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Effectiveness of Methods for Reducing Acrylamide in Bakery Products

PETER A. SADD,* COLIN G. HAMLET, AND LI LIANG

RHM Technology, The Lord Rank Centre, Lincoln Road, High Wycombe HP12 3QR, United Kingdom

Pilot-scale bread, biscuit, and cracker doughs have been baked to assess how well recipe changes could reduce acrylamide in commercial bakery products. Removing ammonium-based raising agents was beneficial in biscuits. In doughs, long yeast fermentations were an effective way of reducing asparagine levels and hence acrylamide. At moderate fermentation times fructose levels increased, but the yeast later absorbed this, so the net effect on acrylamide was beneficial. Metal ions such as calcium reduced acrylamide when added as the carbonate or chloride. Hence, the fortification of flour with calcium carbonate, over and above its natural mineral content, has an additional benefit. However, some other possible methods of adding calcium to bakery doughs, for example, via the permitted preservative calcium propionate, were not beneficial. Amino acid addition to doughs gave modest reductions in acrylamide. Lowering the dough pH reduced acrylamide, but at the expense of higher levels of other process contaminants such as 3-monochloropropane-1,2-diol (3-MCPD).

KEYWORDS: Bread; biscuits; crackers; baking; acrylamide; reduction; yeast; calcium; 3-MCPD

INTRODUCTION

Although the levels of acrylamide in most U.K. bakery products are relatively low (*1*), levels in biscuits can cover a very wide range (*2, 3*) and levels of >1000 μg/kg have been reported. A number of possible mitigation strategies have been proposed for reducing acrylamide in baked goods; however, in most cases they have only been tested on model systems or recipes untypical of U.K. products. The most common strategies have focused on reducing or diluting precursors such as free asparagine:

1. Consuming the asparagine by either adding an enzyme or using yeast or another microorganism (*4, 5*).
2. Adding other amino acids (*6, 7*).
3. Binding the asparagine with a complexing agent, for example, by adding divalent metal ions (*8, 9*).
4. Removing accelerants such as ammonium salts (*10–12*).
5. Reducing the pH to modify the Maillard reactions.
6. Minimizing heating temperatures and times.

Ammonium-based raising agents are widely used in biscuit manufacture, either individually or as part of a mixed chemical/yeast raising system (they have good bench tolerance at room temperature, increase browning, and provide a crisp, porous crumb). In addition, there is no effect on final flavor as the strong odor developed during baking dissipates in the final product so long as it is low-moisture, such as biscuits or crackers. Sodium-based alternatives do not offer all of these advantages.

This work was undertaken to evaluate the available strategies under as realistic conditions as possible by using pilot-scale bread, sweet biscuit, and savory cracker systems. In particular, it explored the effect of commercial variables such as dough age, yeast, fermentation times, and pH and possible recipe modifications such as the addition of amino acids or metal ions and the use of sodium instead of ammonium raising agents.

* Author to whom correspondence should be addressed (e-mail peter.a.sadd@rhm.com).

MATERIALS AND METHODS

Bakery fat, flours, castor sugar, salt, sugar syrups, tartaric acid, and yeast (compressed, "blue") were obtained from commercial suppliers. Ammonium carbonate, ammonium hydrogen carbonate, calcium carbonate, calcium chloride ($CaCl_2 \cdot 2H_2O$), and sodium hydrogen carbonate, all of 97−99% purity, were obtained from Aldrich (Gillingham, U.K.). Fructose (>98% purity) was obtained from Sigma (Poole, U.K.).

Three types of bakery products were studied: bread, savory biscuits (cream crackers), and "rich tea" style sweet biscuits. Their acrylamide levels covered a 25-fold range: typical levels in control samples were 14.3 μg/kg for bread, 20−250 μg/kg for savory biscuits (depending on fermentation time), and 377 μg/kg for sweet biscuits.

Sweet Biscuit Samples. *Mixing.* Dough samples were prepared on a 1000 g of flour basis according to the recipe given in **Table 1**. Raising agents and other components were added in various amounts (addition levels are given in the main text): ammonium hydrogen carbonate (when used) and invert sugar syrup was predissolved in a small quantity (25 mL) of the dough water. All ingredients, except the flour, were added to the bowl of a Z-blade mixer (Hobart model A120) and mixed for 10 s on slow, 10 s on fast, and then 100 s on slow to give a cream with a temperature of approximately 20 °C. Flour (1 kg) was then added and mixed for a further 240 s on the slow speed setting. The resultant dough (temperature = 23 °C) was transferred to a polyethylene bag and left to stand for a minimum of 30 min at 20 °C.

Sheeting. Dough pieces of 250 g were reduced to a thickness of 2.75 mm (between baking parchment sheets) using a Rondo pastry brake. Disks of dough 72 mm diameter were cut to a target weight of

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Table 1. Product Recipes (Grams per 1000 g of Flour)

	bread	savory biscuits[a]	sweet biscuits[a]
flour	1000	1000[b]	1000
salt	20	12.6	10.8
water	600	360	138
fat	10	150	300
yeast	27	13.5	
vinegar	12.5		
improver	20[c]		
castor sugar			320
invert syrup			20[d]
$NaHCO_3$			4
NH_4HCO_3			2.7
tartaric acid			0.9

[a] These recipes were derived from ref *13*. [b] The flour was a blend of 75% hard (11% protein content) and 25% soft (8% protein content) flours. [c] The improver was a commercial blend incorporating ascorbic acid, amylases, and soya flour. [d] The invert syrup contained glucose, 35.7 g/100 g; fructose, 31.8 g/100 g; and sucrose, 2.6 g/100 g.

16 ± 1 g prior to docking (25 holes). Aliquots of all doughs were placed in a freezer (−18 °C) prior to baking and retained for amino acid, pH, and sugar analyses as required.

Baking. Disks of experimental and control doughs were baked simultaneously on a custom-built rectangular baking tray (45.3 × 75.5 cm) fabricated from z47 (mesh) oven band (the metal belt used to transport the product through a commercial oven): disks of experimental and control doughs (nine disks from each dough) were arranged in alternate rows (three disks per row) prior to baking on a rising oven profile using a Spooner traveling oven: the set point temperatures of the oven zones were 170, 190, and 215 °C.

Baked biscuits were immediately cooled to approximately 20 °C in a commercial bread cooler (ca. 3 min) and stored in sealed polyethylene bags at room temperature until required for analysis.

Savory Biscuit Samples: Straight Dough Process. *Mixing.* Dough samples were prepared on a 6 kg of flour basis according to the recipe given in **Table 1**. All ingredients were added to the bowl of a spiral mixer (Kemper, 15 kg) and mixed as follows: 30 s on slow and then 6.5 min on fast. The dough (temperature ex mixer = 24 °C) was divided into six equal aliquots, contained in loosely sealed polythene bags, and stored at 34 °C in a commercial bread prover. Proof time was varied to cover the range most commonly used commercially (0.5−5 h).

Sheeting/Cutting. Dough (ca. 1 kg) was sheeted to 2.5−3.0 mm (ca. 300 mm width) using a Rondo pastry brake and then processed according to the following sequence: a length of cut dough (ca. 1 m) was folded into three, trimmed, and divided; half of the divided dough piece was turned through 90° and placed on top of the remaining dough; relaxed for 1 min; sheeted to 2.5 mm; relaxed for 45 s prior to cutting (65 mm disks; target weight = 9.5 g ± 0.5 g) and docking (17 × 3 mm holes made through the dough using a spiral action cutter). Aliquots of each dough were placed in a freezer (−18 °C) prior to baking and retained for amino acid, pH, and sugar analyses as required.

Baking. Nine disks of cracker dough were arranged in rows of three on a custom-built rectangular baking tray (45.3 × 75.5 cm), fabricated from z47 (mesh) oven band, prior to baking in a Spooner traveling oven on a falling oven profile: the set point temperatures of the three oven zones were 215, 205, and 190 °C; residence time was 5.9 min. The crackers were immediately cooled to approximately 20 °C in a commercial bread cooler (ca. 3 min) and stored in sealed polyethylene bags at room temperature until required for analysis.

Bread Samples. *Mixing.* Dough was prepared on 15 kg scale by the Chorleywood Bread Process (using a high-speed Tweedy 35 mixer) according to the recipe given in **Table 1** and proved for 50 min in commercial units at 45 °C and 70−80% humidity. Divalent metals were incorporated by applying a commercial tin release agent (which had been made into a 1:1 emulsion with a 20% calcium chloride solution) to the inner baking pan surfaces and the loaf top.

Baking. Proved dough samples were baked in batches of 12 loaves to a target loaf center temperature of 94−97 °C using a Spooner traveling oven. Depanned loaves were immediately conveyed to a cooler prior to analysis. When full loaves were not required, dough samples were cooked using a custom-built pressure-cooking apparatus that simulated a 20 min bake (*14*). The generation reaction was quenched by plunging the sealed tubes into water at 20 °C.

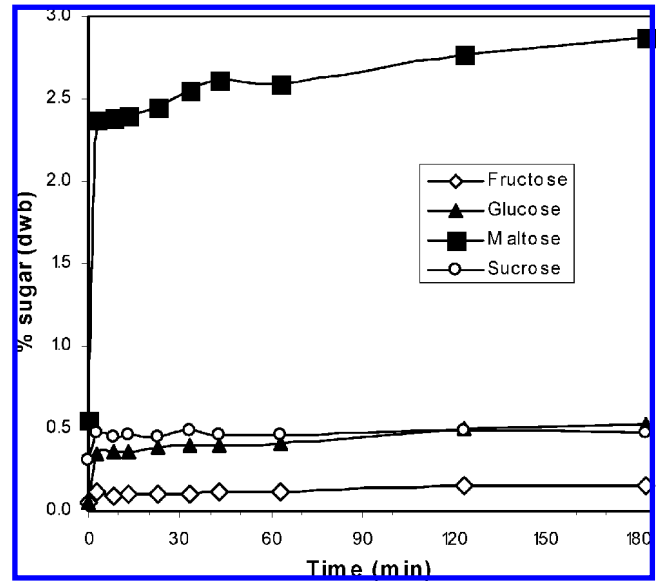

Figure 1. Release of sugars in FSW bread doughs.

Postbake Sample Treatment. Biscuits (six from each experimental bake) were reduced to a powder using a BL 300 domestic blender/food processor (Kenwood, Havant, U.K.). Samples were retained in sealed containers in the dark at room temperature until required for analysis.

For bread crusts the outer 1−2 mm surface layers were removed from each loaf and separated into combined top surfaces and base plus sides plus ends. Samples were air-dried overnight to approximately 10% moisture.

All chemical analysis of doughs and final products for amino acids, sugars, and acrylamide was as in ref *15*. Sixteen amino acids were measured in addition to asparagine (alanine, arginine, aspartate, cystine, glutamate, glutamine, glycine, isoleucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine) and for sugars fructose, glucose, maltose, and sucrose were measured. Except where stated otherwise, uncertainty of determination was ±8% for sugars and ±10% for amino acids and acrylamide.

RESULTS AND DISCUSSION

With simple flour, salt, and water doughs (FSW) sugar levels were relatively stable over time with maltose dominant (**Figure 1**). An important factor in the levels of free sugars reached is the amount of damaged starch generated during the milling process.

With dough made to a full commercial recipe (see **Table 1**) the sucrose was rapidly consumed by the yeast and more fructose was produced than would be expected from the conversion of sucrose by the yeast (possibly from breakdown of glucofructans by the yeast). The other sugars were stable for an hour or so, but declined after extended proof times as the yeast began to consume them as well (**Figure 2**).

Essentially the same pattern was seen with savory cracker doughs: sucrose rapidly consumed by the yeast; rises in glucose, fructose, and maltose; and then declines at long proof times as the yeast consumed first glucose and fructose and then maltose (**Figure 3**). The apparent dip in maltose at 77 min was not considered to be significant as it was within the uncertainty of the individual sugar measurements (shown by the error bars; successive samples were taken for analysis from the same proving dough, so there was no dough-to-dough variation).

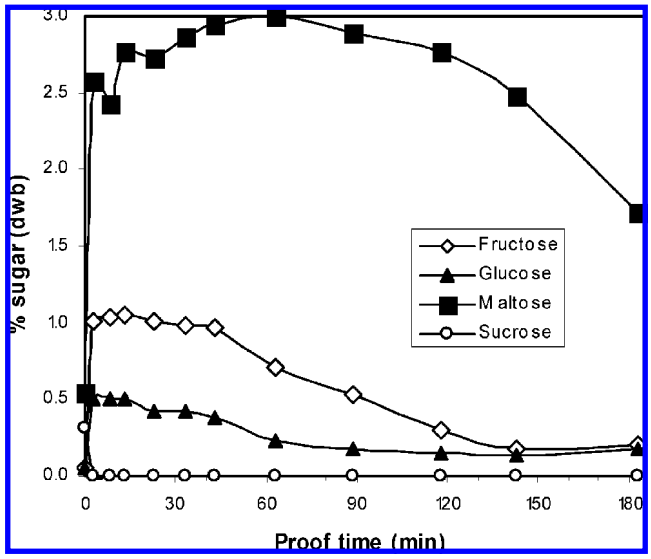

Figure 2. Release and consumption of sugars in commercial bread doughs.

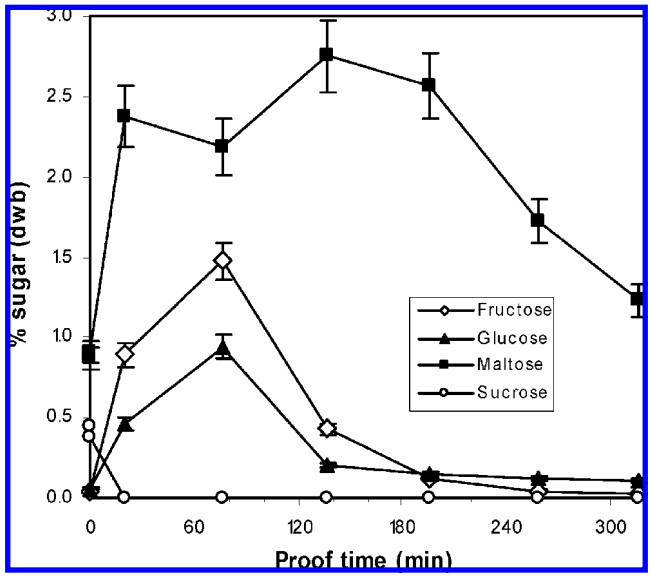

Figure 3. Release and consumption of sugars in fermented cracker doughs.

Virtually all of the sugars were gone after 18 h of fermentation (as might be used in sponge and dough processes) with only 0.1% of total sugars present (data not shown).

With FSW doughs a slight rise in the level of total free amino acids was observed during bench proving at 20 °C (**Figure 4**). However, the level dropped over time once yeast was incorporated in the recipe and began to feed. When full commercial recipes and commercial proving temperatures were used, the behavior was more complex. Initially, the yeast feeding effect dominated, but as the dough warmed up (reflecting heat from the prover as it gradually penetrated into a loaf-sized piece of dough) the level of free amino acids rose. This may have been promoted by melting of the fat in the dough increasing mobility and, hence, enzyme activity.

Asparagine did not follow the general trend and was preferentially consumed by the yeast. This difference was even more marked under commercial proving conditions as there was no rise in asparagine at higher dough temperatures, although the rate of fall was still lower than with a simple yeasted dough.

Figure 5 summarizes how the recipe affects asparagine levels over time, and **Figure 6** shows normalized asparagine measurements for the different dough recipes. Yeast is always beneficial, but it removed less of the asparagine with a fully commercial bread recipe and proving conditions. During the first hour of

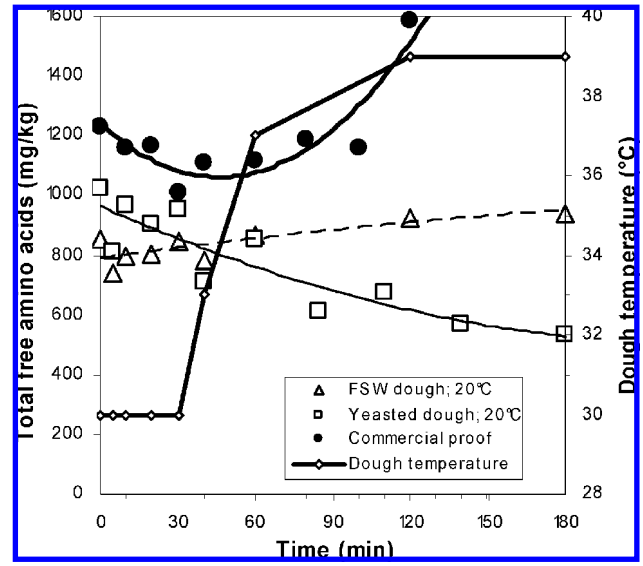

Figure 4. Changes in total free amino acid levels in proving bread doughs.

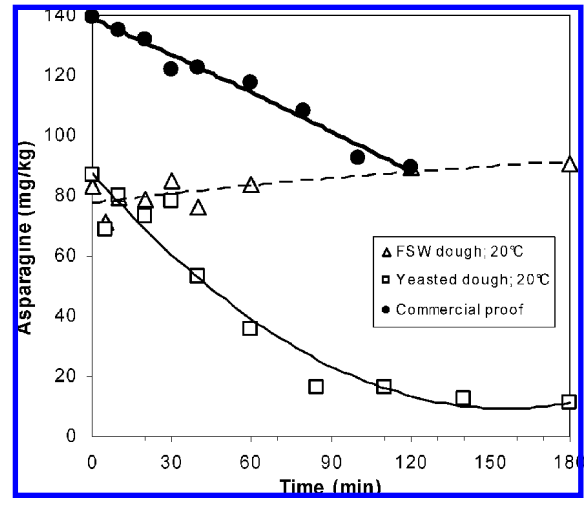

Figure 5. Changes in free asparagine levels in proving bread doughs.

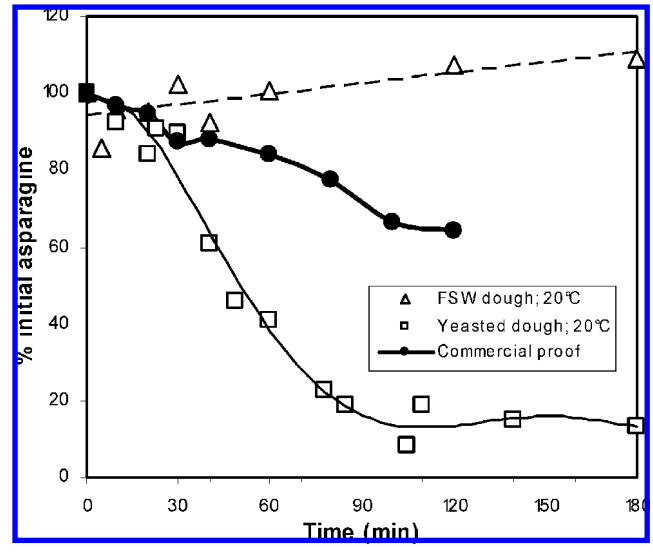

Figure 6. Effect of proof on asparagine in bread doughs.

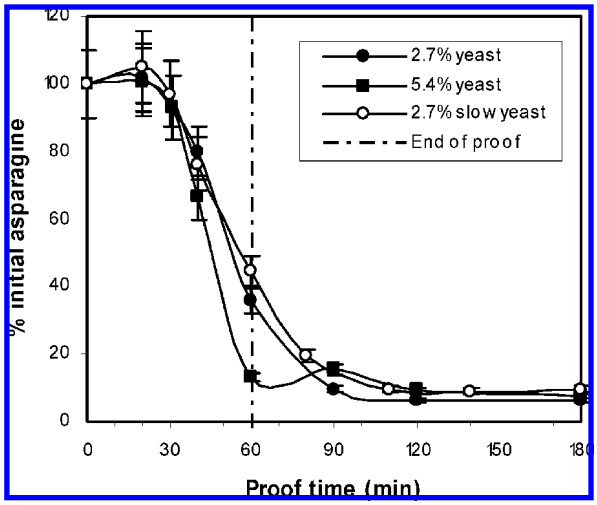

Figure 7. Effect of different yeasts on asparagine in bread dough.

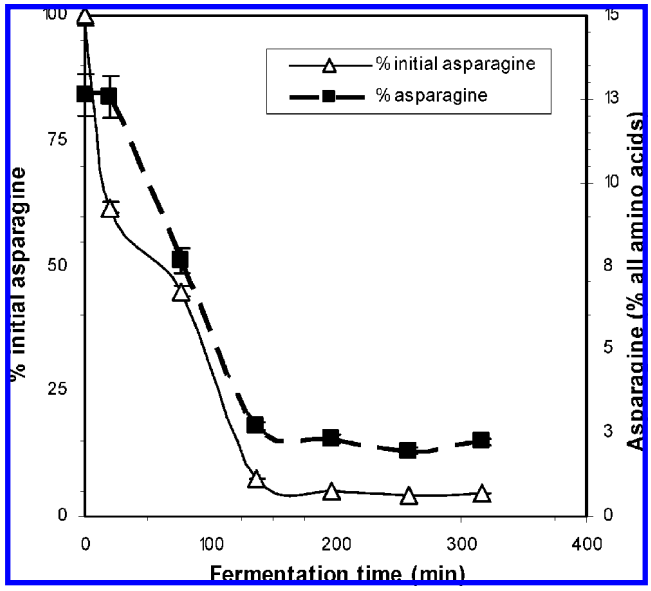

Figure 8. Effect of yeast on asparagine in cracker doughs.

proving the total level of free amino acids changed little, and so this graph also gives an indication of by how much yeast might reduce acrylamide: acrylamide production is essentially proportional to asparagine in this system (*15*).

Doubling the level of yeast from its normal 2.7 to 5.4% cut the level of residual asparagine at the end of proof (**Figure 7**; error bars show the estimated uncertainty of single measurements). A brewing yeast (*Saccharomyces carlsbergensis*, supplied by Brewing Research International, Redhill, U.K.) was found to produce gas too slowly at normal dough temperatures for effective proving, but a low-gassing baker's yeast was almost as effective as the standard yeast in consuming asparagine. This means that in principle asparagine reduction and carbon dioxide production can operate independently. Hence, it might be possible to reduce acrylamide by changing to high levels of low-gassing yeast (subject to the usual need to maintain product quality, of course).

Fermenting cracker doughs showed similar trends to bread doughs: again, the yeast consumed asparagine preferentially over other amino acids (**Figure 8**; error bars show the estimated uncertainty of single measurements). With extended (18 h) fermentation times, such as might be used to make a sponge in a sponge and dough process, all of the asparagine was consumed

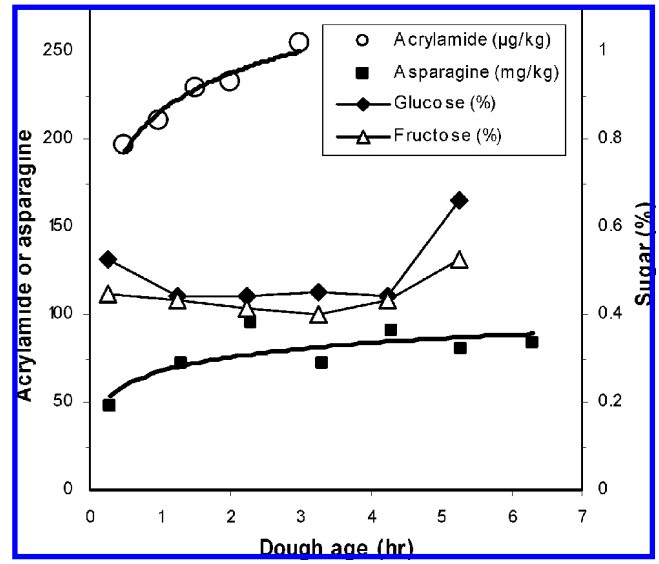

Figure 9. Effect of dough age in sweet biscuits.

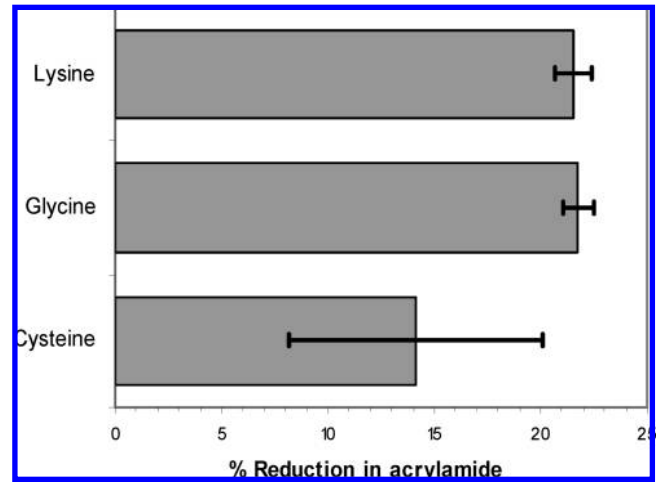

Figure 10. Effect of amino acid addition on bread doughs.

(data not shown), and so no acrylamide would be expected to form when such a dough was baked.

Even in the case of sweet biscuits, where there was no yeast in the recipe, there were changes over time (**Figure 9**). In this case more acrylamide was formed in biscuits baked from older doughs (an increase of approximately 35% over 3 h). The extra acrylamide was not due to a rise in sugar levels, but could be accounted for by an increase in free asparagine.

To try diluting the free asparagine in the dough, 5.6 μmol/kg of three other amino acids each was added separately to FSW doughs, and the resulting doughs were each cooked in duplicate. This addition represented a marked (ca. 40%) dilution of the amino acids naturally present, but in practice only a 15−20% drop in acrylamide formation was seen (**Figure 10**; error bars show the range of the duplicate measurements).

One possibility for the relatively small reduction in acrylamide was that the added amino acids were also interfering with the decay of acrylamide. To test this, FSW doughs with and without 1556 mg/kg of added glycine were spiked with 626 mg/kg [$^{13}C_3$]acrylamide, and the fate of the carbon-labeled acrylamide followed during cooking at 160 °C. There was no loss of acrylamide during bench proof (6 h at 20 °C), but as expected substantial losses of acrylamide occurred during baking. The dough with added glycine lost less of its acrylamide after 20 min of cooking than the unspiked control, suggesting that the

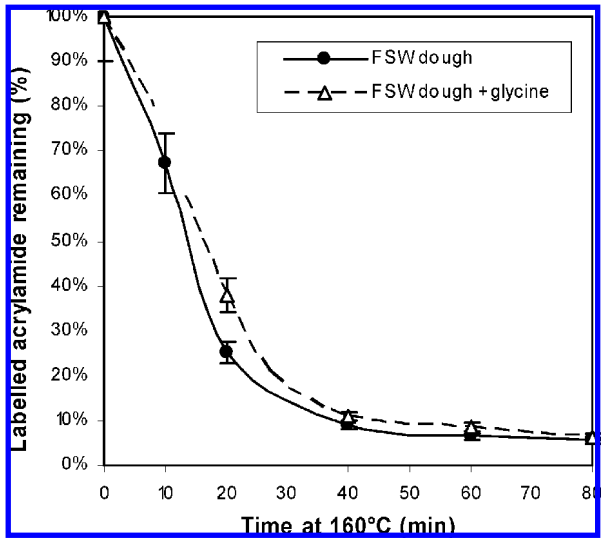

Figure 11. Effect of glycine addition on acrylamide decay in bread dough.

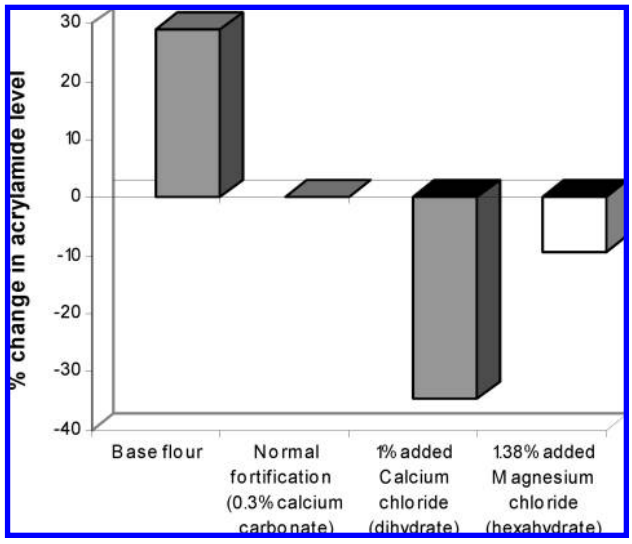

Figure 12. Effect of metal ions on acrylamide formation in FSW bread doughs.

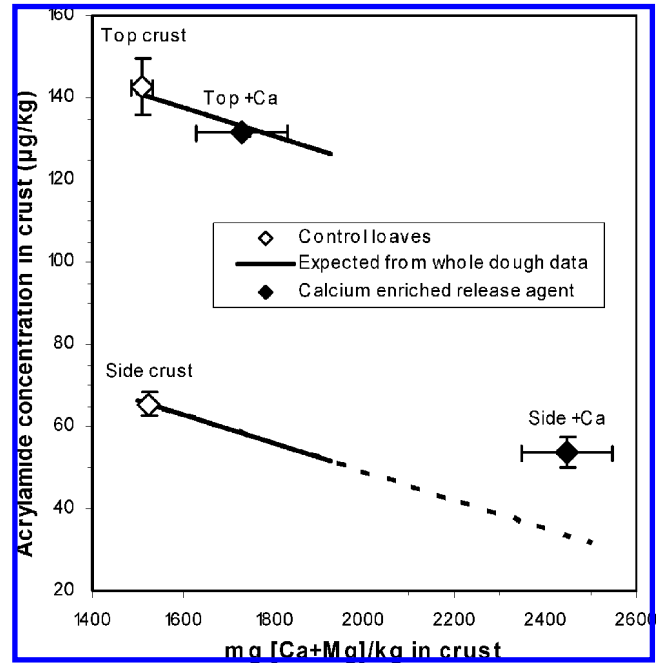

Figure 13. Effectiveness of calcium addition via tin release agent.

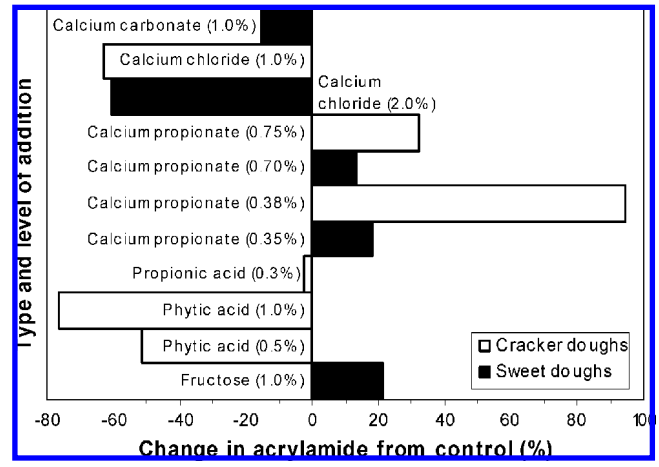

Figure 14. Effect of additives on acrylamide in sweet and savory biscuits.

glycine was helping the acrylamide to survive baking (**Figure 11**; error bars show the estimated uncertainty of single measurements).

It has been suggested (*8*) that adding divalent metal ions could give additional high-temperature stability to asparagine/matrix interactions (stable polymer network), thereby rendering the latter species unavailable for reaction with carbonyl precursors to produce acrylamide. To test this in bread systems, a series of doughs were cooked and the final acrylamide levels measured (**Figure 12**). The highest levels of acrylamide came from doughs made from pure base flour. The standard calcium fortification of 0.3% required by U.K. law for nutritional reasons gave a reduction in acrylamide, and additional calcium fortification reduced the level still further. A similar effect was seen with magnesium addition (both metals produced comparable effects for equal quantities of their metal ions, i.e., after allowing for the different levels of water of crystallization in each salt).

Adding metal ions to the whole dough is inefficient as most of the dough never gets hot enough to generate acrylamide. Ideally, any extra calcium should be applied only to the outer surface of the dough. As a way of exploring this, a calcium chloride solution was made into a water-in-oil emulsion with a commercial tin release agent. The emulsion was then applied to the surfaces of some bread doughs, and the crusts of the baked bread were analyzed for acrylamide and metal ion concentration. Duplicate crust samples were analyzed for both metals and acrylamide, and the range of values measured is shown by the error bars in **Figure 13**. There was no visible difference in appearance or crust color between the treated and untreated loaves, but there was a reduction in acrylamide. To compare with the previous whole dough experiments (**Figure 12**), the expected drops in acrylamide based on the measured changes in metal ion concentrations in each type of crust have been shown as lines on the graph. The drop in top crust acrylamide was in line with the expected trend, but the effect was lower than expected with the side crusts (dotted line). This may be because the bread was baked in an open tin without a top lid, so the top crust will have dried out and heated up more quickly, and this may have encouraged earlier release of the calcium from the emulsion into the dough.

When incorporated in biscuit doughs, fructose increased acrylamide and phytic acid reduced it, as expected (**Figure 14**). So did calcium when added as the chloride or carbonate (although it should be noted that at the levels used calcium

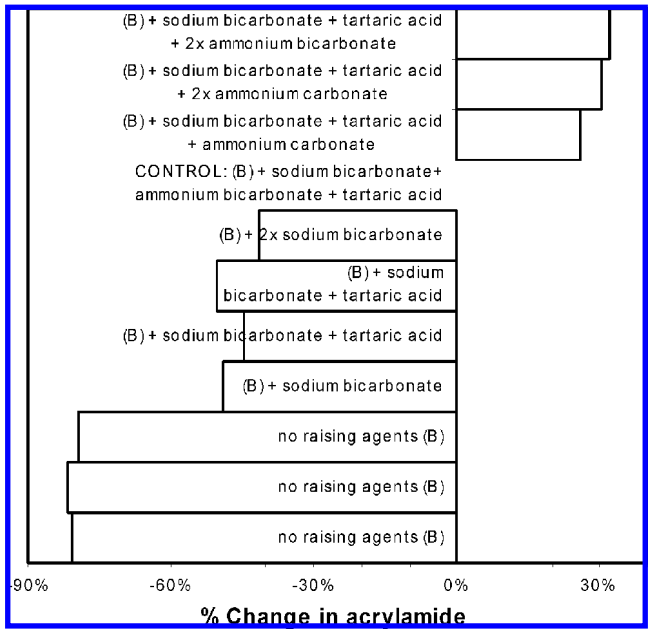

Figure 15. Effect of raising agents in sweet biscuits.

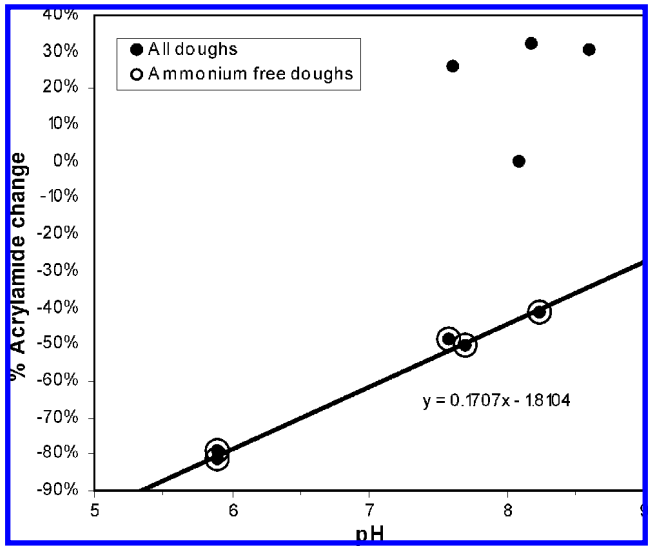

Figure 16. Effect of pH and ammonium salts on acrylamide formation in sweet biscuits.

chloride hindered the rise of sweet biscuits and the products were unpalatable when tasted). Calcium propionate is already added to bakery goods in the United Kingdom as a preservative (up to 0.2%), and so might have offered a convenient route for calcium addition. In practice, however, it was detrimental instead of beneficial for both types of biscuits, especially at lower levels of addition. The reasons for this are not obvious as propionic acid alone had little effect, but it is clear that calcium is not a panacea and that recipe changes aimed at reducing acrylamide will need proper evaluation. The trends were always the same, but when both types of biscuit were tested with the same additive, savory doughs were typically twice as sensitive as sweet.

Sweet biscuits were baked with a range of raising agent combinations to see what effect they had on acrylamide (**Figure 15**). It was clear that any raising agent increased acrylamide, but ammonium-based agents gave the highest levels of acrylamide.

The effect of the non-ammonium-based raising agents could be explained entirely in terms of shifts in pH (**Figure 16**). This is in agreement with previous reports that increased levels of

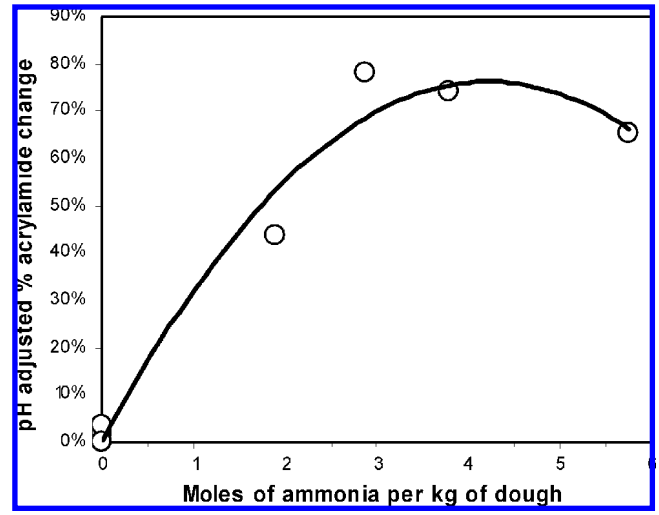

Figure 17. Effect of ammonia-based raising agents on acrylamide in sweet biscuits.

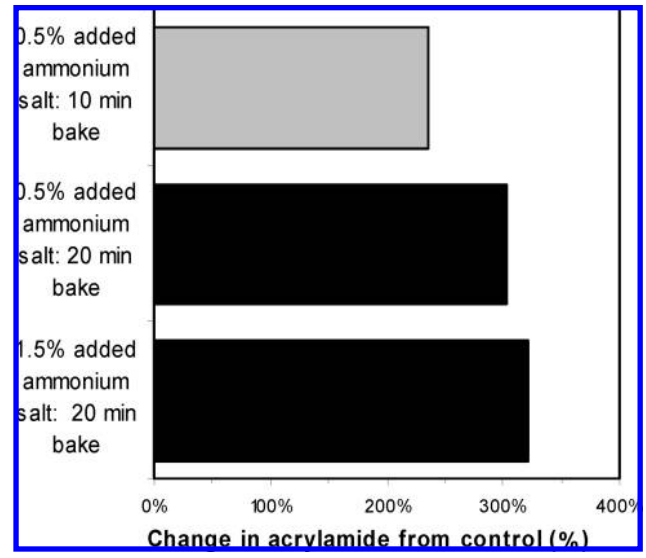

Figure 18. Effect of ammonium bicarbonate on acrylamide in savory biscuits.

tartaric acid lowered acrylamide (*16*), but easier to apply as a practical rule as the effect of pH on acrylamide was linear. The pH sensitivity of the sweet biscuits was similar to that seen with bread (17% reduction per unit drop in dough pH vs a 12% reduction for bread; see **Figure 19**).

Using the linear trend to subtract the effect of pH and separate the effect of ammonia addition produced **Figure 17**. Initially, the ammonium salts strongly increased acrylamide, but then the increase reached a plateau, possibly due to greater decay of acrylamide at high ammonia levels (*17, 18*).

The ammonium-mediated increases in acrylamide in sweet biscuits were relatively modest; however, when ammonium bicarbonate was added to a savory biscuit formulation, larger increases were seen (**Figure 18**) for comparable addition levels (as was seen earlier with calcium salt addition). The increases were also relatively insensitive to either baking time or addition level, but were smaller than those seen in model systems (*10, 11*).

Lowering the pH reduces acrylamide in bread as in sweet biscuits; however, lowering the pH also promotes the formation of other process contaminants such as 3-MCPD (see **Figure 19**). A similar trend of 3-MCPD with pH was seen with savory doughs. Negligible amounts of 3-MCPD were detected in sweet

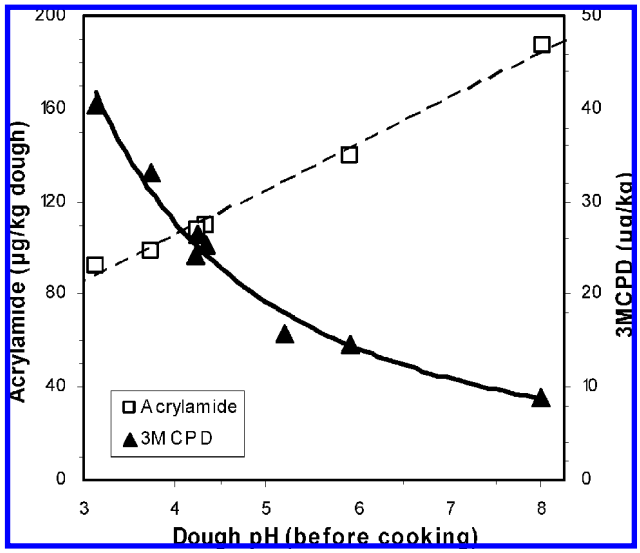

Figure 19. Effect of bread dough pH on acrylamide and 3-MCPD.

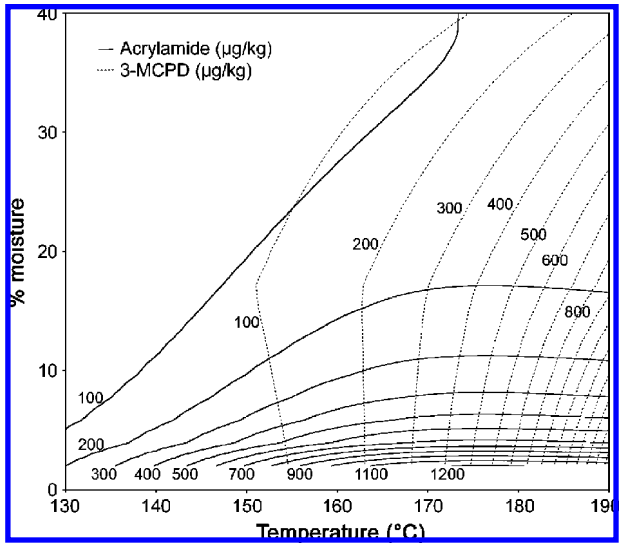

Figure 20. Effect of dough temperature and moisture on acrylamide and 3-MCPD.

doughs because there is no yeast in the recipe to form glycerol, which is the major 3-MCPD precursor in bakery goods (*19*). Therefore, whereas lowering the pH looks superficially attractive as a route for acrylamide reduction, it is important to be aware of potential unwanted side effects. It should also be said that the practical pH range for bakery goods is small if undesirable flavors are to be avoided.

Another question that needs to be considered is the effect of baking conditions. A kinetic model fitted to a matrix of 24 cooking experiments (4 moistures × 6 temperatures) has been used to show that higher moistures and lower temperatures reduce acrylamide (*1*), but such conditions might increase 3-MCPD. To answer this, a kinetic model of 3-MCPD formation (*19*) based on the same experimental protocol has been used to calculate the effect of temperature and moisture on 3-MCPD levels in bread so as to match the model predictions from ref *1*. The results are given in **Figure 20**, which shows contours of the predicted level of each process contaminant after 20 min of heating. They confirm that there is no region of the moisture−temperature map where reducing acrylamide does not leave 3-MCPDs at least unchanged, and normally both reduce together.

In conclusion, the effect of yeast in real bakery processes is more wide ranging than has been hitherto appreciated. It has a protective effect giving lower levels of acrylamide in the final food than would be expected from the levels of precursors in the recipe ingredients. As a consequence of this, recipe or process changes that affect yeast may have unexpected effects on final acrylamide levels as the yeast acts as a filter/amplifier for acrylamide precursors: it may consume asparagine, and reducing sugars can be either released or consumed depending on circumstances. In some products the use of high levels of low-gassing bakers yeasts may offer a reduction route, but extra amino acids have only a modest reducing effect on acrylamide.

With chemically raised products, ammonium salt replacement should be the priority, and best practice should avoid allowing biscuit doughs to age. Calcium supplementation looks promising, but interactions with other ingredients (especially propionate) need investigation.

Low pH is beneficial for acrylamide, but is limited in practice by dough buffering and the risk of promoting other contaminants such as 3-MCPD. Fortunately, changes to process conditions to reduce acrylamide such as reducing oven temperatures or baking times will not promote 3-MCPD.

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LITERATURE CITED

(1) Sadd, P.; Hamlet, C. The formation of acrylamide in UK cereal products. *Adv. Exp. Med. Biol.* **2005**, *561*, 415–429.
(2) Leung, K. S.; Lin, A.; Tsang, C. K.; Yeung, S. T. K. Acrylamide in Asian foods in Hong Kong. *Food Addit. Contam.* **2003**, *20*, 1105–1113.
(3) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R. H.; Gondé, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; ller, D.; Tallmadge, D.; O'Brien, J.; Thompson, S.; Silvani, D.; Whitmore, T. A review of acrylamide: an industry perspective on research, analysis, formation, and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323–347.
(4) Fredriksson, H.; Tallving, J.; Rosen, J.; Man, P. Fermentation reduces free asparagine in dough and acrylamide content in bread. *Cereal Chem.* **2004**, *81*, 650–653.
(5) Hamlet, C. G.; Baxter, D. E.; Sadd, P. A.; Slaiding, I.; Liang, L.; Muller, R.; Jayaratne, S. M.; Booer, C. *Exploiting Process Factors To Reduce Acrylamide in Cereal-Based Foods, C03032 and C03026*; report prepared on behalf of the U.K. Food Standards Agency; RHM Technology: High Wycombe, U.K., 2005.
(6) Bråthen, E.; Kita, A.; Knutsen, S. H.; Wicklund, T. Addition of glycine reduces the content of acrylamide in cereal and potato products. *J. Agric. Food Chem.* **2005**, *53*, 3259–3264.
(7) Claeys, W. L.; De Vleeschouwer, K.; Hendrickx, M. E. Effect of amino acids on acrylamide formation and elimination kinetics. *Biotechnol. Prog.* **2005**, *21*, 1525–1530.
(8) Lindsay, R. C.; Jang, S. Chemical intervention strategies for substantial suppression of acrylamide formation in fried potato products. *Adv. Exp. Med. Biol.* **2005**, *561*, 393–404.
(9) Gökmen, V.; Senyuva, H. Z. Acrylamide formation is prevented by divalent cations during the Maillard reaction. *Food Chem.* **2007**, *103*, 196–203.
(10) Amrein, T. M.; Schonbachler, B.; Escher, F.; Amado, R. Acrylamide in gingerbread: critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **2004**, *52*, 4282–4288.
(11) Amrein, T. M.; Andres, L.; Manzardo, G. G.; Amado, R. Investigations on the promoting effect of ammonium bicarbonate on the formation of acrylamide in model systems. *J. Agric. Food Chem.* **2006**, *54*, 10253–10261.

(12) Biedermann, M.; Grob, K. Model studies on acrylamide formation in potato, wheat flour and corn starch; ways to reduce acrylamide contents in bakery ware. *Mitt. Lebensmitteluntersch. Hyg.* **2003**, *94*, 406–22.
(13) Whitely, P. R. *Biscuit Manufacture; Fundamentals of in-Line Production*; Elsevier: London, U.K., 1971.
(14) Hamlet, C. G.; Sadd, P. A. Effects of yeast stress and pH on 3-MCPD producing reactions in model dough systems. *Food Addit. Contam.* **2005**, *22*, 616–623.
(15) Hamlet, C. G.; Sadd, P. A.; Liang, L. Correlations between the amounts of free asparagine and saccharides present in commercial cereal flours in the UK and the generation of acrylamide during cooking. *J. Agric. Food Chem.* **2008**, *56*, 6145–6153.
(16) Graf, M.; Amrein, T. M.; Graf, S.; Szalay, R.; Escher, F.; Amado, R. Reducing the acrylamide content of a semi-finished biscuit on industrial scale. *LWT* **2006**, *39*, 724–728.
(17) Claeys, W. L.; De Vleeschouwer, K.; Hendrickx, M. E. Kinetics of acrylamide formation and elimination during heating of an asparagine−sugar model system. *J. Agric. Food Chem.* **2005**, *53*, 9999–10005.
(18) Stadler, R. H.; Verzegnassi, L.; Varga, N.; Grigorov, M.; Studer, A.; Riediker, S.; Schilter, B. Formation of vinylogous compounds in model Maillard reaction systems. *Chem. Res. Toxicol.* **2003**, *16*, 1242–1250.
(19) Hamlet, C. G.; Sadd, P. A.; Gray, D. A. Generation of monochloropropanols (MCPDs) in model dough systems. 1. Leavened doughs. *J. Agric. Food Chem.* **2004**, *52*, 2059–2066.

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