Formation and Decomposition of 3-Chloropropane-1,2-Diol in Model Systems

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Abstract: Model experiments were carried out using mixtures of 3-chloropropane-1,2-diol (3-MCPD), its precursor glycerol, NaCl, water and an emulsifier. The aim of this study was to simulate formation and decomposition of 3-MCPD at the surface layers of thermally processed foods containing naturally present or intentionally added salt. The formed 3-MCPD levels depend on temperature and reach the maximum value at 230°C. One kg of glycerol gives rise to about 50 mg of 3-MCPD, while about 0.6 mg of 3-MCPD arise at 100°C. The rate constants k_1 of 3-MCPD formation from glycerol and constants k_2 of 3-MCPD decomposition were calculated employing the first order reaction kinetics. The rate constants k_2 of 3-MCPD degradation are higher than the respective constants of its formation. Other kinetic parameters (time of maximum concentration) were also calculated and the achieved results were discussed with respect to levels of 3-MCPD in foods.

Keywords: 3-chloropropane-1,2-diol (3-MCPD); reaction kinetics; GC/MS

INTRODUCTION

Chloropropanols are group of endogenous food contaminants, which have been found in hydrolyzed vegetable proteins (HVP) in 1978 [1]. The most common chloropropanol 3-MCPD (3-chloropropane-1,2-diol) was identified in model experiments with lipids hydrolyzed by hydrochloric acid in 1979 [2, 3] and in HVP in 1981 [4]. Recent studies [5, 6] have demonstrated the presence of 3-MCPD at levels of 10–30 μg/kg in a range of foods and food ingredients. The precursors of chloropropanols are lipids and glycerol present in foods. Chloropropanols are then formed by reaction with hydrochloric acid in the case of acid-HVP manufacturing or by reaction with chloride anions naturally present or intentionally added as sodium chloride during the food processing.

Recent studies [7] followed the formation of 3-MCPD in model mixtures consisting of sodium chloride and either glycerol or various lipids (phospholipids, monoacylglycerols, diacylglycerols, triacylglycerols) derived mainly from palmitic and oleic acid. The average amount of 3-MCPD formed from these precursors after 30 min of heating at 200°C was 9.7 (lecithin), to 5.1 (diacylglycerols), 4.7 (glycerol), 3.1 (triacylglycerols) and 2.9 (monoacylglycerols) μmole/mole, respectively. The formation of 3-MCPD from glycerol was also studied in the presence of glutathione, cysteine, disodium carbonate and sodium bicarbonate, i.e. compounds having the potential to decompose 3-MCPD or to prevent its formation. The compound most active in preventing the formation of 3-MCPD was sodium bicarbonate followed by disodium carbonate, cysteine and glutathione. The addition of glutathione lowered the level of 3-MCPD produced from glycerol and NaCl to approximately 80%, cysteine to 42%, disodium carbonate to 14% and sodium bicarbonate to as little as 8% in comparison to samples with no additive.

In other study [8] was monitored the influence of various factors (NaCl, water content, and temperature) on the yield of 3-MCPD. The formation of 3-MCPD strongly depended on the concentration of NaCl and reached a maximum level at about 4–7% NaCl. The highest amount of 3-MCPD was formed in media containing approximately 13–17% water. The amount of 3-MCPD increased with increasing temperature over the range 100–230°C and rea-

ched its highest value at 230°C. The production of 3-MCPD was also followed in models very closely related to selected foods which had been shown to have a high potential to yield 3-MCPD during processing (salami, beefburgers, processed cheese, biscuits, crackers, doughnuts). The highest levels of 3-MCPD were formed in models simulating salami as they had the highest content of both fat and salt of all the samples. The lowest amount of 3-MCPD was formed in the models simulating biscuits and crackers as they had a low salt content and, at the same time, their water content was below the optimum level.

Kinetic data on reaction of chlorides with glycerol in systems with low water activity and at high temperatures are missing. Therefore, the aim of this work was to calculate the rate constants of 3-MCPD formation and decomposition in such models using systems with low water activity and heated to high temperatures corresponding to those encountered during the thermal processing of foods.

EXPERIMENTAL

Chemicals. 3-Chloropropane-1,2-diol (98%) was obtained from Merck (Germany), phenylboronic acid was from Fluka Chemie (Switzerland). Glycerol, Tween 80, sodium chloride and paraffin were from Lachema (Czech Republic).

Formation of 3-MCPD. Glycerol (200 mg), NaCl (10 mg, i.e. 3.47% (w/w) or 5% (w/w) with respect to the glycerol as 3-MCPD precursor) and an inert emulsifier (Tween 80, 30 mg) were placed in a 5 ml glass tube, 48 μl water was added. The tube was sealed and heated in an oven at temperatures 100°C, 140°C, 170°C, 200°C and 230°C for 0; 0.5; 1; 2; 4; 8; 16 a 24 h and then cooled to room temperature. The cold tube was opened and 0.2 ml of the internal standard propane-1,2-diol (200 μg) in water were added. The tube contents were transferred to a separating funnel and the tube was rinsed with three portions of hexane (one 4 ml portion and two 3 ml portions) and the washings transferred to the

Table 1. Formation of 3-MCPD (mg/kg glycerol)

Temperature (°C)		Time (h)						
		0	1	2	4	8	16	24
230	1		19.71	24.60	48.19	46.29	43.76	33.18
	2		18.96	22.44	48.00	45.88	43.59	32.58
	average	0	19.34	23.52	48.10	46.09	43.68	32.88
	s_R		0.66	1.91	0.17	0.36	0.15	0.53
200	1		8.26	17.81	21.19	23.47	26.87	36.94
	2		8.21	17.64	20.87	23.04	25.65	33.76
	average	0	8.24	17.73	21.03	23.26	26.26	35.35
	s_R		0.04	0.15	0.28	0.38	1.08	2.82
170	1		1.35	1.97	2.35	6.94	8.39	9.57
	2		1.23	1.91	2.31	6.65	8.26	9.25
	average	0	1.29	1.94	2.33	6.79	8.33	9.41
	s_R		0.11	0.05	0.03	0.26	0.11	0.28
140	1		0.84	0.82	0.57	1.44	5.91	7.63
	2		0.74	0.79	0.51	1.34	5.75	7.44
	average	0	0.79	0.81	0.54	1.39	5.83	7.54
	s_R		0.09	0.02	0.05	0.09	0.14	0.17
100	1		0.60	0.64	0.63	0.46	0.09	0.13
	2		0.60	0.63	0.61	0.43	0.09	0.10
	average	0	0.60	0.63	0.62	0.45	0.09	0.11
	s_R		0.00	0.01	0.02	0.02	0.00	0.03

separating funnel. The tube was then rinsed with 2 ml of water and these washings were also added to the separating funnel and its contents shaken. The aqueous phase was collected in a 25 ml distillation flask. The washing and extraction steps were repeated with three × 2 ml portions of water. The final aqueous extract was evaporated to dryness. The residue was dissolved in 2 ml of 20% sodium chloride solution and used for determination of 3-MCPD by gas-liquid chromatography.

Decomposition of 3-MCPD. In analogous experiments a mixture of paraffin (200 mg) and 3-MCPD (0.2 mg/kg paraffin) was used instead of glycerol.

Determination of 3-chloropropane-1,2-diol. The method of Plantinga *et al*. [9] was used with slight modifications. Two ml of the residue dissolved in 2 ml of 20% NaCl solution (see above) were heated for 20 min at 90°C with 0.2 ml phenylboronic acid in acetone (250 mg/ml) in a 25 ml flask. The flask was cooled and the 3-MCPD extracted into 2 ml hexane by shaking. One µl of the sample (hexane layer) was analyzed by GLC at a split rate of 2:1 using an SPB-I fused silica capillary column (30 m × 0.20 mm i.d., 0.80 µm film thickness, Supelco, USA) fitted to a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector. The oven temperature was initially set to 80°C, raised to 300°C at a rate of 5°C/min and kept at 300°C for 16 min. The injector and detector port temperatures were set to 250°C and 300°C, respectively. The helium carrier gas flow rate was 0.5 ml/min. Two parallel determination of each sample were made. The results were expressed in mg 3-MCPD per kg of precursor and in µmole of 3-MCPD per mole of precursor.

Table 2. Decomposition of 3-MCPD (mg/kg paraffin)

Temperature (°C)		Time (h)								
		0	1	2	3	4	5	8	16	24
230	1		0.125	0.099	0.074	0.041	nd.	nd.	nd.	nd.
	2		0.160	0.091	0.086	0.036	nd.	nd.	nd.	nd.
	average	0.200	0.143	0.095	0.080	0.038				
	s_R		0.031	0.007	0.011	0.004				
200	1		0.193	0.169		0.123		0.076	0.010	nd.
	2		0.190	0.132		0.122		0.069	0.009	nd.
	average	0.200	0.192	0.151		0.123		0.072	0.010	
	s_R		0.002	0.033		0.001		0.006	0.001	
170	1		0.190	0.164		0.140		0.081	0.021	nd.
	2		0.172	0.148		0.104		0.064	0.019	nd.
	average	0.200	0.181	0.156		0.122		0.072	0.020	
	s_R		0.016	0.014		0.032		0.015	0.002	
140	1		0.195	0.178		0.133		0.082	0.069	0.020
	2		0.194	0.164		0.116		0.061	0.042	0.018
	average	0.200	0.194	0.171		0.125		0.071	0.056	0.019
	s_R		0.001	0.013		0.015		0.019	0.023	0.002
100	1		0.199	0.183		0.144		0.115	0.083	0.050
	2		0.193	0.182		0.127		0.095	0.065	0.040
	average	0.200	0.196	0.183		0.135		0.105	0.074	0.045
	s_R		0.005	0.001		0.015		0.017	0.016	0.009

nd. = not detected

RESULTS AND DISCUSSION

The formation of 3-MCPD from its precursor glycerol was followed at temperatures 100°C, 140°C, 170°C, 200°C and 230°C in reaction mixtures simulating the surface layers of processed foods (200 mg glycerol, 10 mg NaCl, i.e. 3.47% (w/w), 48 mg water, i.e. 16.67% (w/w), 30 mg Tween 80). The samples were heated for 0; 1; 2; 4; 8; 16 and 24 h. All measurements were repeated two times. The obtained results are given in Table 1.

In analogous experiments a mixture of paraffin (added as an inert material, 200 mg) and 3-MCPD (0.2 mg/kg paraffin) was used instead of glycerol. These experiments were aimed to study the decomposition of 3-MCPD at the given conditions. The results are summarized in Table 2.

The rate constants k_2 of 3-MCPD decomposition were calculated as the first order reaction from the following equation:

$$c_{\text{3-MCPD}} = c_{\text{3-MCPD}} \left(e^{-k_2 t} \right)$$

where: k_2 = rate constant of 3-MCPD decomposition (s^{-1})
$c_{3\text{-}MCPD}$ = concentration of 3-MCPD in time t
$c_{(3\text{-}MCPD)0}$ = starting concentration of 3-MCPD

Using obtained constants k_2 in following equation:

$$c_{\text{3-MCPD}} = c_{\text{glycerol}} \frac{k_1}{k_2 - k_1} \left(e^{-k_1 t} - e^{-k_2 t} \right)$$

where: k_1 = rate constant of 3-MCPD formation from glycerol (s^{-1})
k_2 = rate constant of 3-MCPD decomposition (s^{-1})
$c_{\text{3-MCPD}}$ = concentration of 3-MCPD in time t
c_{glycerol} = starting concentration of glycerol, could be calculated the rate constants of 3-MCPD formation k_1 (Table 3)

Acquired kinetic data can also be used for the calculation of time needful for achieving maximum concentration of 3-MCPD by application of the equation:

$$t_{\max} = \frac{1}{k_2 - k_1} \ln \frac{k_2}{k_1}$$

CONCLUSIONS

All the data presented in the above tables are the result of simultaneous formation and decomposition of 3-MCPD. More then 20 h is needed to achieve the maximum concentration at 230°C and up to 216 h at 100°C. Based on these results, it seems that levels of 3-MCPD in foods could be lower but still can grow up with the thermal treatment.

Table 3. Kinetic parameters of 3-MCPD formation/decomposition

Temperature (°C)	k_1 (s^{-1}) × 10^{12}	k_2 (s^{-1}) × 10^5	$t_{max.}$ (h)
230	3770	9.9	28.6
200	1120	3.7	78.1
170	281	3.5	93.1
140	147	2.6	129.1
100	7.19	1.9	216.2

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