions: EI^+, selected ion monitoring (SIM); source: 250°C; detector 500 V; emission current 350 μA; dwell time 0.067 min; SIM masses were m/z 257 and 456 for the internal standard 3-MCPD-d_5, 253 and 453 for 3-MCPD, and 257 for 1,3-DCP, respectively.

RESULTS AND DISCUSSION

Bread doughs were prepared varying fat content (0–5%) and type (peanut oil or butter), salt content (1.6–2.4%), addition of sour dough, and baking agent, respectively. Standard slices from each bread were toasted in a customary toaster yielding different stages of browning. This approach was chosen to imitate preparation of toast under domestic conditions, due to the varying consumer preferences for light brown, golden brown or dark brown toasts.

Compared to a fat free recipe the addition of peanut fat (1% in relation to the flour content) increased the formation of 3-MCPD in toast significantly (933 μg/kg compared to 351 μg/kg at toasting level 7). Higher fat contents (2–5%), however, as well as the fat type did not have any additional influence. Hamlet *et al.* [11] could not determine any effect of bakery fats on the formation of 3-MCPD in model doughs. However, their test conditions differed from ours crucially. During the toasting process very high temperatures (> 350°C) act on the bread surface which allow for high temperature hydrolysis of triacylglycerols. The resulting glycerol is an excellent precursor for 3-MCPD [11].

Varying the salt content within commonly used levels (1.6–2.4%) did not have any significant impact on 3-MCPD formation (data not shown). Addition of 4% sourdough, however, could reduce 3-MCPD

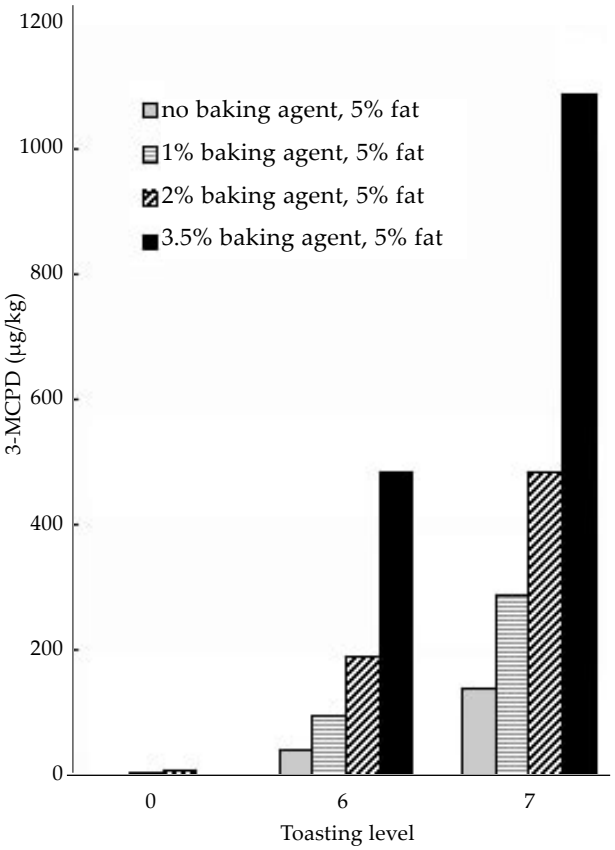

Figure 1. Influence of the amount of baking agent in bread dough on the formation of 3-MCPD in toasted bread slices (given are the mean values of four analyzed slices in μg/kg)

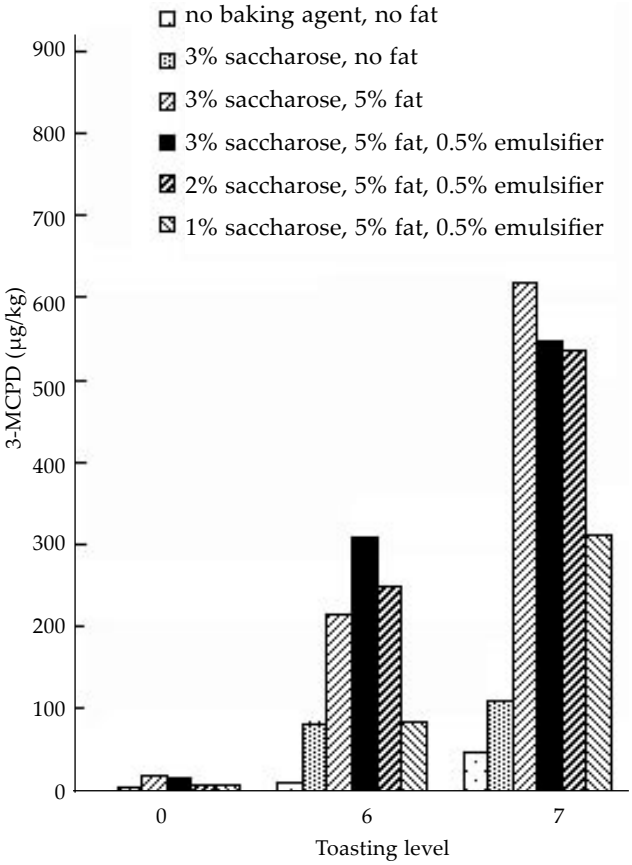

Figure 2. Influence of saccharose and emulsifier (mono- and diacylglycerols) in bread dough on the formation of 3-MCPD in toasted bread slices (given are the mean values of four analyzed slices in μg/kg)

formation to some degree (724 μg/kg compared to 1086 μg/kg at toasting level 7).

Whereas common baking ingredients like fat or salt did not have significant influence on 3-MCPD formation, the impact of a commercially used baking agent was striking. In toasts produced without baking agent only a small amount of 3-MCPD is formed in the bread slices even at the highest toasting level (47 and 26 μg/kg in recipes using wheat flour and wholemeal flour, respectively). In presence of 3.5% baking agent the 3-MCPD amounts increased dramatically (351 and 227 μg/kg, respectively), even without any addition of fat. Furthermore, a correlation between the amount of baking agent added and 3-MCPD formed during toasting could be established (Figure 1).

The applied baking agent consists of **sugar**, flour, soy flour, calcium sulfate, **emulsifier** (mono- and diacylglycerols of edible fatty acids; E 471), enzymes, and ascorbic acid.

Since mono- and diacylglycerols constitute good precursors of 3-MCPD [14], 0.25, 0.5 or 1.0% of these emulsifiers were added to the toast doughs instead of the baking agent, 0.1–0.5% being the manufacturer's recommended amount. Although addition of mono- and diacylglycerols did marginally increase 3-MCPD formation (data not shown), it could not account for the dramatic increase observed with the baking agent. The addition of lecithin did not have any significant influence on 3-MCPD formation which is in accordance with the observations by Hamlet *et al.* [14].

Since saccharose comprised the major ingredient of this baking agent (29 g/100 g), it was added to some recipes. Compared to sugar free recipes, significantly higher amounts of 3-MCPD were formed in toasts made from dough containing saccharose (Figure 2). A positive correlation between the sugar amount (1–3% saccharose) and 3-MCPD formation could be established. When saccha-

rose was substituted with glucose the same effect could be observed. Despite the obvious influence of saccharose and a less distinct effect of mono- and diacylglycerols, these substances could not account for the over-all effect of the baking agent on 3-MCPD formation during toasting. Therefore, other ingredients of the baking agent seem to have synergistic effects. So far, a mechanism for the formation of 3-MCPD starting from saccharose or glucose has not been published.

The brightness of all toasted slices was measured by means of a chromameter in accordance with the L*a*b*-system, L = 100 being black and L = 0 white [15]. Between the different recipes no significant difference in brightness could be observed. An exception was the recipe without any addition of baking agent or sugar which showed less browning even at the highest toasting level. Plotting the decreasing brightness against the 3-MCPD amount shows good correlation, with the 3-MCPD contents increasing exponentially towards deeper browning (data not shown).

The correlation between browning and 3-MCPD formation suggests that Maillard reaction products could be a source for 3-MCPD. We will conduct further experiments to see into this phenomenon.

The regioisomer 2-MCPD could be detected in all samples. Its amount was calculated preliminarily using the 3-MCPD calibration curve since 2-MCPD standard substance was not commercially available. The relative proportions between 3-MCPD and 2-MCPD were an average of 3:1 in all samples.

Fortunately, the toxicologically more critical dichloropropanols (e.g. 1,3-dichloropropan-2-ol) were not detectable even in darkly roasted toast slices.

CONCLUSIONS

Whereas "traditional" dough ingredients like fat or salt showed only minor influence on 3-MCPD formation during toasting, a commercially used baking agent turned out to be the crucial factor in promoting 3-MCPD formation. Concerning the baking agent itself, saccharose seems to be the major 3-MCPD source. It is therefore possible to affect 3-MCPD formation in toasted bread through specific dough components. Furthermore, consumers can avoid high 3-MCPD amounts by roasting their toasts to a golden brown colour at the most.

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