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Generation of Monochloropropanediols (MCPDs) in Model Dough Systems. 1. Leavened Doughs

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The effect of dough recipe ingredients and processing on the generation of monochloropropanediol isomers (MCPDs) in leavened wheat doughs has been investigated. Commercial ingredients having no effect on MCPD formation were acetic acid and baking fats (triacylglycerols). Ingredients making a significant contribution to MCPD levels were yeast and flour improver [ascorbic acid, diacetyl tartaric acid esters of mono- and diglycerides (DATEM), and soya flour]. The results showed that free glycerol is a key precursor of MCPDs in leavened doughs. This glycerol is primarily generated by the yeast during proving but is also present in the flour, the yeast, and the improver. Under conditions of high dough moisture content (45%), MCPD formation was approximately proportional to glycerol concentration but showed a weaker dependence on chloride level, suggesting that the mechanisms of formation involved at least some reversible stages. MCPD generation increased with decreasing dough moisture to a point where the formation reaction was limited by chloride solubility and competing reactions involving glycerol and key precursor intermediates. These results could be predicted by a kinetic model derived from the experimental data. Glycerol was shown to account for 68% of MCPDs generated in proved full recipe dough.

KEYWORDS: Chloride; dough; glycerol; kinetic model; 3-MCPD; monochloropropanediol; wheat; yeast

INTRODUCTION

The compounds 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) are glycerol chlorohydrins, the occurrence of which in foodstuffs was first reported (*1*) for the savory ingredient hydrolyzed vegetable protein (HVP). The structure of MCPDs (**Figure 1**) suggests that glycerol and/or glycero lipids could be precursors in processed foodstuffs. Previous studies have established that MCPDs can be generated as minor products from the reaction of hydrochloric acid with glycerol, acylglycerols, and phospholipids (*2*−*4*) and that these mechanisms accounted for levels found in HVP and soy sauces made by acid hydrolysis (*5*). More recently, MCPDs have been found in processed foods (*6*−*8*), notably a range of cereal products that have been subjected to heat treatments such as baking, frying, roasting, or toasting. The occurrence of MCPDs in products that do not utilize HVP or soy sauce as an ingredient has recently been reviewed (*9*), and the role of chloride, glycerol, and glycero lipids in generating these contaminants is not completely understood.

The toxicological effects of glycerol chlorohydrins are well-known, and the European Commission's Scientific Committee on Food classified 3-MCPD as a potential genotoxic carcinogen.

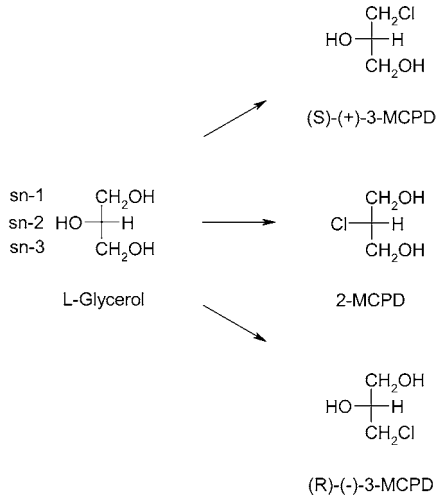

Figure 1. Monochlropropanediol isomers (MCPDs) and their relationship to L-glycerol (shown in Fischer projection). Enantiomers of 3-MCPD are formed when –OH is replaced by –Cl at the sn-1 or sn-3 positions on the glycerol backbone.

A review of the carcinogenicity of 3-MCPD (*10*) and more recent evaluations by expert bodies have concluded that there is a lack of evidence for in vivo genotoxicity. On the basis of these latest toxicological assessments, a provisional maximum tolerable daily intake of 2 μg kg^{-1} body weight has been

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proposed (*11*, *12*). The EC has set a regulatory limit of 0.02 mg kg^{-1} for 3-MCPD in HVP and soy sauce (*13*), and legislation for other foodstuffs is expected to follow.

In a previous report (*14*), we showed that MCPD generation in leavened dough was consistently greater than that in unleavened dough and that the ratio of the isomers formed, 3-MCPD:2-MCPD, was greater in the latter. These observations could be tentatively assigned to elevated levels of glycerol from added yeast and also indicated that MCPD formation could be the product of more than a single precursor.

Glycerol production by yeast has been known since the investigations of Pasteur (*15*), and glycerol generation from bakers yeast (*Saccharomyces cerevisiae*) has been studied extensively (*16*, *17*). Glycerol is believed to account for two important functions in yeast: (i) as an osmolyte in response to water stress determined specifically by salt and sugar concentrations (*18*) and (ii) to compensate for an overproduction of NADH during the anaerobic production of acetate and succinate (*19*). Glycerol may also be present in flour as a result of enzyme activity/lipid degradation.

The objectives of this study were 2-fold: (i) to quantify the contribution to MCPD levels from individual ingredients in a leavened dough system and (ii) to account for the levels formed in terms of the potential precursors glycerol and chloride.

MATERIALS AND METHODS

Bakery Ingredients. Acetic acid, bakery fat (triacylglycerols), flour improver [ascorbic acid, diacetyl tartaric acid esters of mono- and diglycerides (DATEM), and soya flour], wheat kernels (hard and soft), white and wholemeal bread flours, and yeast were obtained from commercial suppliers.

Chemicals and Reagents. Glycerol (>99%) was obtained from Sigma (Poole, U.K.). Acetic anhydride, bromophenol blue, 1,1,2,3,3-pentadeuterioglycerol, and 1-methylimidazole were from Aldrich (Poole, U.K.). Ethyl acetate was from Romil (Cambridge, U.K.). Sodium hydroxide (AR grade) was obtained from Fischer (Loughborough, U.K.). Ultrapure deionized water (resistivity > 18.0 MΩcm) was prepared in-house (NANOpure DIamond, Thame, U.K.).

Preparation of Dough Samples. Full recipe (white flour, salt, water, acetic acid, baking fat, flour improver, and yeast) and model (white flour, salt, and water) bread dough were vacuum mixed to a work input of 36 kJ kg^{-1} on a 1 kg (flour) scale by the Chorleywood bread process (*20*, *21*). Individual ingredients were added to model dough at 1× and 2× standard levels of addition. Glycerol-spiked model dough was prepared by adding glycerol to the dough water prior to mixing.

Dough Proving. Dough was incubated (proved) at 23 ± 1 °C in the laboratory or under simulated commercial conditions in a small-scale prover (Polin, Italy) set to 45 ± 3 °C and 70% relative humidity. Where required, aliquots (20−30 g) were removed periodically for cooking experiments and/or analysis: samples for glycerol analysis were snap cooled in liquid nitrogen and stored at −18 °C until required. The standard proof time was 50 min.

Low Moisture Dough Samples. Dough (200−250 g), prepared according to the procedure described above, was freeze-dried to a target moisture content of 2−8% in a Supermodulyo 12K freeze-dryer (Edwards, Crawley, U.K.). The dried sample was reduced to a fine homogeneous powder in a BL 300 domestic blender (Kenwood, Havant, U.K.).

Generation of MCPDs. Dough samples were cooked using a custom built pressure-cooking apparatus described previously (*14*). Briefly, samples were contained in stainless steel high-performance liquid chromatography (HPLC) tubes of 75 mm length with an internal diameter (i.d.) of 7.75 mm (Hichrom Ltd., Reading, U.K.) and secured with stainless steel end caps modified to accept 1.6 mm o.d. calibrated type K thermocouple probes (Labfacility, Bognor Regis, U.K.).

MCPDs were generated by cooking the samples in a Carlo Erba Mega series (Milan, Italy) gas chromatograph (GC) oven at 180 °C (165 °C for low moisture samples) for 20 min. The temperatures from two thermocouples in each tube and a further oven thermocouple were recorded at 4 s intervals using a Squirrel 1200 series data logger (Cambridge, U.K.). The reaction was quenched by plunging the tubes into water at ≤20 °C. The tubes were additionally cooled overnight at −18 °C before removing the contents for analysis.

Analytical Methods. *Moisture Determinations*. The moisture content of samples was determined gravimetrically following heating overnight at 105 °C.

Measurement of pH. Replicate pH measurements were made using a Gelplas calibrated double junction flat tip probe, model 309/1070/09 (BDH/Merck, Lutterworth, U.K.) to an accuracy of ±0.02 pH unit. The pH of uncooked samples was measured directly at the dough surface; cooked samples were determined as a slurry using deionized water (1:1 w/w).

Chloride Analysis. Samples (1−3 g) were dispersed in freshly boiled deionized water (80 mL, ≥90 °C) prior to making to volume with deionized water (100 mL). Chloride was measured in cooled and filtered samples by potentiometric titration using a 926 Chloride Analyzer (Corning Ltd, Halstead, U.K.).

Analysis of MCPDs. The isomers, 2-MCPD and 3-MCPD, were determined as the heptafluorobutyryl esters by a procedure reported previously (ibid). GC-MS was carried out using a Saturn 2000 ion trap (Varian, Walnut Creek, CA) equipped with a BPX5 (SGE, Milton Keynes, U.K.) capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness).

Analysis of Glycerol. Stock solutions of glycerol and glycerol-d_5 (internal standard, ISTD) were prepared at concentrations of 5 mg mL^{-1} in deionized water. Calibration standards were prepared by serial dilution of the stock solutions with deionized water to give standards in the range of 50−1000 μg mL^{-1} each with an ISTD concentration of 200 μg mL^{-1}.

Yeast and uncooked yeasted dough were ground thoroughly under liquid nitrogen. Samples (4 g) were weighed quickly from a frozen condition (−18 °C) into a 50 mL centrifuge tube. Glycerol-d_5 (1 mL of 5 mg mL^{-1}) was added followed by freshly boiled deionized water (24 mL), and the tubes were loosely capped and incubated for 15−20 min in a thermos flask containing boiling water (≥90 °C). Samples were macerated in situ using a 10T shaft (Ystral, Sweeden) prior to centrifugation (1942g_{av}, 20 min). The clear aqueous layer (0.5 mL) or calibration standard (0.5 mL) was transferred to a 25 mL McCartney bottle (slender neck). The reagents 1-methylimidazole (0.5 mL) and acetic anhydride (5 mL) were added, and the bottle was capped, mixed gently, and left to stand for ≥10 min at ambient temperature. Bromophenol blue solution (0.5 mL of 0.375 mg mL^{-1} in deionized water) was added (to visualize final phase separation) followed by ethyl acetate (2 mL) and water (approximately 7 mL), mixing gently after each addition. Sodium hydroxide solution (7.5 M) was added (2 × 5 mL) taking care to cap and cool (very exothermic) the bottle under cold water for 2−3 min following each addition. When cool, further water was added to bring the upper organic layer into the narrow neck of the bottle. The phases were left for ≥30 min before removing the upper organic layer for analysis.

GC-MS was carried out using a Saturn 3 GC-MS system (Varian Inc.) fitted with a 30 m × 0.25 mm i.d. Rtx-50 (Restek, Bellefonte, PA) column with a 0.25 μm film thickness. Split injections were made under the following conditions: injector temperature, 250 °C; temperature program, 1 min at 120 °C then 4 °C min^{-1} to 170 °C followed by 20 °C min^{-1} to 280 °C, hold for 3 min; transfer line, 270 °C; scanned data acquisitions were made over the mass range of 50−300 at 1 s $scan^{-1}$. Glycerol was quantified in samples from the glycerol (*m*/*z* 103)/glycerol-d_5 (*m*/*z* 106) response, and the slope of a least squares line was fitted to a calibration data set.

RESULTS AND DISCUSSION

Contribution to MCPD Levels from Acetic Acid, Bakery Fat, Flour Improver, and Yeast. Individual ingredients (acetic acid, bread fat, improver, and yeast) were added to model dough (flour + salt + water, 100:2:60 w/w) to see which promoted

Table 1. Mean Levels of 3-MPCD (μg kg^{-1} ± SE) and Relative Isomer Production in Cooked Dough at 45% Moisture Content: Individual Ingredients Were Added at Standard and 2× Standard Levels; Samples Were Cooked (180 °C/20min) at 20 and 60 min (23 ± 1 °C) after Mixing

	3-MCPD (μg kg^{-1})					
dough + ingredient	standard level (t = 20 min)	3:2[a]	standard level (t = 60 min)[b]	3:2[a]	2× standard level (t = 20 min)	3:2[a]
model[c]	14.7 ± 0.6	7.7:1	15.6 ± 0.2	7.1:1		
+ acetic acid	15.8 ± 0.7	7.7:1	16.0 ± 0.6	7.8:1	16.9 ± 0.6	6.3:1
+ bakery fat	15.6 ± 0.2	6.7:1	16.2 ± 0.3	6.9:1	14.5 ± 0.3	6.2:1
+ improver	20.0 ± 0.5	6.9:1	22.4 ± 1.3	7.4:1	25.3 ± 0.7	6.6:1
+ yeast	20.5 ± 0.5	4.7:1	32.4 ± 0.5	4.5:1	22.3 ± 0.1	4.7:1
full recipe	25.1 ± 0.4	5.3:1	34.0 ± 0.8	4.0:1		

[a] Isomer ratio, 3-MCPD:2-MCPD. [b] Samples were proved at 23 ± 1 °C. [c] Flour + salt + water, 100:2:60 w/w.

MCPD formation. The effects of ingredient concentration and incubation time (proof) were assessed by cooking prepared samples at 180 °C for 20 min and measuring the level of MCPDs generated (**Table 1**). The cooking apparatus was a sealed system (pressure cooker) that had been used previously to simulate the conditions of baking in the outer crust region of bread products (*14*). The key features of the pressure-cooking model system were as follows: (i) reproducible generation of MCPDs, (ii) operation over a wide temperature and moisture range, and (iii) independent and accurate control of temperature, moisture, and composition.

Ingredients having no effect on MCPD formation were acetic acid and bakery fat. The bakery fat comprised vegetable-based triacylglycerols with a fatty acid composition consisting of principally palmitic, oleic, and linoleic fatty acids. These data suggest that triacylglycerols do not participate in the formation reaction and are consistent with the similar levels of MCPDs reported for commercial cereal products despite the wide variation in their lipid contents (*9*).

Yeast and Flour Improver. The independent addition of yeast and improver (ascorbic acid, DATEM, and soya flour) to model dough significantly increased the production of MCPDs, and preincubation of model dough with yeast further increased MCPD production, similar to that observed in full recipe dough (**Table 1**). Examination of the 3- and 2-MCPD isomer production from each dough showed that the addition of yeast shifted the isomer ratio to be more like that of the proved full recipe dough (4.0:1), whereas the isomer ratio from the addition of improver resembled model dough (7.7:1). These effects were also shown not to be attributable to pH changes in the dough since based on previous studies (*14*), the measured variation in final pH (mean = 4.59, SD = 0.099) was insufficient to affect the stability of 3-MCPD and 2-MCPD. The addition of yeast and improver to model dough was found to account quantitatively and independently for the level of MCPDs measured in full recipe dough, so removing the need to consider possible synergistic effects arising from combinations of all ingredients.

Glycerol as a Precursor of MCPDs in Dough. Glycerol is a normal byproduct of yeast metabolism and may also be present in older flour and flour improver as a result of enzyme activity/lipid degradation. To confirm this hypothesis, glycerol was added to model dough to explore if it promoted MCPD formation.

The addition of glycerol to model dough over the concentration range of 0−2.4% gave a first order increase in 3-MCPD production (**Table 2**): apparent first-order rate constant, 1.290 × 10^{-8} s^{-1} (mol/mol). A reduced formation was observed when sodium chloride was omitted confirming that both glycerol and chloride were required to generate 3-MCPD. The lower level of 3-MCPD formed under these conditions could also be explained because of endogenous chloride present at 0.07% in the white flour. Furthermore, the ratio of 3-MCPD:2-MCPD produced at each level of addition of glycerol closely paralleled that obtained from proved full recipe dough analyzed previously, i.e., 3.3:1 (*14*).

Table 2. Mean Levels of MCPDs (μg kg^{-1} ± SE) Generated (180 °C/20 min) in 45% Moisture Content Dough with Added Glycerol[a]

dough type	chloride[b] (%)	added glycerol (%)	3-MCPD (μg kg^{-1})	2-MCPD (μg kg^{-1})	3:2[c]
model	0.79	0	14.8 ± 0.6	2.3 ± 0.3	6.4:1
model	0.79	0.61	139 ± 6.5	44.4 ± 1.4	3.1:1
model	0.79	1.22	240 ± 0.2	72.2 ± 1.9	3.3:1
model	0.79	2.41	415 ± 3.6	140 ± 2.6	3.0:1
flour + water	0.05	0	<1.0	<1.0	
flour + water	0.05	2.44	46.5 ± 0.5	12.2 ± 0.5	3.8:1

[a] The equation of the linear regression line through the model dough + glycerol data was as follows: $y = 164.0x + 28.1$ μg kg^{-1} ($R^2 = 0.994$). [b] Added + measured contribution from flour and water. [c] Isomer ratio, 3-MCPD:2-MCPD.

Glycerol Evolution in Yeasted Dough. To establish a link between MCPDs and glycerol in leavened dough, added yeast model dough, full recipe dough, and model dough (control) were bench proved at 23 ± 1 °C over approximately 100 min. Aliquots of dough were removed periodically for glycerol analysis and MCPD generation (cooking).

The concentration of glycerol in the yeasted doughs increased linearly with time, and the rate of evolution was similar in both dough types. Glycerol levels did not change in the unleavened model dough. The correlation between 3-MCPD and glycerol obtained by cooking the preincubated dough samples at 180 °C (**Figure 2**) showed good agreement with that obtained from the addition of glycerol to model dough (**Table 2**).

The increased level of 3-MCPD in the full recipe dough was consistent with an additional contribution from the flour improver (ascorbic acid, DATEM, and soya flour). The divergence of the two fitted regression lines toward higher glycerol concentrations (and increased time) suggests that the improver contribution may also be time-dependent. This effect, although small, was also evident from the preincubation of model dough with added improver (**Table 1**) and may be due to an increase in glycerol levels from the action of lipases present in the soya flour. Extrapolation of each data set to a zero glycerol concentration suggests that additional precursors are present in the flour and the improver, respectively.

Effect of (Sodium) Chloride. The rate of generation of MCPDs showed a nonlinear dependence on chloride levels (**Figure 3**) in dough at 45% moisture content. One possible explanation

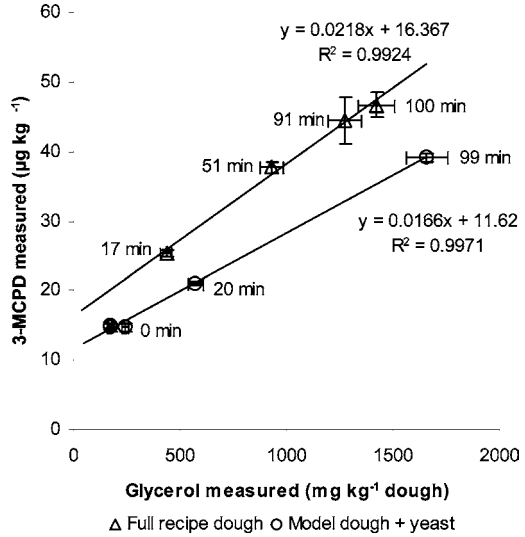

Figure 2. Correlation between yeast-evolved glycerol and 3-MCPD obtained by cooking preincubated yeasted model dough at 180 °C for 20 min. Data points are means of at least duplicate experiments; horizontal and vertical bars are ±SE.

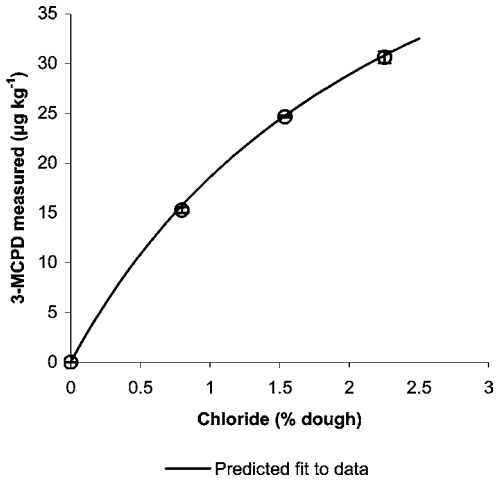

Figure 3. Effect of added chloride on 3-MCPD generation in dough cooked at 180 °C for 20 min. The fit to the data was derived from eq 4 and the data given in **Table 3**. Data points are means of duplicate experiments, and vertical bars are ±SE.

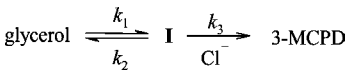

Figure 4. Proposed kinetic model for the formation of 3-MCPD from glycerol and Cl^- at 45% moisture. The intermediate **I** is formed reversibly from glycerol.

for this could be the formation of 3-MPCD from an unknown intermediate that is in equilibrium with glycerol according to the kinetic model given in **Figure 4**.

To test this hypothesis, the reaction scheme was translated into a mathematical model by setting up differential equations for the formation of 3-MCPD and the intermediate **I**, using the law of mass action:

$$\frac{\mathrm{d[3\text{-}MCPD]}}{\mathrm{d}t} = k_3[\mathbf{I}][\mathrm{Cl}^-] \quad (1)$$

$$\frac{\mathrm{d[I]}}{\mathrm{d}t} = k_1[\mathrm{glycerol}] - k_2[\mathbf{I}] - k_3[\mathbf{I}][\mathrm{Cl}^-] \quad (2)$$

Table 3. Experimental Data Used to Test the Validity of the Kinetic Model Given in **Figure 4**[a]

			measured			predicted[b]
dough type	chloride[c] (%)	glycerol[d] (mg kg^{-1})	3-MCPD (μg kg^{-1})	2-MCPD (μg kg^{-1})	3:2[e]	3-MCPD (μg kg^{-1})
flour + water	0.05	850	<1.0	<1.0		1.6
model	0.79	850	15.3 ± 0.3	2.3 ± 0.3	6.6:1	15.2
model	1.52	850	24.7 ± 0.1	3.7 ± 0.2	6.7:1	24.8
model	2.23	850	30.6 ± 0.6	3.9 ± 0.3	7.9:1	31.9

[a] Experimental values for MCPDs are means ± SE, and the dough moisture content was 45%. Samples were cooked at 180 °C for 20 min. [b] From eq 4 and the recorded temperature data. [c] Added + measured contribution from flour and water. [d] Extrapolated value, from data in **Table 2**. [e] Isomer ratio, 3-MCPD:2-MCPD.

At equilibrium, [**I**] does not change significantly, i.e., d[**I**]/dt = 0 (*22*); hence, [**I**] can be replaced by:

$$[\mathbf{I}] = \frac{k_1[\mathrm{glycerol}]}{k_3[\mathrm{Cl}^-] + k_2} \quad (3)$$

Substituting in eq 1 for [**I**] and k_1 ($=Ae^{\frac{-E_a}{RT}}$) and dividing through by k_3 gave the following mathematical model for the formation of 3-MCPD:

$$\frac{\mathrm{d[3\text{-}MCPD]}}{\mathrm{d}t} = \frac{[\mathrm{glycerol}][\mathrm{Cl}^-]}{\frac{k_2}{k_3} + [\mathrm{Cl}^-]} \cdot Ae^{\frac{-E_a}{RT}} \quad (4)$$

The pre-exponential factor A ($= 1.101 \times 10^{14}$ s^{-1}) and activation energy E_a ($= 182$ kJ mol^{-1}) were obtained from supplementary data (*14*); R ($= 8.314$ J mol^{-1} K^{-1}) is the molar gas constant; T is the absolute temperature (K). The concentration of glycerol in model dough ($= 850$ mg kg^{-1}) was obtained from the data in **Table 2**. A value for k_2/k_3 ($= 2.5 \times 10^7$ μg kg^{-1}) was obtained by fitting eq 4 to the experimental data given in **Table 3**.

This gave an expression that could be integrated numerically, based on the recorded temperature data. Hence, the predicted values of 3-MCPD as a function of chloride and glycerol could then be compared with the experimental data. The proposed kinetic model, and hence eq 4, is a simplification that represents a net formation since 3-MCPD is known to slowly decay under the conditions employed (ibid).

Figure 5 shows that the experimental data from both the added (**Table 2**) and the evolved glycerol (**Figure 2**) dough experiments were in good agreement with that predicted by eq 4 and hence the proposed kinetic model. The agreement between the experimental and the predicted data for the flour + water + 2.4% glycerol dough, i.e., no added chloride, provides additional confirmation of the proposed glycerol and chloride dependence of MCPD.

Effect of Moisture. To simulate the conditions of MCPD formation in the crust of cereal products, a series of added glycerol dough samples were prepared in the standard way (45% moisture content) and freeze-dried to a target moisture content of 2−8%. For a given temperature, MCPD generation was greater over the range 2−8% moisture as compared to 45% moisture, but the formation reaction was found to be very sensitive to individual variations in water content. This moisture

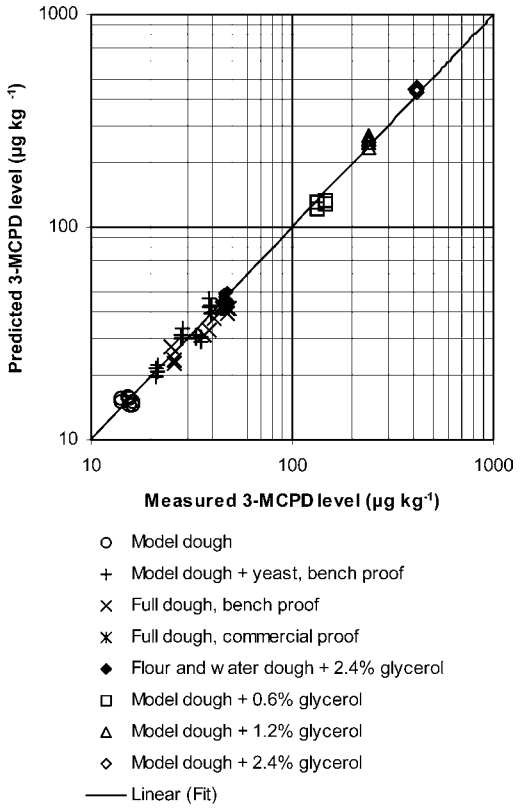

Figure 5. Summary of 3-MCPD generation from added and evolved glycerol in dough (45% moisture). The data from both the added and the yeast-evolved glycerol dough experiments were in good agreement with that predicted by eq 4 and the recorded temperature data.

dependence was confirmed by the linear relationship obtained from the generation of MCPDs in added glycerol doughs that had each been equilibrated to a moisture content of 0.5% (**Figure 6**).

These data could not be predicted by eq 4 and were therefore inconsistent with the proposed kinetic model given in **Figure 3**. Two possible reasons for this were considered as follows: (i) formation of the intermediate glycidol and water from glycerol and (ii) reaction of glycerol with the proposed intermediate glycidol (*23*) and the formation of glycerol dimers (**Figure 7**).

To test this hypothesis, the kinetic model was translated into a mathematical model by setting up differential equations for the formation of 3-MCPD and the intermediate **I***, described previously

$$\frac{\mathrm{d[3\text{-}MCPD]}}{\mathrm{d}t} = k_3'[\mathbf{I}^*][\mathrm{Cl}^-] \quad (6)$$

$$\frac{\mathrm{d}[\mathbf{I}^*]}{\mathrm{d}t} = -k_2'[\mathbf{I}^*][\mathrm{H_2O}] - k_4'[\mathbf{I}^*][\mathrm{glycerol}] - k_3'[\mathbf{I}^*][\mathrm{Cl}^-] + k_1'[\mathrm{glycerol}] = 0 \quad (7)$$

Equation 7 can therefore be rearranged to:

$$[\mathbf{I}^*] = \frac{k_1'[\mathrm{glycerol}]}{k_2'[\mathrm{H_2O}] + k_3'[\mathrm{Cl}^-] + k_4'[\mathrm{glycerol}]} \quad (8)$$

Substituting in eq 6 for **I*** and k_1' ($= A'e^{\frac{-E_a}{RT}}$) and dividing through by k_3' gave the following mathematical model for the

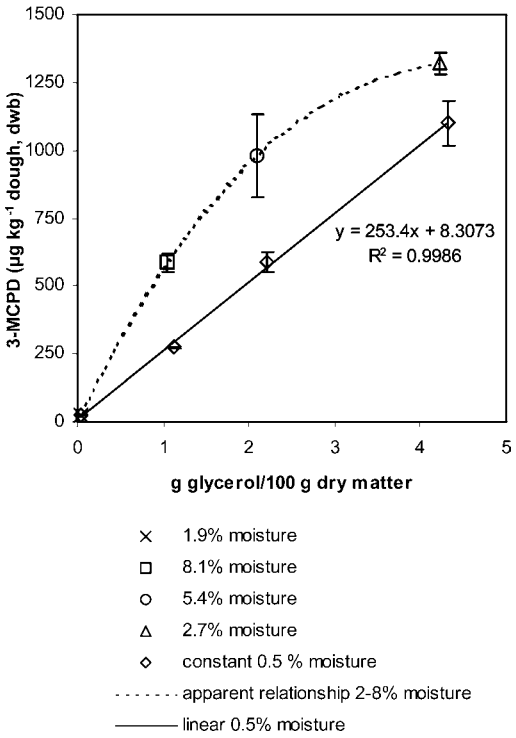

Figure 6. Effect of water on 3-MCPD generation in low moisture content-added glycerol dough. Samples were cooked at 165 °C for 20 min. The apparent nonlinear relationship (upper trace) was attributed to differences in the individual moisture contents of each dough. Data are means of at least duplicate experiments, and vertical bars are ±SE.

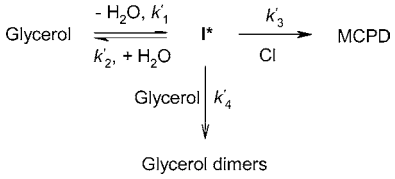

Figure 7. Modified kinetic model for the formation of 3-MCPD from glycerol and chloride at low moisture. The proposed intermediate (**I***) is the epoxide glycidol, which can react with glycerol (*23*).

formation of 3-MPCD:

$$\frac{\mathrm{d[MCPD]}}{\mathrm{d}t} = \frac{[\mathrm{Cl}^-][\mathrm{glycerol}]A'e^{\frac{-E_a}{RT}}}{\frac{k_2'}{k_3'}[\mathrm{H_2O}] + [\mathrm{Cl}^-] + \frac{k_4'}{k_3'}[\mathrm{glycerol}]} \quad (9)$$

At low concentrations of glycerol, $k_4'/k_3'[\mathrm{glycerol}] \approx 0$, so eq 9 has the same form as eq 4 and values for A ($= 1.101 \times 10^{14}$ s^{-1}) and k_2'/k_3' ($= 0.056$) could be obtained from previous data (**Table 3**). Equation 9 was then integrated numerically so that the last unknown parameter, k_4'/k_3' ($= 0.197$), could be obtained by a fit to the experimental data (**Table 4**). The measured values for 3-MCPD given in **Table 4** showed reasonable agreement with those predicted by eq 9 and hence the kinetic model given in **Figure 7**.

Figure 8 shows that eq 9 predicts a very different chloride dependence at low moisture contents to that observed at high moisture but still agrees with the observed glycerol dependence at both high and low moistures. The predicted moisture dependence and plateau, obtained by imposing a limit for the solubility of chloride in dough water of 20%, is consistent with data reported previously (*14*). The kinetic model, and hence eq 9, does not however account for the overall magnitude of the

Table 4. Experimental Data Used to Test the Validity of the Kinetic Model Given in **Figure 7**[a]

dough type	glycerol[c] (%)	water (%)	chloride[d] (%)	measured 3-MCPD (μg kg^{-1})	predicted[b] 3-MCPD (μg kg^{-1})
model	0.09	1.9	1.39	26.3 ± 0.2	35.7[e]
model	1.09	8.1	1.30	538 ± 30.9	492[e]
model	2.15	5.4	1.34	982 ± 145	872[e]
model	4.28	2.7	1.38	1285 ± 39.4	1229[e]
flour + water	4.25	3.5	0.08	380 ± 36.1	338[e]
model	0.09	0.5	1.41	26.1 ± 0.9	25.8[f]
model	1.18	0.5	1.41	272 ± 1.9	325[f]
model	2.26	0.5	1.41	587 ± 37.6	606[f]
model	4.37	0.5	1.41	1093 ± 81.5	997[f]
flour + water	4.37	0.5	0.08	225 ± 2.8	200[f]

[a] Experimental values for 3-MCPD are means ± SE. Samples were cooked at 165 °C for 20 min. [b] From eq 9 and the recorded temperature data. [c] Added + estimated contribution from flour. [d] Added + measured contribution from flour and water. [e,f] Additional factors of ×5.5 and ×2.3, respectively, were required to fit the data.

increase in MCPD production observed at low moisture, which is clearly not due solely to precursor concentration effects. A factor in this increase may be the reduced decay of 3-MCPD at low moisture (*14*), which has been omitted from the model for simplicity.

Hence, the factors limiting MCPD formation at low moisture are most probably the solubility of chloride and to a lesser extent, competing reactions between the proposed intermediate glycidol (**I**) and the glycerol (*22*). The observed dependence (**Figure 8A**,**B**) exhibited by chloride and glycerol suggests that the latter is likely to be a much better indicator of MCPD generation in dough at low moistures.

Figure 9 suggests one possible mechanism for the formation of MCPDs from glycerol via the unstable intermediate epoxide glycidol (**I**) and is consistent with the observed selectivity, i.e., isomer distribution, and the proposed kinetic model given in **Figure 7**.

Glycerol and Chloride Analysis of Doughs and Ingredients. Comparison of the concentrations of glycerol (%) in yeasted dough samples immediately before and after pressure cooking (180 °C for 20 min) showed that levels do not change significantly (gradient of linear correlation regression line = 1.048; $R^2 = 0.998$), i.e., the flour lipids are heat stable. This result is consistent with the observation that added triglyceride (baking fat) did not promote the formation of MCPDs when added to model dough. Incubation of full recipe dough under commercial proof conditions, i.e., a prover temperature of 45 ± 3 °C and 70% relative humidity, showed a moderate increase in the rate of glycerol evolution as compared with that generated under laboratory conditions at 23 ± 1 °C.

Although significant levels of glycerol and chloride were found in the flour improver (**Table 5**), these were too low to promote MCPD formation in dough at the dilutions employed

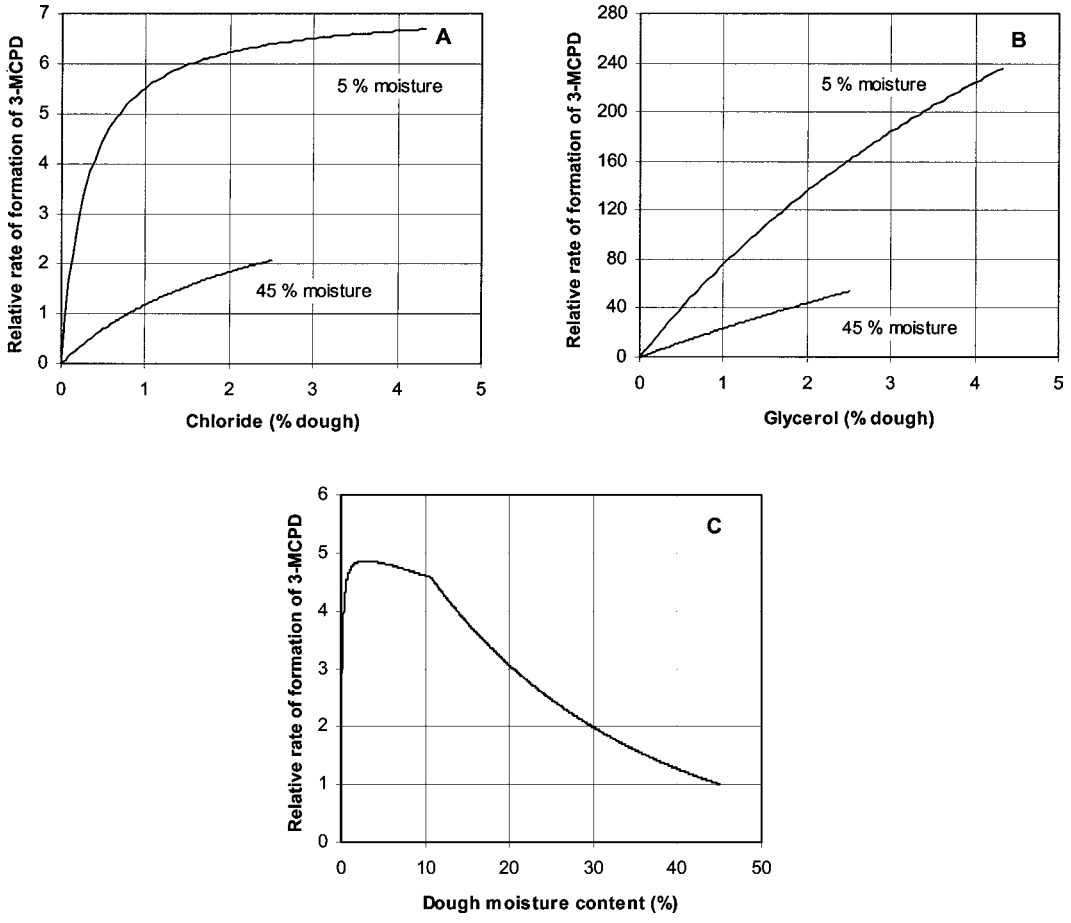

Figure 8. Relative 3-MCPD generation in dough (165 °C and 20 min) as a function of chloride, glycerol and moisture (normalized to model dough at 45% moisture, 0.8% chloride and 400 mg kg^{-1} glycerol). The curves were derived from eq 9 and the kinetic parameters given in the text: (A) effect of chloride at constant (400 mg kg^{-1}) glycerol, (B) effect of glycerol at constant (0.8%) chloride and (C) effect of moisture at constant (0.8%) chloride and (400 mg kg^{-1}) glycerol.

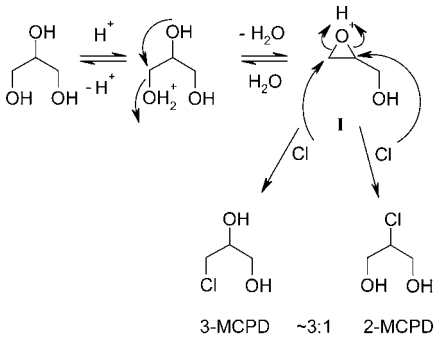

Figure 9. Proposed mechanism of formation of MCPDs from glycerol via the intermediate epoxide, glycidol (**I**).

Table 5. Mean Glycerol and Chloride Levels (mg kg^{-1} ± SE) in Wheat Dough Ingredients

ingredients	moisture (%)	glycerol (mg kg^{-1})	N[a]	chloride (mg kg^{-1})	N[a]
		dough recipe samples			
white bread flour	13.1	443 ± 26	5	747 ± 15	3
improver[b]		661 ± 42	2	13 200 ± 933	2
yeast		262 ± 19	2		
		wheat kernels			
hard wheat[c]	15.9	49.8 ± 3.5	2	654 ± 54	2
soft wheat[c]	14.7	59.8 ± 4.2	2		
		wholemeal bread flour			
mid storage life	14.0	386 ± 33	2		
end storage life	14.0	711 ± 55	2		

[a] Number of replicates. [b] Contains ascorbic acid, DATEM, and soya flour. [c] Laboratory milled immediately before analysis.

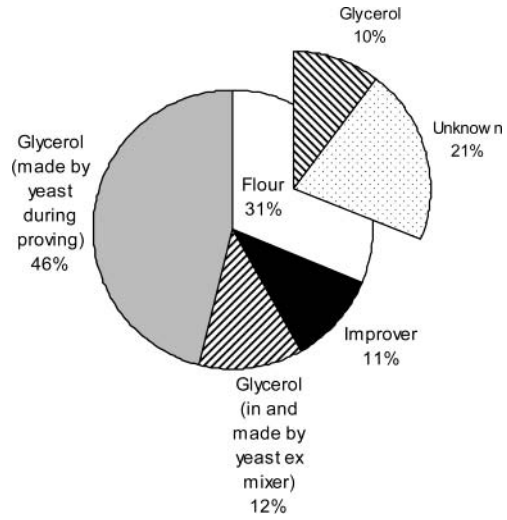

Figure 10. Contributions to 3-MCPD levels in proved, full recipe dough; data are normalized to commercial proof conditions.

thereby confirming that additional precursors were present in this ingredient (ascorbic acid, DATEM, and soya flour). The level of glycerol in the white flour was sufficient to account for 33% of the measured contribution to MCPDs from this ingredient (**Figure 10**). Presumably, the remaining contribution was due to additional precursors in flour that had not yet been identified.

Analysis of freshly milled wheat kernels showed that glycerol concentrations are relatively low (**Table 5**) in the grain. Glycerol levels remained constant over the storage life of commercial white flour while those in wholemeal flour increased, presumably due to the action of lipases present in the bran.

Figure 10 summarizes the contributions to MCPDs levels in proved full recipe dough. The contributions were calculated from the data in **Table 1**, the measured glycerol and chloride levels, and kinetic data from the glycerol addition experiments (**Table 2**).

Supporting Information Available: Change in pH of pressure-cooked dough samples; glycerol dose/response relationship in 45% moisture content dough; comparison of glycerol levels before and after cooking. This material is available free of charge via the Internet at http://pubs.acs.org.

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