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Effect of salts on the formation of acrylamide, 5-hydroxymethylfurfural and flavour compounds in a crust-like glucose/wheat flour dough system during heating

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A R T I C L E   I N F O

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Maillard reaction
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Baking
Aroma
Strecker aldehydes
Pyrazines
Calcium

A B S T R A C T

Among many strategies known to mitigate acrylamide formation, addition of cations, particularly calcium, is effective and can be used in bakery products. In this study, the effects of NaCl, KCl, CaCl₂, MgCl₂, sodium lactate, calcium lactate, and magnesium lactate on aroma and acrylamide formation were investigated in glucose/wheat flour dough systems during heating. Addition of salts inhibited Maillard reaction in favour of caramelisation, with divalent cations found to be most effective. The impact of salts on acrylamide reduction became less effective with increasing temperature. Most Strecker aldehydes and pyrazines decreased in the presence of salts, however CaCl₂ and calcium lactate increased the concentration of furans, furfurals, and diketones. Calcium lactate also increased some ethyl-substituted pyrazines at high temperatures. Reduction of acrylamide with salts is associated with higher amounts of furan derivatives and decreased amounts of Strecker aldehydes and pyrazines. The mechanisms behind these changes are discussed.

1. Introduction

In bakery products, salt controls the rate of fermentation, strengthens the dough, provides microbial safety, prolongs shelf life as well as affecting colour and flavour formation (Miller & Hoseney, 2008; Silow, Axel, Zannini & Arendt, 2016). Reduction of NaCl from 20 to 10 g/kg, without any replacement, was reported to negatively affect toasted aroma and crust colour of bread (Passalone, Caponio, Pagani, Summo, & Paradiso, 2019). Maillard reaction and caramelisation are important reactions that contribute to the generation of flavour and browning in bakery products during baking (Pozo-Bayón, Guichard, & Cayot, 2006). However, these reactions also induce the formation of process contaminants, such as acrylamide and 5-hydroxymethylfurfural (HMF) (Capuano & Fogliano, 2011; Gökmen, Açar, Köksel, & Acar, 2007). These compounds and/or their metabolites are considered as probably or potentially carcinogenic to humans (Capuano & Fogliano, 2011). Bread and baked products were reported to have the highest acrylamide content after coffee and fried potato products (EFSA, 2015). Of these, biscuits, crackers, crispbread and similar products had the highest average acrylamide level (265 µg/kg), followed by breakfast cereals (161 µg/kg), processed cereal-based baby foods (73 µg/kg) and soft bread (42 µg/kg) (EFSA, 2015). The content of HMF was reported to range from 4.1 to 151.1 mg/kg in bakery products (Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2000).

There are several strategies to reduce the levels of acrylamide and HMF in foods (Capuano & Fogliano, 2011; Sadd, Hamlet, & Liang, 2008). One method is the incorporation of salts in the recipe to reduce acrylamide formation during baking. This approach can be combined with the replacement of NaCl with non-sodium salts and fortification of the products with desirable metal cations (Açar, Pollio, Di Monaco, Fogliano, & Gökmen, 2012; Sadd et al., 2008). In several studies, salts were shown to reduce acrylamide formation in different bakery products but they either increased or had no effect on HMF levels depending on the type of cation (Açar et al., 2012; Kukurová, 2016; Mesias, Holgado, Márquez-Ruiz, & Morales, 2015). Calcium sources (calcium chloride and Puracal Act 100) added to cookie dough up to 0.5 % dosages were reported to reduce acrylamide by 70 % without changing the sensory properties, but HMF increased (Açar et al., 2012). Mesias et al (2015)

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achieved up to 58% and 35–40% reduction in the acrylamide content of cookies containing either CaCl$_2$ or a mix of CaCl$_2$ with other commercial salt-replacers, respectively. They also reported that HMF and furfural did not show great variations when the recipe contained either different salts or commercial salt-replacers, except for calcium chloride, which increased furfural significantly (Mesias et al., 2015). Moreover, divalent cations were found to be more effective compared to monovalent cations in acrylamide reduction in fructose-asparagine model systems (Gökmen & Şenyuva, 2007a).

Whilst the effect of salts on acrylamide and HMF formation is already known, it is important to also understand the effect of mineral salts on the formation of flavour compounds and the simultaneous formation of process contaminants. This raises the question as to whether it is possible to reduce acrylamide without sacrificing the flavour of the bakery product. Therefore, the aim of this study is to investigate the effect of the application of different salts (sodium chloride, potassium chloride, calcium chloride, calcium lactate, sodium lactate (NaLactate) and magnesium lactate (MgLactate) and process temperatures in low moisture crust-like samples, made from glucose and wheat flour, on the formation of flavour compounds and process contaminants (acrylamide and HMF).

2. Material and methods

2.1. Chemicals and consumables

Wheat flour, not enriched with calcium, was supplied from a local market in Turkey. D(-)+-glucose (≥99.5%), calcium chloride (≥93%), magnesium lactate hydrate (≥95%), sodium lactate (≥98%), and magnesium chloride hexahydrate (99–102%) were supplied from Sigma-Aldrich (Gillingham, UK). Sodium chloride (≥99%) was from Fischer Scientific (Loughborough, UK), potassium chloride (min 99%) was from VWR (Lutterworth, UK), and CaLactate (food grade) was from Special Ingredients Ltd. (Chesterfield, UK).

Analytical standards of 2-methylpyrazine (99+%), 2,3-dimethylpyrazine (99%), 2,5-dimethylpyrazine (99%), 2,6-dimethylpyrazine (98%), 2-ethylpyrazine (98%), 2-ethyl-3-methylpyrazine (98+%), and benzoacetalddehyde (98%) were purchased from Acros Organics (Waltham, MA, USA). 2-Methylbutan-al (≥95%), 3-methylbutan-al (≥98%), and methional (≥97%) were supplied by Tokyo Chemical Industry (Tokyo, Japan). Pyrazine (99+%) was purchased from Lancaster (Ward Hill, MA, USA) and 2-isopropylpyrazine (99.5%) was from Alfa Aesar (Waltham, MA, USA). 3-Methyl-2-butanone (99%), 2-furaldehyde (furfural) (99%), 2-furaldehyde (97+%), 2-methylpropanal (99+%), 2,3-butanedione (diacetyl) (97%), 2,3-pentanedione (97%), 2-ethyl-5(6)-methylpyrazine (≥98%), as well as the alkanes standards C$_6$-C$_{22}$ (100 µg/mL in diethyl ether) were obtained from Sigma-Aldrich (Gillingham, UK). Analytical standards of acrylamide (99%) and 5-hydroxymethylfurfural (≥98%) were purchased from Sigma-Aldrich (Gillingham, UK). Acetonitrile (VWR, Lutterworth, UK), formic acid (Fischer Scientific, Loughborough, UK) and water (VWR, Lutterworth, UK) employed for LC-MS/MS analysis were of mass spectrometry grade.

2.2. Preparation of dough systems

To prepare the glucose/wheat flour/salt dough systems, 50 mmol glucose and 20 mmol salt (either sodium chloride, potassium chloride, calcium chloride, calcium lactate, sodium lactate, magnesium lactate, magnesium chloride) were dissolved in 50 mL deionised water. Then, the solution was added to 100 g wheat flour and mixed in a lab-scale mixer (Kenwood, Woking, UK) to obtain a homogenous dough. A glucose/wheat flour dough system without salt served as control and was prepared by mixing 100 g wheat flour and 50 mmol glucose in 50 mL deionised water. The doughs were frozen at −80 °C, for 24 h and dried in a freeze-dryer (Christ Gamma 2–16 LSC, Martin Christ, Osterrode, Germany). After freeze-drying, they were ground in a coffee grinder (De’Longhi, Treviso, Italy). To avoid the physical effect of salts on dough rheology, which could alter heat transfer and thermal load, it was necessary to obtain dry systems prior to heating, rather than directly heating the doughs. Thus, it was possible to observe chemical effects only. The powders had an initial moisture content below 2% with no significant differences between the samples of different recipes (p > 0.05).

The ground samples (0.5 ± 0.05 g) were weighed into Duran culture tubes (16 mm × 160 mm, 20 mL capacity from Fischer Scientific, Loughborough, UK) and their caps were tightly closed. The control and dough systems with NaCl, KCl, CaCl$_2$, and CaLactate were heated at 160 °C for 2, 3, 4, 5, 7, 9, and 11 min; at 180 and 200 °C for 1, 2, 3, 4, 5, 6, and 7 min in an oil bath (Memmert, Germany). In addition, NaLactate, MgLactate and MgCl$_2$ were heated only at 160 °C to compare other lactate salts and another divalent cation under the conditions most likely to show an effect. Immediately after heating, the tubes were submerged in an ice bath to prevent any further reaction. For each time-temperature combination six tubes were heated simultaneously. Two of them were directly used for volatile analysis by transferring the contents into headspace vials and the other tubes were stored at −20 °C until further analysis.

2.3. Aqueous extraction

A portion of the sample (0.5 ± 0.05 g) was extracted with a total of 10 mL deionised water in two stages. Initially, 5 mL deionised water was mixed with the sample in a 15 mL falcon tube which was placed in an ultrasonic bath at 40 °C for 15 min. After centrifugation at 4000 × g for 5 min, the supernatant was transferred to another tube. The same procedure was repeated for the second stage of the extraction and the supernatants were combined. The combined supernatants were transferred to Eppendorf tubes, were centrifuged at 10000 × g for 5 min and an aliquot of the clear supernatant was passed through a 0.2 µm nylon syringe filter (Fisher Scientific, Loughborough, UK) and transferred to an high-performance liquid chromatography (HPLC) vial for HM and acrylamide analysis. The remaining part was transferred to an Eppendorf tube to measure colour and pH.

2.4. Analysis of acrylamide

Aqueous extracts were analysed using an Agilent (Santa Clara, CA, USA) 1200 HPLC system coupled to an Agilent 6410 triple quadrupole mass spectrometer (MS) with electrospray ion source (ESI) in positive mode. Chromatographic separation was carried out using a Hypercarb column (100 × 3.0 mm i.d., 5 µm) with a Hypercarb precolumn (10 × 3.0 mm i.d., 5 µm, ThermoFisher Scientific, Waltham, MA, USA). The mobile phase was 0.1 % aqueous formic acid at a flow rate of 0.3 mL/min at 30 °C. The injection volume was 5 µL. The ESI source parameters were: gas temperature 350 °C, gas flow 10 L/min, nebuliser pressure 35 psi, capillary voltage 3000 V and fragmentor voltage 40 V. The MRM transitions measured for acrylamide were m/z 72>55 (N$_2$ collision energy (CE), 8 eV) and 72>27 (CE, 16 eV). The quantification of acrylamide was performed based on the 72>55 transition. An external calibration curve of acrylamide was built between 0.5 and 100 µg/L.

2.5. Analysis of 5-hydroxymethylfurfural

Aqueous extracts of the control dough and those with added NaCl, KCl, CaCl$_2$, and CaLactate were analysed according to the method previously described by Gökmen & Şenyuva (2007) using an Agilent 1200 HPLC system (Santa Clara, CA, USA) and a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm, 100 Å; Phenomenex Inc., Torrance, CA, USA). The mobile phase was an isotropic mixture of water:acetonitrile (90:10, v:v) and the flow rate was 1 mL/min. The total run time was 12 min. The column temperature was 30 °C and the injection volume was 10 µL. The diode array detector collected signals at 285 nm. Quantification was
performed with an external calibration curve where the standard solutions of HMF were prepared in concentrations ranging from 1 to 250 mg/L.

2.6. Analysis of volatile compounds

Heated samples were transferred into 20 mL screw cap glass headspace vials. In order to equalise the ionic strength of the model systems and enhance the release of the volatile compounds to the headspace, 1 mL of saturated NaCl solution (35 % NaCl) was added and the mixture was vortexed for 1 min. The saturated NaCl solution contained a mixture of internal standards; 3-methyl-2-butanal (for Strecker aldehydes) and 3-furaldehyde (for furans) each at a concentration of 0.5 mg/L, and isopropylpyrazine (for pyrazines) at a concentration of 0.05 mg/L. Solid-phase microextraction (SPME) was performed using a CTC PAL headspace autosampler (CTC Analytics AG, Switzerland) coupled to an Agilent 7890A gas chromatography (GC) system (Agilent Technologies, Santa Clara, CA, USA) attached to a 5975C inert MSD triple-axis detector. A 50/30 μm DVB/CAR/PDMS SPME fibre (Supelco, Bellefonte, PA, USA) was used for the extraction of the volatile compounds. Agitation was applied to the samples during the equilibration (50 °C, 30 min) and extraction (50 °C, 20 min) stages. The fibre was desorbed in the injection port where the temperature was held at 250 °C. Helium was used as the carrier gas with a constant flow rate of 1.2 mL/min throughout the analysis. A DB-WAX column (30 m × 0.25 mm × 0.5 μm film thickness, Agilent, Santa Clara, CA, USA) was employed for the separation of the volatile compounds. During analysis, the GC oven temperature was initially set at 35 °C for 10 min. Then, it was raised to 250 °C at a rate of 4 °C/min and remained at 250 °C for 10 min. The mass spectrometer operated in electron impact mode (70 eV), the source temperature was 200 °C and the scanning range was from m/z 29 to m/z 400. Analysis was performed in duplicate for each sample point.

Peaks were identified by comparing their mass spectra against those in the NIST 11 Mass Spectral Database. The linear retention index (LRI) was calculated for each compound. To achieve that, 1 μL of a standard mixture of n-alkanes (C₇-C₂₂) was injected and analysed under the same chromatographic method as the samples. Authentic compounds run under the same conditions were used for confirmation of identities of most of the volatiles. The identities of molecules were confirmed if their mass spectra and LRI matched those of the authentic compounds.

For the quantitation and semi-quantitation of the volatile compounds, stock solutions of the standards were prepared in water:acetonitrile (50:50, v/v) and diluted by using saturated NaCl solution to obtain working solutions. A set of mixtures of standard solutions was prepared containing 2-methylfuran, 2-methylbutan-3-ol, 3-methylbutanal, diacetyl, 2,3-pentanedione, methional, 2-furfural and benzoic acid at concentrations ranging from 0.05 to 200 μg/L; 2,5-dimethylpyrazine and 2,6-dimethylpyrazine at concentrations ranging from 0.005 to 2 mg/L; 2-ethyl-3-methylpyrazine at concentrations ranging from 0.00018 to 0.018 mg/L; 2-ethyl-6-methylpyrazine at concentrations ranging from 0.00032 to 0.032 mg/L. Standard mixtures also contained internal standards 3-methyl-2-butanol (for Strecker aldehydes) and 3-furaldehyde (for furans) each at a concentration of 0.5 mg/L, and isopropylpyrazine (for pyrazines) at a concentration of 0.05 mg/L. Dimethyl disulfide, dimethyl trisulfide, 2-acetolpyridine, and 2-acetylpyrrole were approximately quantified by using the calibration curve built for 2-furfural. The standard solution mixtures (1 mL) were transferred into GC-MS vials containing 0.5 g unheated wheat flour/glucose system in order to account for the matrix effect.

2.7. Colour analysis

The aqueous extracts were transferred to a 96 well-plate and a Spark multimode microplate reader (Tecan Group Ltd., Mannedorf, Switzerland) was used to measure the absorbance of the samples at 420 nm.

2.8. Measurement of pH

Clear extracts of samples were used for pH measurement. The pH was determined by using a benchtop Orion pH-meter (Thermo Scientific, USA) connected to an Inlab pH electrode (Mettler Toledo, USA). The electrode was calibrated by using buffers at pH 4, 7 and 10.

2.9. Analysis of moisture content

The moisture content of dried wheat flour/glucose systems was determined by using an infrared moisture analyser (Sartorius MA150, Göttingen, Germany) at 105 °C.

2.10. Statistical analysis

One-way ANOVA and Tukey’s post hoc test were carried out using SPSS Statistics software Version 23.0 (IBM, USA) to determine the significant differences (p < 0.05) between samples. The principal component analysis (Pearson correlation) was carried out using XLSTAT software Version 2021.1.1 (Addinsoft, Paris, France).

3. Results and discussion

3.1. Effect of salts on acrylamide formation

Acrylamide concentration of the heated dough systems with or without salts is given in Fig. 1. At 160 °C, the control samples had the highest acrylamide concentration throughout the heating period (Fig. 1a). It was followed in decreasing order by KCl, NaCl, CaLactate, NaLactate, MgLactate, CaCl₂ and MgCl₂ containing systems. Acrylamide formation was mitigated 14, 24, 44 and 45 % after heating at 160 °C for 7 min when KCl, NaCl, NaLactate, and CaLactate were incorporated in the dough recipe, respectively. Even more drastic decreases were found in MgLactate (60 %), CaCl₂ (64 %) and MgCl₂ (67 %) systems under the same conditions. By increasing cooking temperature, acrylamide concentration increased in all samples as expected. The acrylamide formation trend at 180 °C was similar to 160 °C: the control had higher concentrations than the salt containing systems throughout the heating period (Fig. 1b). However, it was notable that the acrylamide reduction effect of mineral salts decreased when temperature was raised to 180 °C. The acrylamide formation curves of NaCl, KCl, and CaLactate systems converged and were closer to the control. An acrylamide reduction of only 9 % for NaCl and CaLactate and 20 % for KCl systems heated at 180 °C for 7 min was achieved (p < 0.05), and the greatest reduction was achieved using CaCl₂ (49 %).

The mitigating effect of salts was even less apparent at 200 °C, where the curves of the acrylamide formation for control, NaCl, and CaLactate converged at most of the heating times (Fig. 1c). Acrylamide reduction was achieved, but after 7 min it was only 15 % for CaLactate with respect to control (p < 0.05). The trend of acrylamide formation in the KCl containing system was slightly lower than the control but not significant at the later time points. The samples with CaCl₂ showed 43 % of acrylamide reduction compared to control for 200 °C at their final heating points. Overall, mitigation of acrylamide formation by mineral salts showed a temperature-dependent behaviour and CaCl₂ was the most promising salt compared to NaCl, KCl, and CaLactate, even under the harshest thermal conditions applied. Van Der Fels-Klerx et al. (2014) also found a similar temperature-dependent effect in acrylamide reduction of biscuits during baking. In their kinetic study, NaCl reduced acrylamide in biscuits during heating at 180 °C for up to 15 min. Moreover, there was still an apparent difference in the acrylamide concentration of biscuits with and without NaCl during baking at 190 °C, although they found no difference at 200 °C (Van Der Fels-Klerx
Numerous studies have focused on the effect of salts on acrylamide reduction in both dry or aqueous model systems and foods, including bakery products. In general, salts were found to be effective in acrylamide reduction, the degree of which depended on the conditions applied. Gökmên & Şenyuva (2007b) found a 97 % or more reduction in relation to the control when an equimolar mixture of asparagine and glucose containing equimolar amounts of monovalent, divalent and trivalent cations such as K⁺, Ca²⁺, Mg²⁺, Zn²⁺, and Fe³⁺ were heated at 150 °C for 20 min. Levine & Ryan (2009) reported an acrylamide reduction of up to 36 % and 23 % in cooked wheat dough model systems by replacing water with aqueous 0.04 M CaCl₂ or NaCl, respectively. However, they did not observe any reduction when CaCO₃ was added directly to wheat flour to simulate calcium enriched flour. Furthermore, addition of 0.5 % (flour basis) of a mix of CaCl₂ and MgCl₂ in biscuits was reported to reduce acrylamide formation by 60 % (Quarta & Anese, 2010).

Several studies investigated the mechanism of the effect of salts on acrylamide reduction in both dry or aqueous model systems and foods, including bakery products. In general, salts were found to be effective in acrylamide reduction, the degree of which depended on the conditions applied. Gökmên & Şenyuva (2007a), Gökmên & Şenyuva (2007b) reported that cations mitigated acrylamide formation in equimolar fructose (or glucose)-asparagine-salt model systems by suppressing formation of the Schiff base of asparagine. They concluded that the cations alter the reaction pathway towards the dehydration of glucose leading to HMF and furfural rather than Schiff base formation (Fig. 2). In addition to that, presence of salts blocks the nucleophilic properties of the amino group of the amino acids (Fig. 2). For example, Ca²⁺ stabilises the zwitterionic form of the amino acids by forming a complex, hence the amino group is protonated and is rendered incapable of nucleophilic attack on the carbonyl compounds (Bush, Oomens, Saykally, & Williams, 2008; Göncüoğlu Taş, Hamzalıoğlu, Kocadağlı, & Gökmên, 2016; Qin, Zhang, & Lu, 2013; Remko & Rode, 2006).

### 3.2. Effect of salts on pH drop and its relation to acrylamide formation

The initial pH of the control sample was 5.82 ± 0.04 and the addition of NaCl, KCl, CaLactate, NaLactate and MgLactate did not significantly change the initial pH (p > 0.05) (Table 1). Addition of CaCl₂ and MgCl₂ decreased the initial pH by 0.35 and 0.41 units, respectively (p < 0.05). It has been reported that by adding citric acid to a model cookie dough, in which approximately 70 % of sucrose was replaced by glucose, the dough pH decreased from 7.40 to 3.28 and acrylamide formation was mitigated by approximately 50 % (Gökmên et al., 2007). However, in our study, acrylamide reduction by up to 65 % (when CaCl₂ was added) and 74 % (when MgCl₂ was added) cannot be explained by the small drop (up to 0.41 units) of the initial pH.

![Fig. 1. Formation of acrylamide and HMF in glucose/wheat flour (control) and glucose/wheat flour/salt dough systems during heating at (a) 160 °C, (b) 180 °C and (c) 200 °C.](image-url)
Table 1
Changes in the pH of glucose/wheat flour (control) and glucose/wheat flour/mineral salt doughs during heating at 160, 180 and 200 °C. *

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>0</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>pH drop²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5.82 ± 0.04abcd</td>
<td>5.60 ± 0.02abc</td>
<td>5.21 ± 0.00ab</td>
<td>5.03 ± 0.01abc</td>
<td>5.03 ± 0.01abc</td>
<td>4.88 ± 0.01abcd</td>
<td>4.80 ± 0.04ab</td>
<td>4.67 ± 0.10abc</td>
<td>1.15abcd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>5.93 ± 0.08abcd</td>
<td>5.53 ± 0.01abcd</td>
<td>5.22 ± 0.00abcd</td>
<td>5.20 ± 0.02abcd</td>
<td>5.18 ± 0.04abcd</td>
<td>4.95 ± 0.06abcd</td>
<td>4.89 ± 0.03abcd</td>
<td>4.79 ± 0.02abcd</td>
<td>1.14abcd</td>
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<tr>
<td></td>
<td>KCl</td>
<td>5.96 ± 0.07abcd</td>
<td>5.68 ± 0.11abc</td>
<td>5.35 ± 0.01abcd</td>
<td>5.16 ± 0.05abc</td>
<td>5.18 ± 0.01abc</td>
<td>4.97 ± 0.03abcd</td>
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<td>0.95abcd</td>
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<tr>
<td></td>
<td>CaCl₂</td>
<td>5.47 ± 0.01abcd</td>
<td>5.67 ± 0.13abc</td>
<td>5.57 ± 0.05abc</td>
<td>5.22 ± 0.02abc</td>
<td>5.03 ± 0.01abc</td>
<td>5.04 ± 0.06abc</td>
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<td>4.75 ± 0.01abc</td>
<td>0.72abcd</td>
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<tr>
<td></td>
<td>CaLactate</td>
<td>5.68 ± 0.02abcd</td>
<td>5.71 ± 0.02abc</td>
<td>5.48 ± 0.01abc</td>
<td>5.41 ± 0.01abc</td>
<td>5.36 ± 0.01abc</td>
<td>5.23 ± 0.03abc</td>
<td>5.17 ± 0.00abc</td>
<td>5.15 ± 0.01abc</td>
<td>0.53abcd</td>
<td></td>
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<tr>
<td></td>
<td>NaLactate</td>
<td>5.71 ± 0.01abc</td>
<td>5.31 ± 0.00abc</td>
<td>4.95 ± 0.03abc</td>
<td>4.69 ± 0.01abc</td>
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<td>1.07abcde</td>
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<td>0.70abcd</td>
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<tr>
<td></td>
<td>MgLactate</td>
<td>5.61 ± 0.10abcd</td>
<td>5.05 ± 0.08abcd</td>
<td>5.04 ± 0.01abcd</td>
<td>5.03 ± 0.01abcd</td>
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<td>MgCl₂</td>
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</tr>
</tbody>
</table>

Table 1.

In general, the trends for pH with heating time between the control, NaCl, and KCl recipes were not different at the temperatures studied (Table 1). The most striking pH drop at the end of heating at 160 °C was 1.47 units on the MgCl₂ system, followed by NaLactate, control, NaCl, KCl, CaCl₂, MgLactate and CaLactate. Even though MgCl₂ and control had two of the highest pH drops, their acrylamide formation trends were very different: MgCl₂ containing samples had the lowest acrylamide formation trend, while control samples had the highest acrylamide formation rate. This shows that the trend of the pH during heating cannot be correlated with the rate of formation of acrylamide.

The incorporation of CaLactate and MgLactate in the recipe resulted in a notable buffering effect against pH drop during heating (Table 1). However, this was not observed in NaLactate, which has one lactate anion per molecule compared to two lactate anions per molecule for its Ca and Mg salts. Lactate in these salts is a weak conjugate base according to Bronsted-Lowry acid base theory. It may be a proton receptor upon dissociation in accordance with its pKa and thus act as a buffer where Maillard reaction produces acids during heating. The buffering effect of the lactate anions and the subsequent prevention of the high pH protonation of the amines diminishes the inhibitory effect of the cations on the Maillard reaction, which might explain the different behaviour between the systems containing CaCl₂ and CaLactate. Those differences were apparent in the formation trends of acrylamide. Acrylamide formation was halved in the presence of CaCl₂ at 200 °C whereas CaLactate was not effective in acrylamide reduction, following the same trend as the control (Fig. 1). The similar observations were apparent also in the trends of the flavour compounds, as discussed later in this paper. It seems that when CaLactate is added, the effect of Ca⁺² on stabilising the zwitterionic form of the amino acids is counterbalanced by the buffering effect of the lactate, especially at higher temperatures.
3.3. Effect of salts on 5-hydroxymethylfurfural formation

The addition of salts had the opposite effect on the formation of HMF compared to the formation of acrylamide. In contrast to acrylamide, the addition of salts in the dough formulation increased the rate of formation of HMF in the dough systems during heating at 160, 180 and 200 °C (Fig. 1a-c). Additionally, although the acrylamide reduction effect of salts was temperature-dependent and decreased at high temperatures (especially at 200 °C), the effect of mineral salts on HMF formation was more pronounced as the process temperature was increased. The trend of the generation of HMF followed the order CaCl₂ > NaCl > KCl > Calactate > control and that was independent of the heating temperature. HMF concentration in the CaCl₂ system reached a plateau after heating at 200 °C for 5 min, having a very high initial formation rate, and remained constant after that (Fig. 1c). Rapid depletion of glucose in the presence of CaCl₂ at 200 °C was the reason for this plateau (data not shown). The effect of salts as additives that increase HMF formation was also observed previously in bakery products. Van Der Fels-Klerx et al. (2014) reported an increase in HMF concentration in biscuits containing NaCl compared to biscuits without NaCl during baking at 180, 190 and 200 °C.

When the pH of the dough systems (with or without added salt) was considered (Table 1), it was concluded that although HMF was expected to form at comparatively lower pH, there was no correlation between pH drop and HMF formation. Kocadağlı and Gökmen (2016a) also reported that the increase in HMF in cookies was not related to pH in the presence of NaCl, KCl, and CaCl₂.

Under caramelisation conditions of glucose, NaCl was reported to increase the rate constants of glucose to fructose interconversion about 2.5-fold and the rate constants of HMF formation from fructose about 4-fold (Kocadağlı and Gökmen, 2016b). Zhao, Holladay, Brown, & Zhang (2007) showed that the interaction of metal halides with glucose catalyses mutarotation and isomerisation of glucose to fructose. Mayes, Nolte, Beckham, Shanks, & Broadbelt (2015) demonstrated the catalytic effect of Na⁺ on the conversion of glucose to fructose by applying computational methods. Additionally, under pyrrolic conditions, the rate constants of HMF formation from the dehydration of the intact fructofuranose ring was shown to increase in the presence of NaCl (Mayes et al., 2015). They also reported in another study that HMF formation from glucose through isomerization to fructose and dehydration of cyclic intermediates has lower energy barriers than other pathways (Mayes, Nolte, Beckham, Shanks, & Broadbelt, 2014). The importance of fructose dehydration through cyclic intermediates to form HMF was also elucidated quantitatively during the multisresponse kinetic modelling of the Maillard reaction and caramelisation in glucose/wheat flour systems (Kocadağlı and Gökmen, 2016c). Moreover, Gökmen & Şenyuva (2007b) reported that glucose decomposed more rapidly in the presence of cations during heating in an asparagine-glucose model system and the Maillard reaction shifted towards glucose dehydration when certain metal ions were present. Overall, it seems that in the presence of salts from strong acids and strong bases, the Maillard reaction is suppressed in favour of caramelisation, whereas HMF formation is favoured (Fig. 2). In the systems with Calactate, the buffering properties of lactate ions seems to minimise this effect at all temperatures (Fig. 1a-c).

3.4. Effect of salts on browning development

Browning in the dough systems increased continuously during heating at 160, 180 and 200 °C (Fig. 1a-c). In general, the absorbance of salt containing systems was higher than control at all temperatures after prolonged heating. Browning development in CaCl₂ and Calactate dough systems were higher compared to NaCl, KCl, and control dough systems at 180 and 200 °C. Lactate ions and their subsequent buffering effect (see previously) moderated the browning development promoted by Ca²⁺, since the CaCl₂ dough systems developed a darker colour that the Calactate ones, followed by NaCl, KCl, and control in decreasing order at 180 and 200 °C. Overall, dough systems containing divalent cation developed more browning. It was previously reported that Group I metal ions (Li, Na, K, Rb, and Cs) lead to a small increase in browning (A₂O₂) while Group II metals have greater effects in aqueous pH 7.2 buffered solutions of amino acids and pentose sugars (Rizzi, 2008). Kwak & Lim (2004) found accelerated browning in the presence of Fe²⁺ and Cu²⁺ in buffered model systems depending on the type of amino acids, heating time and type of metal ion. Interestingly, they also reported inhibition in the presence of a high concentration of NaCl (Kwak & Lim, 2004). Moreau, Bindzus, & Hill (2009) reported that the presence of NaCl in model systems containing pregelatinized starch, glucose, and an amino acid cocktail increased colour formation during heating. They also reported higher colour intensity with higher NaCl concentration. Kocadağlı and Gökmen (2016a) have previously reported that NaCl, KCl, and CaCl₂ increased browning in cookies due to the increased formation of furfurals from caramelisation. Addition of salts decreased browning in Maillard reaction model system of glucose-glycine-NaCl/
KCl/CaCl$_2$ however browning increased in similar systems in the absence of amino acids (i.e.) glycine (Kocadağlı & Gökmen, 2016a). In accordance with the previous model systems and cookies, browning increased in our crust model systems by addition of salts. Overall, addition of salt suppresses the Maillard reaction and enhances caramelisation, leading to an increase in browning.

3.5. Effect of salts on the formation of flavour compounds

The kinetic behaviour of some selected volatile compounds originating mainly from Maillard reaction and sugar degradation was monitored during heating of the dough systems at 160, 180 and 200 °C (see supplementary material). Twenty-six volatile compounds were quantified or semi-quantified in the heated systems. These were 5 Strecker aldehydes, 2 diketones, 9 pyrazines, 4 furans, 2 furfurals, 1 pyridine, 1 pyrrole, and 2 sulfur compounds. Selection was based on examples of compounds with different functional groups, different formation pathways and those that are most likely to be odor-active in biscuits based on literature and unpublished data.

Strecker aldehydes are one of the most important flavour compounds which are formed during the Maillard reaction either via Strecker degradation, i.e. the decarboxylation and deamination of their parent amino acids in the presence of Maillard-derived $\alpha$-dicarbonyl compounds or directly from a Maillard-derived Amadori product without the presence of $\alpha$-dicarbonyl compounds (Hofmann & Schieberle, 2000). 2-Methylpropanal (malty), 2- and 3-methylbutanal (malty), methional (cooked potato-like), and benzenacetaldehyde (honey-like) were the odor-active Strecker aldehydes in the dough systems that were
monitored. They are derived from valine, isoleucine, leucine, methionine, and phenylalanine, respectively. The concentrations of 2- and 3-methylbutanal were higher than any other Strecker aldehyde at 160 and 180 °C (see supplementary material). Similarly, the concentrations of 2- and 3-methylbutanal were higher in comparison to other Strecker aldehydes in a whole slice and crust of wheat bread (Raffo et al., 2018), and in extruded oat flour exposed to temperatures of 150 and 180 °C (Parker, Hassell, Mottram, & Guy, 2000). In general, the concentration of Strecker aldehydes increased when the temperature increased from 160 °C to 180 °C but decreased when the temperature was further increased to 200 °C. This behaviour is attributed to the higher degradation rate of the Strecker aldehydes compared to their formation rate at 200 °C.

The formation of 3-methylbutanal decreased in almost all conditions in the presence of salts (Fig. 4). NaCl decreased the formation of 2-methylpropanal, 2- and 3-methylbutanal by 31 %, 25 %, and 32 % in the samples heated at 160 °C for 11 min, respectively, and this effect was less pronounced at higher temperatures (see supplementary material). On the other hand, KCl was in general less effective on the suppression of those Strecker aldehydes than NaCl. Lactate salts (NaLactate, CaLactate, and MgLactate) suppressed Strecker aldehyde formation more than NaCl and KCl at 160 °C, with the exception of MgLactate on the formation of methional. Among all salts, the addition of CaCl₂ resulted in the generation of the lowest levels of 2-methylpropanal, 2-methylbutanal and 3-methylbutanal. CaCl₂ reduced these Strecker aldehydes 52 %, 36 %, and 32 % for 160, 180, and 200 °C at their final time points, respectively. On the contrary, the samples with CaCl₂ had the highest concentration of methional and benzencetaldehyde. Interestingly, there was a notable increase in the formation of benzenacetaldehyde in the presence of CaCl₂ after the initial heating at all temperatures. It is well established that the formation of Strecker aldehydes is connected with the Maillard reaction. Since the addition of salts in the dough recipes suppress the Maillard reaction, then the concentration of these aldehydes decreased. The rather odd behaviour of benzenacetaldehyde signifies that is not only formed from the Strecker degradation of phenylalanine but also via other non-Maillard pathways.

The concentration of diketones diacetyl (buttery) and 2,3-pentanedione (buttery) increased with heating temperature (see supplementary material). Monovalent cation salts, namely NaCl, KCl and NaLactate, had a small impact on the formation of diacetyl and 2,3-pentanedione in relation to the control (Fig. 4). Divalent cation salts, CaLactate, MgLactate, and CaCl₂ were found to be the most effective salts for increasing diacetyl and 2,3-pentanedione. Interestingly, CaLactate was found to promote the formation of these diketones more than CaCl₂ at all temperatures, which can be attributed to the higher pH observed in the former. CaLactate increased diacetyl and 2,3-pentanedione by up to 3 and 4-fold, respectively. Similar observations were made also for MgLactate. On the contrary, α-dicarbonyl compounds 3-deoxyglucosone, glucosone, glyoxal, methylglyoxal, and diacetyl did not considerably change in cookies (a sucrose rich system) which were formulated with CaCl₂ (Kocadağlı and Gökmen, 2016a). The increase in diacetyl in the divalent cation containing systems could be explained by the reactivity of glucose compared to sucrose which has no free carbonyl group unless it is hydrolysed. As described before, the presence of cations suppressed the Maillard reaction and as a consequence these diketones might have accumulated – due to the lower availability of reactants – in the glucose rich system (Fig. 2).

Furans and furfurals, including 2,4-, 2,5-, 2,6- and 2,3-dimethylpyrazines, were quantified in the dough systems at their final time points, respectively. The concentration of diketones diacetyl (buttery) and 2,3-pentanedione (buttery) increased with heating temperature (see supplementary material). Monovalent cation salts, namely NaCl, KCl and NaLactate, had a small impact on the formation of diacetyl and 2,3-pentanedione in relation to the control (Fig. 4). Divalent cation salts, CaLactate, MgLactate, and CaCl₂ were found to be the most effective salts for increasing diacetyl and 2,3-pentanedione. Interestingly, CaLactate was found to promote the formation of these diketones more than CaCl₂ at all temperatures, which can be attributed to the higher pH observed in the former. CaLactate increased diacetyl and 2,3-pentanedione by up to 3 and 4-fold, respectively. Similar observations were made also for MgLactate. On the contrary, α-dicarbonyl compounds 3-deoxyglucosone, glucosone, glyoxal, methylglyoxal, and diacetyl did not considerably change in cookies (a sucrose rich system) which were formulated with CaCl₂ (Kocadağlı and Gökmen, 2016a). The increase in diacetyl in the divalent cation containing systems could be explained by the reactivity of glucose compared to sucrose which has no free carbonyl group unless it is hydrolysed. As described before, the presence of cations suppressed the Maillard reaction and as a consequence these diketones might have accumulated – due to the lower availability of reactants – in the glucose rich system (Fig. 2).

Furans and furfurals had the highest concentration compared to other volatile compounds formed throughout heating. The furans that were quantified in the dough systems were 2-methylfuran, 2-acetyl furan, 2-bifuran and 2,2'-bifuran (see supplementary material). Either CaLactate or CaCl₂ was found to cause the greatest increase in the concentration of furans (Fig. 4). 2-Furanmethanol formation rate was drastically increased in the presence of CaLactate at all heating temperatures. CaCl₂, NaLactate, and MgLactate were also found to increase 2-furanmethanol concentration at least 1.5-fold compared to control at 160 °C after heating for 11 min. Furfural and 5-methyl-2-furfural were increased in the presence of CaCl₂ at all heating temperatures, similar to HMF. Those three compounds are affected by Ca²⁺ and
its role as a facilitator for the isomerisation of glucose to fructose (as discussed earlier) (Fig. 2). The effect of other mineral salts was less clear at 160 and 180 °C although their effect became obvious at 200 °C. They all increased furfural and 5-methyl-2-furfural in increasing order: Ca\textsuperscript{2+}, KCl, NaCl and CaCl\textsubscript{2} during heating at 200 °C. Furfural and 5-methyl-2-furfural trends confirm the role of lactate ions from CaLactate as moderators to the effects of the addition of Ca\textsuperscript{2+}.

Pyroles are generally generated from a reaction between a 3-deoxyketose and an amino compound followed by dehydration and subsequent ring closure (Dorotram, 1993). 2-Acetylpyrrole (caramel-like) was the only pyrrole found in dough systems. 2-Acetylpyridine (popcorn) was formed in all dough systems. At 160 °C, samples containing divalent ions (Ca\textsuperscript{2+} and Mg\textsuperscript{2+}) had higher formation rate than control, while samples containing monovalent ions had lower (Fig. 4). Interestingly, CaLactate had by far the highest formation rate of 2-acetylpyrrole and 2-acetylpyridine at 180 and 200 °C (see supplementary material). The MgLactate and NaLactate samples were not studied at these temperatures.

3.6. Principal component analysis

The principal component plot (PC vs PC2) shown in Fig. 5 explains 88 % of the variance in the data at 160 °C. PC1 represents heating time, showing all the sample cooked for 11 min situated at the positive end of the x-axis, and associated with the thermally generated volatile compounds, as well as the processing contaminants acrylamide and HMF. The uncooked (and less cooked) samples are situated at the negative end of PC1. Whereas the trajectories over time of the samples containing NaCl (N), KCl (K) were very similar to those of the control (CON), the trajectories for both CaCl\textsubscript{2} (CC) and CaLactate (CL) were quite different, indicating different volatile profiles in the cooked samples, and a likely change in relative contribution from competing formation pathways. PC2 separates CaCl\textsubscript{2} and CaLactate from the other three samples, and the distribution of the volatile along PC2 shows how the volatile profile changes. The control is associated with high levels acrylamide and Maillard reaction products (pyrazines and Strecker aldehydes), whereas the CaCl\textsubscript{2} sample is associated with high levels of HMF and other furans.

The CaLactate sample lies between the two and is associated with the two nitrogen heterocycles 2-acetylpyridine and 2-acetylpyrrole.

3.7. Mechanistic considerations

The changes in volatile profile and processing contaminants can be explained in terms of a shifting balance of the pathways shown in Fig. 2. In a system with a high glucose content, where the glucose concentration is in excess compared to the concentration of free asparagine, the dominant pathway for formation of acrylamide is via the specific amino acid route (Balagiannis et al 2019). When CaCl\textsubscript{2} is added, the Ca\textsuperscript{2+} binds with the free ASN, removing a limiting precursor from the system and resulting in a decrease in acrylamide formation. Ca\textsuperscript{2+} also promotes the glucose to fructose conversion, increasing the formation of furans, HMF and colour via caramelisation (Kocadağlı and Gökmen, 2016a; Kocadağlı and Gökmen, 2016b). Ca\textsuperscript{2+} mediated removal of both glucose and free amino acids from the system decreases the Maillard reaction (both low and high pH routes), resulting in a reduction in the formation of Maillard reaction products such as pyrazines and Strecker aldehydes, which are important for flavour. The addition of CaLactate raises the pH (compared to the other samples) which promotes the 1DG Maillard pathway over the 3DG pathway. This may be the explanation for an increase in dicarbonyls and pyrazines in this sample, and potentially a less drastic effect on flavour. However, CaLactate also moderates the reduction in acrylamide, which might be the result of an increased contribution from the generic amino acid pathway, boosted in the presence of more reactive dicarbonyls. It does not significantly affect the formation of Strecker aldehydes, which might be formed directly from the ARP.

The results indicated that salts showed a temperature-dependent reduction effect on acrylamide formation where there was less reduction at higher temperatures (especially at 200 °C). Additionally, this behaviour of acrylamide and HMF formation could not be explained by pH, except when comparing the effect of CaCl\textsubscript{2} with the effect of CaLactate: lactate ions, through their buffering properties, moderate the effect of Ca\textsuperscript{2+} on the studied reactions.

![Fig. 5. Observation (a) and variable (b) plots of principle component analysis at 160 °C. 2-Methylpropanal (2MP), 2-methylbutanal (2 MB), 3-methylbutanal (3 MB), methional (M), benzenacetaldehyde (B), diacetyl (DIA), 2,3-Pentanedione (23PD), pyrazine (P1), methylpyrazine (P2), 2,5-dimethylpyrazine (P3), 2,6-dimethylpyrazine (P4), ethylpyrazine (P5), 2,3-dimethylpyrazine (P6), 2-ethyl-6-methylpyrazine (P7), 2-ethyl-5-methylpyrazine (P8), 2-ethyl-3-methylpyrazine (P9), 2-methylfuran (2MF), 2-acetylfuran (AF), 2,2′-bifuran (BF), 2-furanmethanol (FM), furfural (FR), 5-methyl-2-furfural (SMFR), 5-hydroxymethylfurfural (HMF), 2-acetylpyridine (APYD), 2-acetylpyrrole (APYL), dimethyl dithiol (DMDS), dimethyl trisulfide (DMTS), acrylamide (ACR).]
4. Conclusion

Formation of acrylamide and HMF in bakery products are a concern for both consumers and industry, due to their negative health effects. Reduction or control strategies of these process contaminants in bakery products should also consider maintaining the quality characteristics of the product. Usage of salts, especially non-sodium ones, is a strategy for both sodium reduction and control of acrylamide in bakery products. However, we show that this approach increases the formation of HMF (another process contaminant) and has a significant impact on the volatile profile of the product and is likely to have an impact on the organoleptic quality of the product. Therefore, in the selection of salts for the control of process contaminants in bakery products, one should also take precautions by considering boosting relevant flavour compounds by maybe considering a combination of different mineral salts at different concentrations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References


