

Effects of Yeast Stress and Organic Acids on Chloropropanols Formation in Cereal Products

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Abstract: A major precursor of monochloropropanediols (MCPDs) in leavened cereal products is glycerol, which is formed as a natural by-product of yeast fermentation. However, yeast metabolism is affected by stresses such as low osmotic pressure from e.g. the incorporation of sugar or salt in the dough recipe. Tests with cooked model doughs have shown that glycerol production was proportional to yeast level and limited by available sugars, but high levels of yeast inhibited MCPD formation. Added glucose did not increase the production of glycerol but did promote the generation of MCPDs. This effect was attributed to the thermal generation of organic acids from added glucose, so the effect of pH and short-chain organic acids on MCPD generation was measured. There was a good correlation between initial dough pH and the level of MCPDs generated. The effect was weaker than that predicted by simple kinetic modelling, suggesting that the involvement of H⁺ and/or the organic acid was catalytic or not rate-determining.

Keywords: 3-MCPD; cereal products; glycerol; organic acids; yeast

INTRODUCTION

Chloropropanols, in particular monochloropropanediols (MCPDs) are food-borne contaminants that can form in the high temperature crust region of cereal products. A major precursor of monochloropropanediols in leavened cereal products is glycerol [1], which is formed as a natural by-product of yeast fermentation. Although the primary role of glycerol production by yeast is the maintenance of cellular redox balance [2], it is also produced in response to stresses such as heat shock [3] and low osmotic pressure [4]. Hence, sudden exposure to extreme temperatures (e.g. proving in hot tins) and the incorporation of additional sugar or salt to leavened dough recipes might be expected to increase the production of glycerol by yeast.

Previous studies have indicated that the reaction of glycerol with added chloride (from salt) to produce MCPDs may also be catalysed by organic acids such as citric acid [5]. Short-chain organic acids are used widely in bakery products, but their effects on the generation of MCPDs in dough are not known.

EXPERIMENTAL

Materials and chemicals. White bread making flour, dextrose monohydrate (bakery grade containing 85% glucose, by HPLC), salt and yeast were obtained from commercial suppliers. Acetic acid, citric acid, L-, and DL-malic acids, oxalic acid, sodium hydrogencarbonate, L-tartaric acid, all 97–99% purity, were obtained from Aldrich (Gillingham, UK).

Preparation of dough samples. All dough samples were vacuum-mixed to a work input of 36 kJ/kg by the Chorleywood baking process [6]. Yeast and glucose were added to a model dough mix comprising white flour (1000 g), salt (20 g) and water (600 g); organic acids or sodium hydrogencarbonate were added at 1% (on flour weight) via the dough water. Dough was incubated (proved) at 23°C ± 1°C in the laboratory, or under simulated commercial conditions in a small-scale prover (Polin, Italy) set to 45°C ± 3°C and 70% relative humidity: samples of dough were taken over 0–100 min for glycerol analysis and MCPD generation.

Generation of MCPDs in dough. Dough samples were cooked at 180°C for 20 min using a custom built pressure-cooking apparatus that simulates the conditions of baking [1, 7, 8].

Controlled heating experiments with glucose and chloride. Glucose (466 mg) and sodium chloride (94 mg) in 0.1M phosphate buffer (2.790 ml, pH 4.5) were heated in sealed glass vials at 160°C (10–40 min), with and without added asparagine (4.8 mg). Positive control samples were prepared by adding 1000 ng of 3-MCPD prior to heating.

Analytical methods. MCPDs were determined as the heptafluorobutyryl esters [9]; glycerol was converted to triacetin using acetic anhydride [1]; all samples were analysed by GC/MS. Replicate pH measurements were made on stirred aqueous dough slurries (1:1 v:w) using a Gelplas calibrated general-purpose electrode, model 309/1050/03 (accuracy ± 0.02 pH unit, BDH/Merck, Lutterworth, UK).

RESULTS AND DISCUSSION

Effect of temperature on glycerol production by yeast

Incubation of yeasted (2.7% on flour weight) model dough under commercial proof conditions i.e. 10 min at 23°C + 50 min at 45°C and 70% relative humidity gave a 43% increase in the rate of glycerol production compared to dough incubated at 23°C. The conditions of commercial proving are close

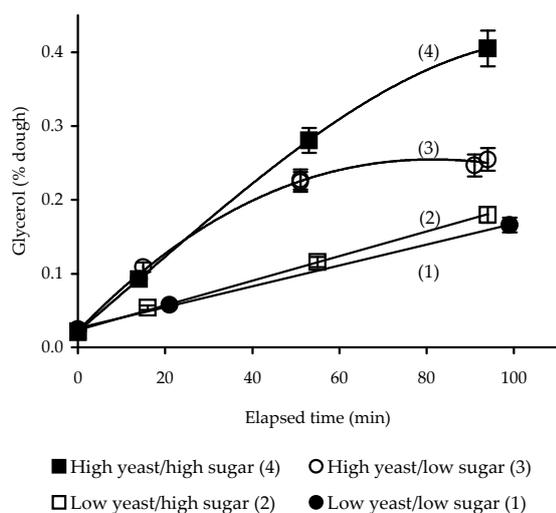


Figure 1. Effect of glucose and yeast on glycerol production in dough

to the maximum temperature tolerable by yeast, and this may account for the modest increase in glycerol production.

Effect of added sugar on glycerol production by yeast

The addition of glucose (8.5% on flour weight) to model dough in the presence of a standard level of yeast (2.7% on flour weight) did not significantly increase glycerol production Figure 1, curves (1) and (2). One possible explanation for this result could be that glycerol production by yeast in dough is already at a maximum due to added salt [3]. Glycerol production was approximately proportional to added yeast level during the early stages of proof and latterly slowed (Figure 1, curves 3 and 4). This effect was more pronounced at low levels of glucose addition curve (3), presumably because the consumption of sugars by yeast was greater than that released from starch by amylase, and hence glycerol production through normal fermentation activity was reduced.

Effect of additional sugar and yeast on MCPD generation

The addition of glucose to model dough at both high yeast (8.1% on flour weight) and standard yeast levels approximately doubled the production of MCPDs (Figure 2, curves 2 and 4). Since curves (1), (2) and (4) shared a similar intercept

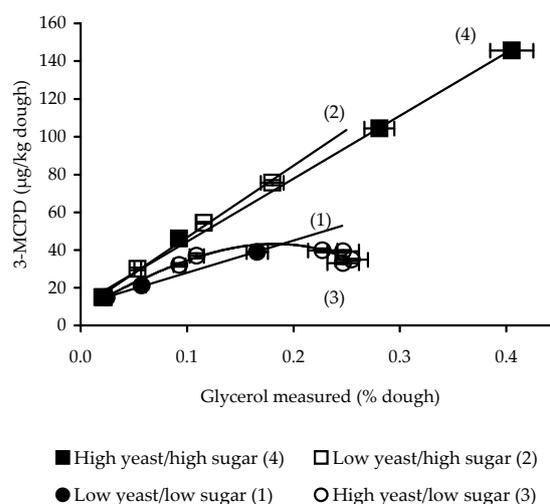


Figure 2. Correlation between yeast evolved glycerol and 3-MCPD generated in added yeast/sugar doughs

Table 1. MCPD generation (\pm SE) and pH in model doughs before and after cooking

Dough	3-MCPD ($\mu\text{g}/\text{kg}$)	Initial dough pH	Final dough pH	pKa of acid
Model dough + oxalic	40.3 \pm 2.0	3.14	2.92	1.27
Model dough + L-tartaric	33.2 \pm 1.6	3.74	3.77	2.98
Model dough + DL-malic	26.6 \pm 1.2	4.24	3.96	3.40
Model dough + citric	25.3 \pm 1.2	4.35	3.95	3.13
Model dough + L-malic	24.3 \pm 1.2	4.23	3.92	3.40
Model dough + acetic acid	15.8 \pm 0.7	5.20	4.60	4.76
Model dough	14.7 \pm 0.6	5.91	4.73	–
Model dough + Na_2HCO_3	9.0 \pm 0.6	8.02	5.57	–

value at zero glycerol (8.7–11.0 $\mu\text{g}/\text{kg}$ 3-MCPD), this suggested that the effect was unlikely to be due to an additional precursor in, or generated from, the added glucose. This was confirmed by the controlled heating of glucose and sodium chloride at 160°C for up to 40 min: no MCPDs were generated under these conditions or from glucose-amino acid (Maillard) intermediates, generated by the addition of asparagine.

The addition of high levels of yeast to model dough (Figure 2, curve 3) had an apparent inhibitory effect on the generation of MCPDs at longer fermentation times. The most likely explanation for this effect is that ammonia, produced by the deamination of amino acids (from the additional yeast), reacted with the 3-MCPD [10]. Under the conditions of falling sugar levels due to normal yeast metabolism, the concentration of ammonia may increase, as it is no longer effectively removed by reaction with sugars.

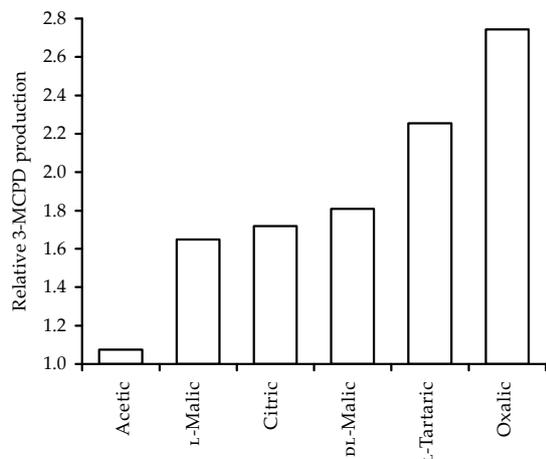
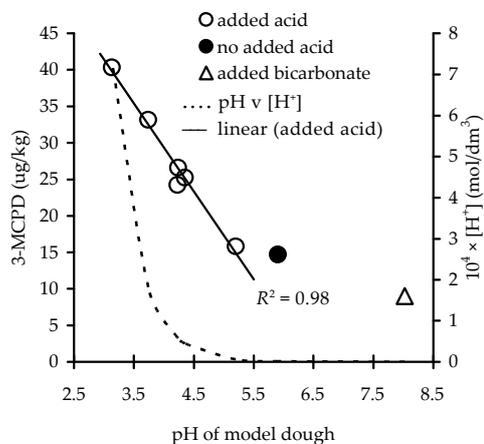


Figure 3. Effect of organic acids on 3-MCPD generation in unleavened dough

Effect of organic acids and pH on MCPD generation

One possible explanation for the effect of glucose on MCPD generation could be catalysis by organic acids [5], formed by thermal decomposition of the added glucose [11]. Hence, short-chain organic acids were added to model doughs to see if they promoted MCPD formation.

The addition of short-chain organic acids at 1% (on flour weight) to a model dough mix did promote the generation of MCPDs (Figure 3). The highest levels of 3-MCPD were formed from the addition of acids having the lowest pKa, and hence lowest initial dough pH. There was a good correlation between the initial pH of acidified dough samples and the level of MCPDs generated (Figure 4) suggesting that H^+ is involved in the generation mechanism from glycerol [1].

Figure 4. Relationship between dough pH, $[\text{H}^+]$ and 3-MCPD generated

To measure the range of this pH effect, model dough was prepared at an initial pH of 8 by adding sodium hydrogencarbonate (1% on flour weight). The higher than expected level of 3-MCPD generated was probably due to the greater pH shift experienced by this sample compared to the acidified dough samples. Overall, the effect of pH was much weaker than that predicted by simple kinetic modelling, i.e. based on $d[3MCPD]/dt \propto [H^+]$, indicating that H^+ was catalytic or not rate-determining (Figure 4). However, since lysophospholipids are the major precursors of MCPDs in unleavened model dough [8], the increased hydrolysis of lipids at reduced pH to yield additional glycerol, and hence MCPDs, cannot be ruled out.

CONCLUSIONS

Added glucose does not increase the production of glycerol in yeasted dough containing standard levels of salt under normal proof conditions. Glucose and its Maillard intermediates are not precursors of MCPDs, but glucose does promote the generation of MCPDs in yeasted dough. High levels of yeast inhibit MCPD generation under the conditions of falling glucose concentration. Although the mechanism of inhibition is not yet known it may be due to the reaction of 3-MCPD with ammonia that is formed by deamination of the additional yeast proteins. Organic acids also promote MCPD formation, probably via catalysis involving H^+ . This mechanism may also explain why added glucose increases MCPD generation, i.e. via the thermal generation of organic acids from the sugar.

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