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Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies

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Abstract

The effects of dough formula and baking conditions on the formations of acrylamide and hydroxymethylfurfural (HMF) were studied in a cookie model system. Increasing the sugar concentration in the dough formula increased acrylamide formation during baking at 205 °C for 11 min. The effect of sugar on acrylamide formation was more pronounced for glucose than for sucrose, expectedly. Addition of citric acid into dough formula comprising sucrose increased the susceptibility of acrylamide formation, while it decreased acrylamide formation in the dough formula comprising glucose. Decreasing the pH of dough formula increased the tendency to surface browning and the formation of hydroxymethylfurfural in cookies during baking. The results suggest that a cookie with acceptable texture and colour, but having less than 150 ng/g of acrylamide, can be manufactured by lowering the baking temperature and avoiding reducing sugars in the recipe.

Keywords: Acrylamide; HMF; Cookie; Baking; Browning; Maillard reaction

1. Introduction

Acrylamide formation was found to occur during a thermal process, by Maillard reaction, of reducing sugars with asparagine at temperatures above 120 °C (Friedman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Yaylayan, Wnorowski, & Perez-Locas, 2003). The formation of key intermediates responsible for acrylamide formation is determined by the concentrations and types of sugars and amino acids present. These intermediates also react with other amino acids to form brown-coloured products and flavour compounds. Meanwhile, some chemical indicators, such as hydroxymethylfurfural (HMF), for assessing the quality of thermally processed foods, also form (Berg & van Boekel, 1994; Gökmen & Acar, 1999; Gökmen & Şenyuva, 2006a; Morales, Romero, & Jiménez-Pérez, 1997). Thus, the formation of acrylamide from asparagine is one of a number of competing processes. The yield of acrylamide is sensitive to both the composition of food and the conditions which are known to promote the Maillard reaction.

The major concern of food producers is to reduce the acrylamide content of foods (concerning the health impact), but to keep quality parameters unaffected by the adjusted processing conditions (concerning the economic impact). Some results clearly indicate that additions of acids, amino acids, or proteins seem to reduce acrylamide formation (Jung, Choi, & Ju, 2003; Kita, Bråthen, Knutsen, & Wicklund, 2005; Pedrechi, Moyano, Kaack, & Granby, 2005; Rydberg et al., 2003). However, it is unclear whether these pretreatments impart undesirable changes to the finished product, because the desired consequences of the Maillard reaction share intermediates with acrylamide formation.

The aim of this work is to understand the effects of dough formula (type and concentrations of sugars and pH) and the baking process (temperature and time) on acrylamide and HMF formations in cookies.

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2. Materials and methods

2.1. Materials

Acrylamide and HMF were purchased from Sigma (Diesenhofen, Germany). A solution of $^{13}C_3$-acrylamide (1 mg/ml) in methanol was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Sodium thiosulfate pentahydrate, hydrobromic acid, hydrochloric acid, potassium bromide (all AnalaR grade) and bromine (99.8%) were purchased from Merck (Darmstadt, Germany). Methanol, acetonitrile, glacial acetic acid, potassium hexacyanoferrate and zinc sulfate (all AnalaR grade) were purchased from Merck (Darmstadt, Germany). Ultra pure water was used throughout the experiments (MilliQ system, Millipore, Bedford, MA, USA).

Oasis HLB (1 ml, 30 mg) solid phase extraction cartridges and the Atlantis dC_{18} analytical column (4.6 × 300 mm 5 μm) were supplied by Waters (Milford, MA, USA). The capillary column (InnoWax, 30 m × 0.25 mm i.d., 0.15 μm film thickness) was purchased from Agilent Technologies (Palo Alto, CA, USA). Macro-spin PVDF centrifuge filters (0.45 μm) were purchased from Alltech (Deerfield, IL, USA).

Carrez I solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 ml of water, and Carrez II solution by dissolving 30 g of zinc sulfate in 100 ml of water.

2.2. Preparation of cookies

Flour and shortening were supplied by local producers, and other ingredients were purchased from local supermarkets. The model cookies were prepared according to a recipe described in AACC (American Association of Cereal Chemists) Method 10-54, with some modifications, in order to study the effects of type and concentrations of sugars, pH and baking conditions on acrylamide and HMF formation. Recipes are listed below.

Recipe 1: 80 g of wheat flour, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate, 17.6 ml of deionized water and sucrose (10, 15, 20, 25, 30 or 35 g), respectively.

Recipe 2: 80 g of wheat flour, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate, 17.6 ml of deionized water, 10 g of sucrose and glucose (0, 5, 10, 15, 20 or 25 g).

Recipe 3: 80 g of wheat flour, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate, 17.6 ml of deionized water, 35 g of sucrose and citric acid (3, 1 or 0 g, corresponding to dough pH of 3.28, 4.37 and 7.40, respectively).

Recipe 4: 80 g of wheat flour, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate, 17.6 ml of deionized water, 10 g of sucrose, 25 g of glucose and citric acid (3, 1 or 0 g, corresponding to dough pH of 3.28, 4.37 and 7.40, respectively).

Recipe 5: 80 g of wheat flour, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate, 17.6 ml of deionized water and 35 g of sucrose.

Recipe 6: 80 g of wheat flour, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate, 17.6 ml of deionized water, 10 g of sucrose and 25 g of glucose.

The ingredients were thoroughly mixed according to the recipe. Dough was rolled out to disks, after which trays of these disks were then baked in the oven. Four dough disks were produced from each recipe. Dough disks of Recipe 1–4 were baked at 205 °C for 11 min. Dough disks of Recipes 5 and 6 were baked at different temperatures and times (5, 10, 15 and 20 min at 160 and 180 °C; 5, 10 and 15 min at 200 and 210 °C and 5, 8 and 10 min at 230 °C).

2.3. Measurement of acrylamide

The cookies were ground, and 2 g were taken for analysis. Acrylamide was analyzed as the dibromo derivative by gas chromatography–mass spectrometry (GC–MS), using the method of Castle, Campos, and Gilbert (1991) with some modifications. The extracting medium was methanol (20 ml), rather than water, because addition of water to the ground cookies resulted in a thick slurry, rendering extraction difficult (Gökmen, Şenyuva, Acar, & Sarıoğlu, 2005). $^{13}C_3$-acrylamide (500 ng) was added to the extract as the internal standard, along with 15 ml of brominating reagent. The bromination was allowed to proceed overnight at room temperature.

The brominated extract (1 μl) was injected onto an Agilent 5973 GC–MS system (Agilent Technologies, Palo Alto, CA) in splitless mode at 200 °C. Helium carrier gas flow rate was maintained at 1 ml/min. An InnoWax capillary column was used. The oven temperature was 80 °C, rising at 10 °C/min to 250 °C and followed by holding for 10 min. The transfer line was held at 250 °C and the ion source at 180 °C. Electron impact mass spectra were obtained at 70 eV. The mass spectrometer was operated in selected ion monitoring mode. Four ions were used to characterize brominated $^{13}C_3$-acrylamide (m/z 108, 110, 153, and 155), and another four ions were used to characterize brominated acrylamide (m/z 106, 108, 150, and 152). The ion m/z 152 was used to quantify brominated acrylamide.

Stock solution of acrylamide, at a concentration of 1.0 mg/ml, was prepared in methanol. Working standards were prepared daily by diluting the stock solution to concentrations of 0.01, 0.02, 0.05, 0.10, 0.25, 0.50 and 1.0 μg/ml with methanol. They were brominated prior to GC–MS analysis using the procedure described above. The limit of detection and the limit of quantitation for acrylamide were 15 and 50 ng/g in cookies, respectively. Signal response was linear over a concentration range between 50 and 1000 ng/g of acrylamide. The coefficient of variation was 10% or lower for three repetitive measurements of acrylamide in cookies.

2.4. Measurement of HMF

The cookies were ground, and 1 g was taken for analysis. HMF was analyzed by high-performance liquid chromatography (HPLC), using the method of Gökmen and Şenyuva (2006a).

The extract was injected onto an Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a quaternary pump, an autosampler, a diode array detector and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC_{18} column, using the isocratic mixture of 0.1% aqueous acetic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 ml/min at 40 °C. Data acquisition was performed, acquiring chromatograms at the detection wavelength of 285 nm.

Stock solution of HMF was prepared at a concentration of 1.0 mg/ml in distilled water. Working standards were prepared daily by diluting the stock solution to concentrations of 0.05, 0.10, 0.25, 0.50 and 1.0 μg/ml with distilled water.

3. Results and discussion

3.1. Effects of dough formula

The major components of cookies are cereal flour, sugars and fats. The dough is conventionally baked at high temperature (~200 °C) for a few minutes (~10 min), in order to obtain a low final water content (<5%) and a brown surface. In this study, the effects of sugars on acrylamide and HMF formation in cookies were investigated. Cookie dough was prepared by varying the concentrations of sucrose (Recipe 1) or glucose (Recipe 2), but the amounts of wheat flour, shortening, non-fat dry milk, salt, leavening agent (mix of sodium and ammonium bicarbonate) and water were kept constant, as described in the AACC Method 10-54. It was noted, during the course of preliminary baking trials, that replacing sucrose with glucose in the recipe adversely affected the cookie structure. Therefore, a fixed amount of sucrose (10 g) was necessarily included in the Recipe 2 to improve the dough consistency and texture of cookies. Cookies were baked at 205 °C for 11 min, as described in the AACC Method 10-54.

As shown in Fig. 1, the type and concentration of sugars showed a strong influence on acrylamide formation in cookies. Sucrose was less efficient than glucose on the yield of acrylamide, expectedly. Increasing the amount of sucrose from 10 to 35 g in the recipe almost doubled the amount of acrylamide formed during baking. Replacing sucrose with glucose in the recipe resulted in a drastic increase in the amount of acrylamide formed in cookies during baking. Upon baking at 205 °C for 11 min, 74.1 ± 5.60 ng/g of acrylamide formed in cookies in which glucose was initially lacking in their recipe (Fig. 1). This was caused by the presence of a fixed amount of sucrose in the Recipe 2 due to the reasons mentioned above. It is

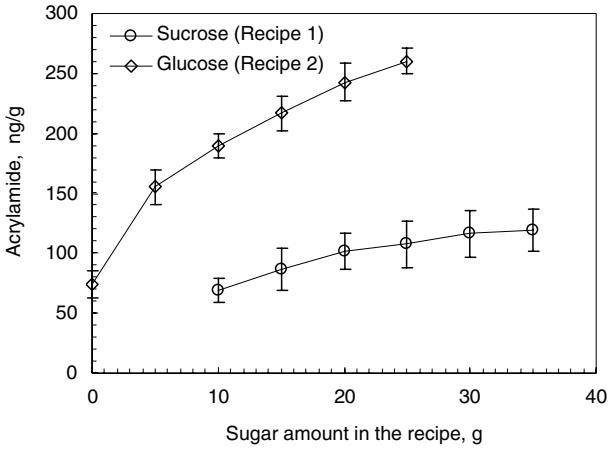

Fig. 1. Effects of the amounts of sucrose (Recipe 1) and glucose (Recipe 2) on acrylamide formation in cookies upon baking at 205 °C for 11 min.

thought that the degree of sucrose hydrolysis determines how much acrylamide is formed in cookies during baking where reducing sugars are initially lacking in dough. Since the amounts of acrylamide formed in cookies comprised of sucrose were significantly lower than those comprised of glucose, the hydrolysis of sucrose might be very limited during baking at 205 °C for 11 min.

It was concluded from these results that acrylamide levels can be reduced by a factor of 50% or more by using sucrose instead of reducing sugars (e.g. glucose). This confirms earlier findings that replacement of reducing sugars by sucrose is an effective way to significantly reduce the acrylamide content of sweet bakery (Amrein, Schönbächler, Escher, & Amado, 2004; Graf et al., 2006; Vass, Amrein, Schönbächler, Escher, & Amadò, 2004).

During baking, complex chemical reactions take place in cookies, leading to the formation of heat-generated toxicants such as acrylamide. Previous findings have confirmed that a reducing sugar is needed to form acrylamide from asparagine (Mottram et al., 2002; Stadler et al., 2002; Yaylayan et al., 2003). It has been shown that kinetics of acrylamide formation in a mixture composed of fructose and asparagine are of first order with respect to fructose and zeroth order with respect to asparagine (Gökmen & Şenyuva, 2006b). Sugars seem to be the most important ingredient in the dough formula from the viewpoint of acrylamide formation, because free asparagine level of wheat flour is relatively low. Noti et al. (2003) reported levels of 0.15–0.4 g/kg of asparagine in 10 samples of wheat flour. Surdyk, Rosén, Andersson, and Åman (2004) measured asparagine levels of 0.17 g/kg in white wheat flour.

HMF is not present in fresh, untreated foods, but it is formed as an intermediate product in the Maillard reaction upon heating at high temperatures (Berg & van Boekel, 1994; Morales et al., 1997). It is also formed from the degradation of sugars as a result of caramelisation (Kroh, 1994). Although the toxicological relevance of HMF is not clear, as *in vitro* studies on genotoxicity and mutagenicity have given controversial results (Cuzzoni, Stoppini,

Gazzani, & Mazza, 1988; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000; Lee, Shlyankevich, Jeong, Douglas, & Surh, 1995), its accumulation in cookies is considered undesirable during baking.

As shown in Fig. 2, there was a linear relationship between the amount of sugar in the recipe and the amount of HMF formed upon baking at 205 °C for 11 min. The effect of glucose on HMF formation was much greater than the effect of sucrose.

It is a fact that both acrylamide and brown-coloured products are formed during the Maillard reaction at high temperatures (Friedman, 2003; Gökmen & Şenyuva, 2006c; Mottram et al., 2002; Stadler et al., 2002; Şenyuva & Gökmen, 2005). The taste is not noticeably affected, but the cookies prepared with sucrose had a somewhat lighter colour due to a lack of reducing sugars, as reported earlier by others (Martins & van Boekel, 2003).

Organic acids, such as citric acid or tartaric acid, are often added to baking agents containing $NaHCO_3$ to enhance the leavening (Amrein et al., 2004). It has been reported by others that the addition of acids, by means of lowering pH, decreased the amount of acrylamide formed in foods during heating (Jung et al., 2003; Kita et al., 2005; Rydberg et al., 2003; Surdyk et al., 2004). The protonation of the α-amino group of asparagine hinders the formation of the *N*-substituted glycosylamine, which may explain the reduced acrylamide content.

In order to investigate the effect of pH of dough on acrylamide and HMF formations, cookie dough was prepared by adding varying amounts of citric acid in the recipes containing sucrose (Recipe 3) and glucose (Recipe 4), separately. The dough with varying amounts of citric acid was baked at 205 °C for 11 min. As shown in Fig. 3a, lowering the pH from 7.40 to 3.28, by adding citric acid to the dough comprising glucose, resulted in a 67% of reduction in acrylamide content of cookies when baked at 205 °C for 11 min. However, the addition of citric acid to dough formula comprising sucrose increased the susceptibility of acrylamide formation in cookies, most probably due to the excessive hydrolysis of sucrose, which increased the concentration of reactive sugars. Decreasing pH from 7.40 to 3.28 in the dough comprising sucrose caused a 1.8-fold increase in acrylamide content of cookies upon baking at 205 °C for 11 min.

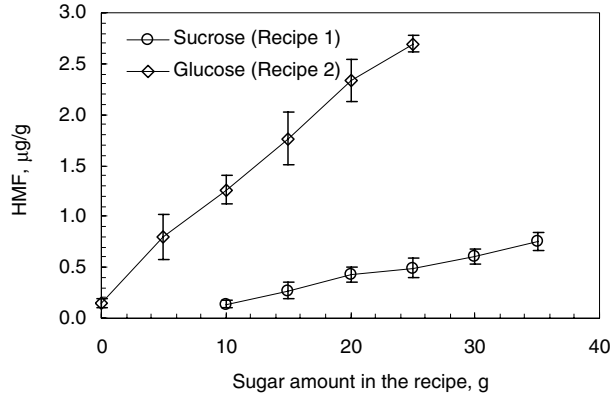

Fig. 2. Effects of the amounts of sucrose (Recipe 1) and glucose (Recipe 2) on HMF formation in cookies upon baking at 205 °C for 11 min.

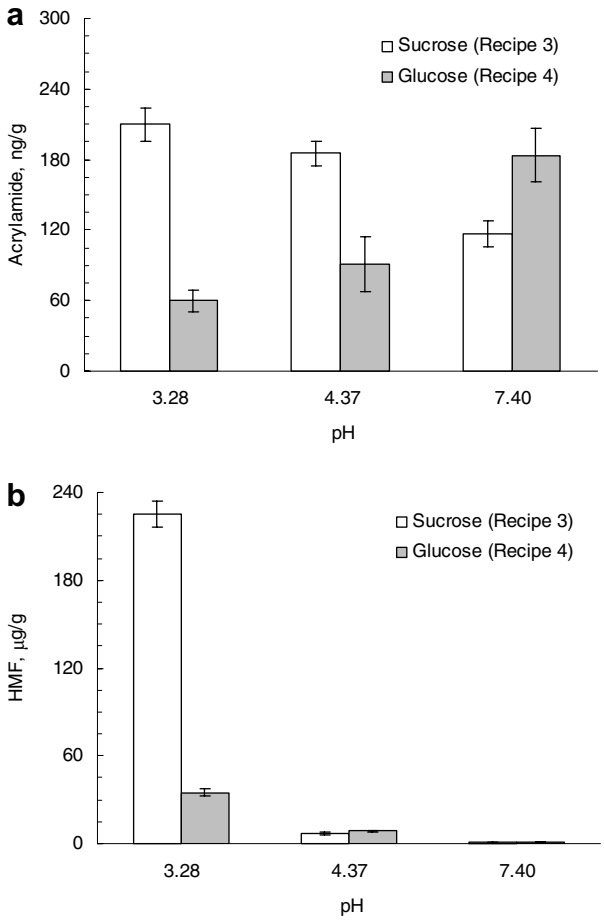

Fig. 3. Effect of initial dough pH on the formation of: (a) acrylamide, and (b) HMF in cookies comprised of sucrose (Recipe 3) and glucose (Recipe 4) upon baking at 205 °C for 11 min.

In general, lowering pH increased the tendency of HMF formation in cookies during baking. This effect was more pronounced for the dough comprising glucose, as shown in Fig. 3b. Addition of citric acid to dough formula also imparted unwanted flavour and colour to the finished product. These cookies were noticeably sour and the progress of browning over the surface was not homogeneous which limited their acceptability.

3.2. Effect of baking conditions

In order to investigate the effects of baking time and temperature on acrylamide and HMF formations, disks of cookie dough (Recipes 5 and 6) were baked at different temperatures and times. Progresses of texture and colour were decisive in defining the baking intervals at different temperatures. Bakings for less than 10 min at 160, 180, 200 and 210 °C, and 8 min at 230 °C were found to be insufficient in terms of cookie structure and surface colour. Excessive surface browning of cookies was prevented by

limiting the baking times to 25 min at 160 and 180 °C, to 20 min at 200 and 210 °C, and to 15 min at 230 °C.

Kinetics of acrylamide formation significantly differed for the recipes comprising sucrose (Recipe 5) and glucose (Recipe 6), as shown in Fig. 4. The dough prepared to study the effects of baking temperature and time was initially ($t = 0$) free of acrylamide. The amounts of acrylamide formed in cookies comprising sucrose were relatively lower after a baking time of 10 min. This initial period was characterized by rapid evaporation of moisture from cookies, but the measured water activities were still 0.4 or higher after 10 min of baking at all temperatures studied. Evaporative cooling, which prevented an excessive increase of cookie temperatures, most probably limited the hydrolysis of sucrose into glucose and fructose and thus the formation of acrylamide within this period. After the initial lower rate period, acrylamide concentration of cookies reached a plateau within a baking time of 15 min at 180 °C or higher temperatures. When sucrose was replaced with glucose in the dough, acrylamide concentration of cookies increased rapidly upon onset of baking, attaining the plateau values earlier. The plateau values were significantly higher in cookies comprising glucose than in those comprising sucrose.

Summa, Wenzl, Brohee, De La Calle, and Anklam (2006) recently demonstrated the effects of the type of sugars on acrylamide formation in cookies, using recipes prepared with sucrose and fructose. They observed a linear increase in the acrylamide concentration of cookies for the recipe in which fructose was applied. But, there was an initial lower rate period extended up to 15 min for the recipe in which sucrose was applied.

Kinetics of HMF formation were somewhat different from that of acrylamide formation for the baking conditions implemented in this study. For the recipes comprising both sucrose and glucose, an initial lower rate period was observed, which was followed by a rapid increase thereafter. This period extended from 8 to 15 min, depending on the baking temperature, but was not affected by the type of sugar, as shown in Fig. 5. One of the feasible pathways responsible for HMF formation is the dehydration of hexose sugars. Since the formation of one mole of HMF from one mole of hexose needs the release of three moles of water, the presence of too much water in the early stages of the baking process might have been inhibited the reaction by the product. The water activity is a fundamental parameter in HMF production (Kroh, 1994). It has been reported that HMF formation in cookies starts at an average water activity

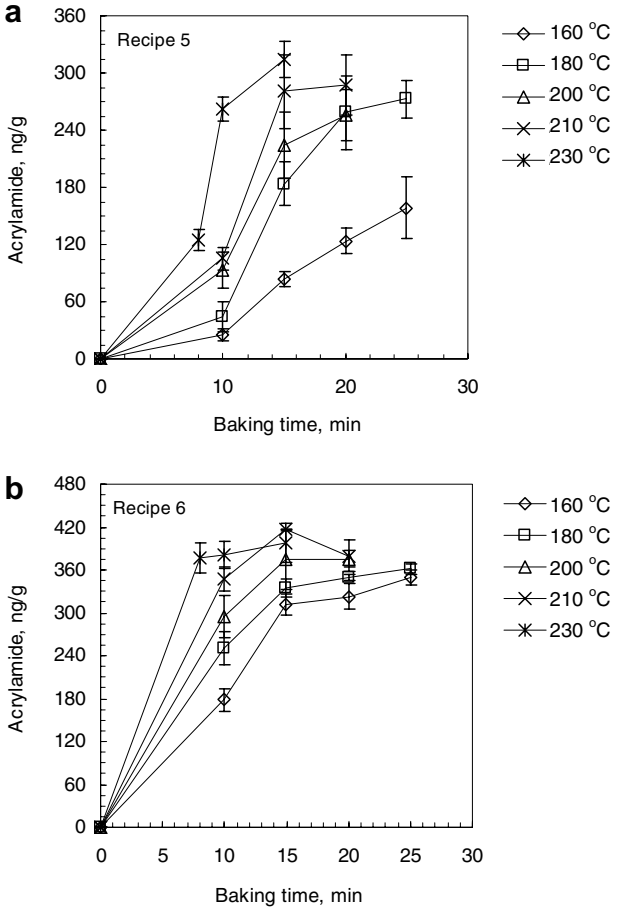

Fig. 4. Effects baking temperature and time on acrylamide formation in cookies comprised of: (a) sucrose (Recipe 5), and (b) glucose (Recipe 6).

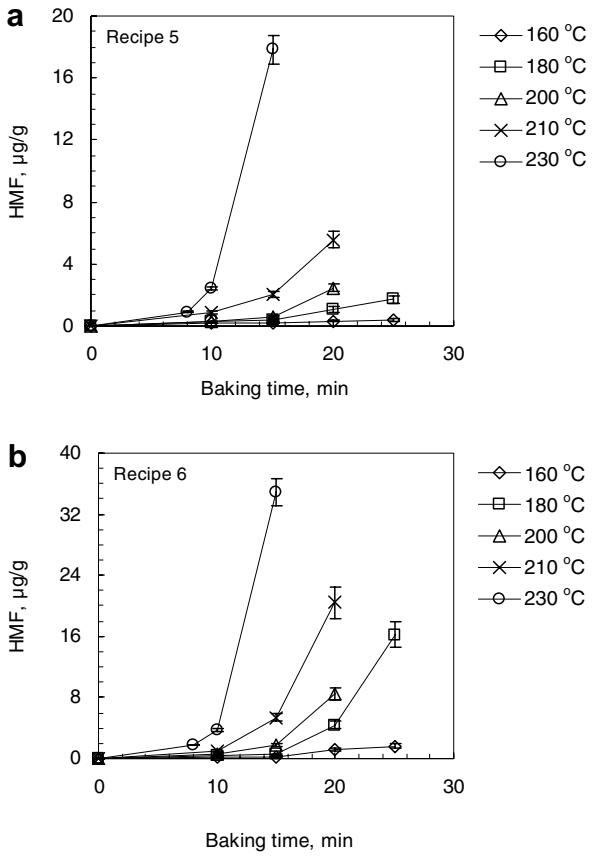

Fig. 5. Effects of baking temperature and time on HMF formation in cookies comprised of: (a) sucrose (Recipe 5), and (b) glucose (Recipe 6).

of 0.40, regardless of the temperature in the cookie (Ameur, Trystram, & Birlouez-Aragon, 2006).

4. Conclusion

Acrylamide formation in foods is a major concern, for both consumers and producers, due to its potential health impact. Any treatment to reduce its content in foods should also keep the quality parameters unaffected by the adjusted processing conditions (concerning the economic impact). Here, the effects of dough composition and baking conditions on the formations of acrylamide and HMF were studied in cookies in order to find a balanced approach for the mitigation of acrylamide. In this respect, the results suggest that replacing reducing sugars by sucrose is a feasible approach for reducing acrylamide content of cookies. Addition of acids brings some disadvantages by increasing the tendency to acrylamide and HMF formations through sucrose hydrolysis, and by adversely affecting sensorial quality; thus, it is not recommended as a viable mitigation strategy. On the other hand, it is possible to produce cookies with an acrylamide concentration of less than 150 ng/g by baking them at 160 °C for 25 min, which will convert the surface colour to fully brownish, but will also prevent any increase in excessive darkening and HMF concentration.

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