Impact of charged ionic species (NaCl and KCl) on the generation of color and volatile aroma compounds during caramelization

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Abstract

During the process of caramelization, volatile and non-volatile flavor and color are generated via the degradation of carbohydrates. This research investigated the effect of the types and concentrations of salts including NaCl and KCl on the generation of volatile aromas and color during caramelization. The solid phase microextraction-gas chromatograph-mass spectrometry (SPME-GC-MS) was used to measure the volatile compounds generated in caramelization. The results demonstrated that increasing content of salt (NaCl or KCl) could significantly improve ($P<0.05$) the generation of some essential volatile compounds during caramelization such as furfural, 5-methylfurfural, 5-hydroxymethyl-furfural (HMF), propionic acid and butyric acid. However, the ascending amount of salt (NaCl or KCl) had no significant impact ($P<0.05$) on the color generation of caramel. In conclusion, the usage of salt was beneficial to the generation of more aromatic compounds during caramelization.

Keywords: caramelization; color; flavor; salt; volatile compounds.

Practical Application: The mechanism found in the study could be used for instructing the food production such as candy, biscuit et al. These five aromas could be either positive or negative for customers. However, the actual odor or taste of a food product is a combined impact of all odorous and flavoring molecules and depends on the relative amount of the positives and the negatives. The producers have to consider all possible factors impacting the food flavor in the actual production procedure so as to meet the requirements of customers.

1 Introduction

Heating sugar and sugar-rich food is one of the oldest ways to generate color and flavor in food (Novotný et al., 2008; Barra & Mitchell, 2013; Li et al., 2021b). During heating reaction procedure, a series of non-enzymatic browning reactions called caramelization occurs and the characteristic caramel-like volatile compounds could be generated (Kroh, 1994; Shah et al., 2018; Zhang et al., 2013; Bressani et al., 2021). The reaction starts with heating of carbohydrates and contributes to the formation of both volatile and non-volatile products associated with food flavor and color (Cirlini et al., 2019; Li et al., 2021a; Lajolo et al., 2021). The properties of the final caramel depend on the initial types and concentrations of sugar as well as the preparation conditions such as heating temperature, duration, water content and pH (Brands & van Boekel, 2001).

The caramelization reaction is temperature-dependent (Benjakul et al., 2005). Different sugars have their own temperature point at which the reactions can start stably (Luna & Aguilera, 2014). For example, the caramelization temperature of fructose is 110 °C (230 °F), while for sucrose, it is 160 °C (320 °F) (Luna & Aguilera, 2014). In addition, the caramelization reactions are sensitive to the chemical environment (Seo et al., 2012; Uğur et al.,

2022). The reaction rate will alter with the changing of acidity (pH) level (Ajandouz et al., 2001). The rate of caramelization is normally the lowest when the pH is around 7 and increases under both acidic (especially pH < 3) and basic (especially pH > 9) conditions (Ajandouz et al., 2001; Chen et al., 2022).

Salt, as a flavor enhancer, has been widely used in many food products such as bread, biscuit, crisp etc. However, the functions of salt are not similar in different food products (Moreau et al., 2011). For example, sodium chloride is beneficial to the generation of color and degradation of starch during the heating process (Moreau et al., 2011). The presence of salt improves depolymerizing the glucose chains to form smaller molecules, such as glucose. Then the released glucose might caramelize and further enhance color formation (Moreau et al., 2011).

Besides the flavor enhancing function, salt could also contribute to the formation of aroma and color in food matrix. During the degradation of carbohydrates, it could promote the depolymerization of polymeric glucose, producing the precursors for further caramelization (Kamuf et al., 2003). However, the effect of salt on simple sugars like disaccharide has not been fully explained. Therefore, the aim of this study was to investigate

the effect of salt types and concentrations on the generation of volatile compounds and color during caramelization of sucrose.

2 Materials and methods

Sucrose was purchased from Sliver Spoon Ltd., Peterborough, UK; sodium chloride (NaCl), potassium chloride (KCl) and 5-Hydroxymethylfurfural (HMF) were purchased from Sigma Aldrich Ltd., Nottingham, UK.

2.1 Preparation of caramel

The staring mixture for caramelization was prepared according to the recipes listed in Table 1. The mixtures in each group were made in triplicate. All mixtures were then heated separately using a digital thermometer (-50-200 °C, E.T.I. Ltd.) to monitor temperature. Once the temperature arrived at 185 °C, the mixture was poured into containers (with labeling) immediately for cooling to room temperature.

2.2 Measurement of caramel color

A colorimeter (Color Quest XE, Hunter Lab Inc.) was used to quantify the color of caramel. The machine was warmed up for one hour and then calibrated according to the hints on the computer screen. After that, caramel solid (thickness 5 mm) wrapped using transparent preservative film was placed on the detection port to acquire data. The measurement was performed by transmission against a white background plate. Results were calculated by the equipment and scaled to the Hunter Lab color scale (0 (black) to 100 (white)).

2.3 Measurement of water activity

Freshly prepared mixtures (syrups) were subjected to water activity (Aw) measurement (the initial Aw). A Dew Point Water Activity meter (4TE, AQUALAB Inc.) was used to measure the Aw of these syrup samples. After heating, the caramel solids were poured into a sealed container to cool to room temperature. The caramel sample was then immediately grinded to powder in 30s by a mini chopper (CH180 mini chopper, 300 watt, Kenwood) placed at a room with controlled humidity at 40%. The powder was quickly transferred into a disposable cup (less than half volume of the cup) and used for measurement of final Aw by the same water activity meter.

2.4 Measurement of volatile compounds

Caramel samples were ground to powder using mini chopper (CH180 mini chopper, 300 watt, Kenwood). 3 g of each sample

Table 1. The recipe of caramel containing either NaCl or KCl.

Group	Sucrose (g)	Water (g)	Salt (g)	Calculated salt conc. (w/w)
1	70.00	20.00	0.00	0.00%
2	70.00	20.00	0.50	0.55%
3	70.00	20.00	1.00	1.10%
4	70.00	20.00	2.00	2.17%
5	70.00	20.00	3.00	3.23%

powder combining 2.5 µL of 1% heptanone as internal standard was placed into GC vials for analysis. A GC-ISQ (Thermo Electron Corporation Inc.) was used to detect the volatile compounds in full scan mode over the mass range 20-200 m/z (ion source 200 °C). The sample was incubated at 60 °C for 5 min with shaking using the autosampler. A 50/30 µm DVB/Carboxen/PDMS fiber was used for sampling the volatiles in the head space of GC vials for 4 min followed by a 1.5 min desorption (inlet temperature 200 °C). A splitless mode was used and the constant carrier pressure was at 103kPa. The GC oven was held at 40 °C for 2 min, and then increased from 40 °C to 210 °C at a rate of 4 °C/min, and held at 210 °C for 2 min. Separation was carried out on a ZB-WAX Capillary GC Column (Length 30 m, inner diameter 0.25 mm, and film thickness 0.25 µm; Phenomenex Inc., Macclesfield, UK). Thermo Xcalibur software was used to identify and quantify the volatile compounds. The identity of volatile compounds was determined by comparing with the library (NIST/EPA/NIH Mass Spectral Library. Version 2.0, Faircom Corporation, U.S.) in Xcalibur software and the retention time of standards. The peak areas of compounds and the internal standard were determined using Thermo Xcalibur software. The following Formula 1 was used to calculate the concentration of volatile compounds:

$$The\ concentration\ of\ volatile\ compounds\ (mg/kg) = \frac{\begin{array}{c}Peak\ area\ of\ volatile\ compound \times \\ Amount\ of\ internal\ standard\ (mg)\end{array}}{\begin{array}{c}Peak\ area\ of\ internal\ standard \times \\ Dry\ weight\ of\ caramel\ powder\ (kg)\end{array}} \quad (1)$$

2.5 Measurement of 5-Hydroxymethyl-furfural (HMF) in caramel solution by High Performance Liquid Chromatography (HPLC)

For demonstrating that the addition of salt improved the caramelization, but not the release of volatile compounds from the matrix, the 5-Hydroxymethyl-furfural (HMF) in caramel solution was measured by High Performance Liquid Chromatography (HPLC). The 5-Hydroxymethyl-furfural (HMF) of caramel groups with 0.00 g (0.00%, w/w) (Group 1) and 3.00 g (3.23%, w/w) (Group 5) of NaCl were compared. The caramel solids from Group 1 and 5 were dissolved into 100 g of water. After that, 3.00 g of NaCl was added into Group 1 solution. Chromatographic separation was performed on a ZORBAX Rx-C18 column (250 mm × 4.6 mm, 5 µm) with Agilent 1200 liquid chromatograph instrument equipped with UV-vis detector. The mobile phase consisted of (A) 0.3% formic acid in water and (B) methanol. The isocratic elution was as follows: 0-20 min, 10% B. The flow rate was set at 1.0 mL/min, the column temperature was maintained at 30 °C, and the detector wavelength was set at 280 nm. The identification of HMF was verified by comparing the relative retention time with authentic compound on the column. Reference standard (5-Hydroxymethylfurfural) was purchased from Sigma Aldrich Ltd. (Nottingham, UK).

2.6 Statistical analysis

The results were presented as mean value ± standard deviation. Each sample was analyzed in triplicate. One-way analysis of variance (ANOVA) was used to analyze the differences

between groups by SPSS version 21.0 (IBM, NY, USA). Letter a-e indicated a significant difference (p<0.05).

3 Results and discussion

3.1 Caramel color

A total of 10 groups, 30 samples was prepared and the browning effect was measured after heating. As shown in Figure 1, neither the type (NaCl and KCl) nor the amount of added salts can cause significant change in the color of caramel solids. The higher the heating temperature, the darker the caramel color (Quintas et al., 2010). In addition, the caramel color was darker in both acidic and alkaline conditions than it was in a neutral environment (Myers and Howell, 1992). In this research, sucrose, water and salt (NaCl or KCl) were used as ingredients. The reaction procedures were kept the same and initiated at neutral pH.

3.2 Water activity of caramel

The initial water activity of syrup (before heating)

The initial water activities of these samples with different salt contents were measured and the results were showed in Figure 2.

Regardless of the type of the added salts, with the rising of salt contents, the initial water activities of syrups were decreased significantly (P<0.05). The observed phenomenon was quite reasonable since it is a quite accepted concept that when salts were solvated in water, the cation attracts to the hydroxyl ion (from water) and the anion attracts to hydrogen ion (from water) by electrostatic attraction (Hou et al., 2013). Furthermore, water could be adsorbed on the surface of dissolved ions. Therefore, the more the salts were in the system, the less the free water would remain and hence the lower the measured Aw.

Final water activity of caramel (after heating)

After heating, water activity was measured again in the caramel solids. As shown in Figure 3, with increasing amount of added NaCl or KCl in the mixture, the final water activity of caramel solid raised significantly (P<0.05). As discussed above, with the increasing of salt contents, more water was combined with salt before heating (syrups). When the temperature of system arrived at 100 °C during heating, free water, which was not combined with salt, would be evaporated from system to air and form steam. Therefore, with the ascending of salt contents, more water was protected and locked by combining salt ions before heating, less free water existed in the systems and less
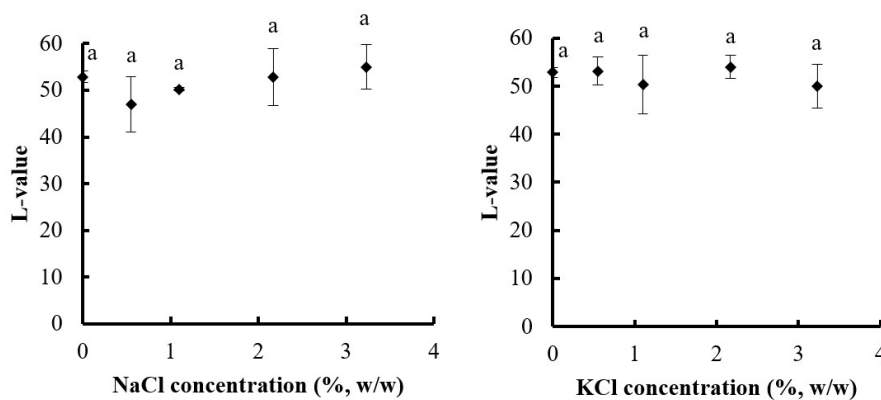

Figure 1. Impact of NaCl and KCl concentrations (%, w/w) on the caramel color (*L* value). [a] Samples with the same letter code in any groups are not significantly different (p < 0.05) Mean ± standard deviation.
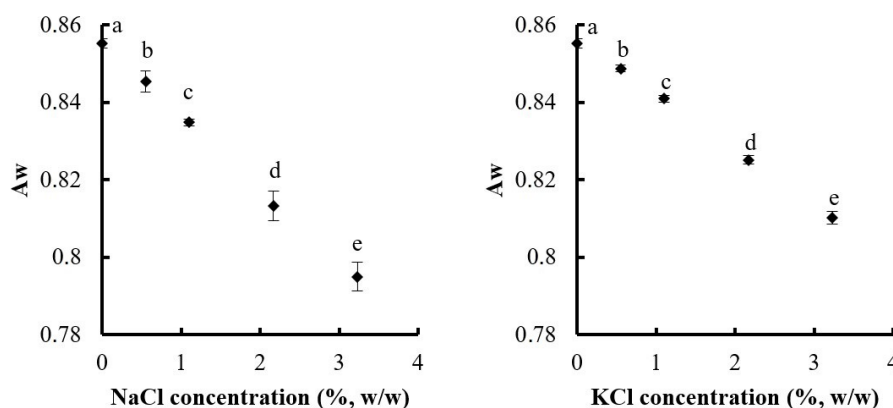

Figure 2. Impact of NaCl and KCl concentrations (%, w/w) on the initial water activity of syrup (before heating, syrup). [abcde] Samples with the same letter code in any groups are not significantly different (p < 0.05) Mean ± standard deviation.
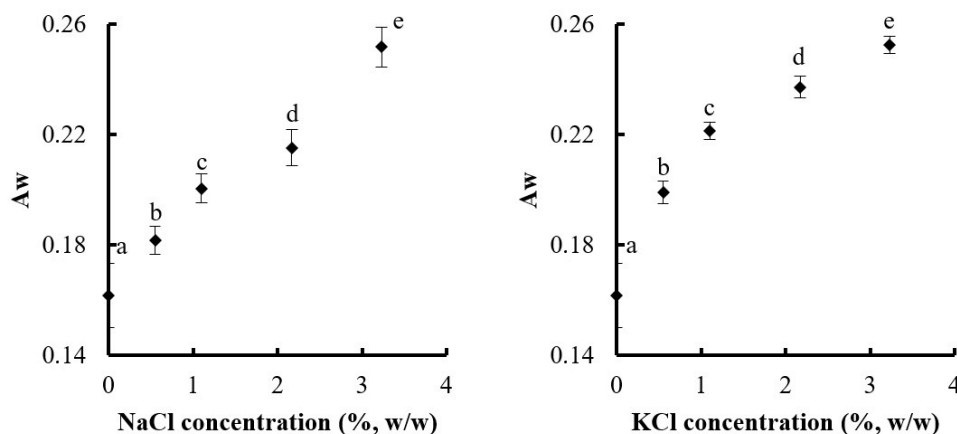
Figure 3. Impact of NaCl and KCl concentrations (%, w/w) on the final water activity of caramel (after heating, caramel solid). [abcde] Samples with the same letter code in any groups are not significantly different ($p < 0.05$) Mean ± standard deviation.

free water was evaporated at 100 °C or above. As a result, more water was kept in the caramel solids and the final water activities of these caramel solids were higher. Again, the trend seemed irrelevant to salt types. However, the possibility that salt type could impact the Aw of caramel solids cannot be excluded in consideration of the quite similar properties of NaCl and KCl.

3.3 Volatile compounds of caramel

Caramelization reaction included many chemical reactions and many volatile compounds were generated during caramelization (Paravisini et al., 2012). In this study, five main volatile caramel compounds were detected and their retention time, chemical names and formulas were showed in Table 2.

These five volatile aromas were found in every caramel sample prepare with NaCl or KCl. Furfural, 5-methylfurfural and 5-hydroxymethyl-furfural (HMF) were the derivatives of furan and related to the formation of caramel color and flavor (Pons et al., 1991). Propionic acid and butyric acid were two main organic acids in the caramel (Brands & van Boekel, 2001).

Impact of salt content on the amount of the derivatives of furan in the caramelization

The amounts of all three furan derivatives, furfural (Figures 4a, 4b), 5-methylfurfural (Figures 4c, 4d) and 5-hydroxymethyl-furfural (HMF, Figures 4e, 4f) ascended significantly with increasing content of salt (P<0.05). During caramelization of sucrose, the first step was the hydrolyzation of sucrose. In this stage, the sucrose molecule reacted with the hydrogen ion (H^+) from water and was hydrolyzed to glucose and fructose (Clarke et al., 1997). Produced glucose or fructose molecule could form 1,2-endiol by the enolization of sugar (Kroh, 1994). The 1,2-endiol could further generate the 3-dexoxyhexosulose by the β-elimination mechanism of water (Kroh, 1994). Finally, the 3-dexoxyhexosulose produced the HMF by the intramolecular cyclisation and further dehydration. Under the condition of high temperature, HMF could be decomposed to the 5-methylfurfural by the reduction reaction and the furfural

Table 2. The retention time, chemical name and formula and identification method of caramel volatile compounds.

Retention time (RT)	Chemical name	Chemical formula	Identification method
23.94	Furfural	$C_5H_4O_2$	NIST library and run standard
27.69	5methylfurfural	$C_6H_6O_2$	NIST library and run standard
26.81	Propionic acid	$C_3H_6O_2$	NIST library and run standard
29.64	Butyric acid	$C_4H_8O_2$	NIST library and run standard
42.82	5-Hydroxymethyl-furfural (HMF)	$C_6H_6O_3$	NIST library and run standard

by the fragmentation reaction, respectively (Myers and Howell, 1992). Figure 5 showed the chemical routes from the sucrose to HMF and further to the furfural and 5-methylfurfural.

According to the reaction mechanisms, the sucrose could not be used as the reactant to generate the intermediates such as 1,2-endiol. Only glucose and fructose (monosaccharide) could generate intermediates by enolization of sugar. In other words, for generating HMF, the sucrose had to be hydrolyzed firstly. However, in this step, water as a reactant could provide hydrogen ion (H^+), which meant that when heating temperature arrived at the caramelization temperature of sucrose, the more water the system contained, the more sucrose would be hydrolyzed. As a result, more glucose and fructose molecules would be generated and further generated more HMF.

As mentioned above, with increasing of salt contents, more water was combined with the salt and locked in the system. Therefore, when temperature arrived at 100 °C, more water was kept in the system. When the temperature increased sequentially to about 160 °C (caramelization temperature of sucrose), more water could be used as reactant to hydrolyze the sucrose and generate more glucose and fructose. Therefore, more furfural, 5-methylfurfural and HMF were generated.
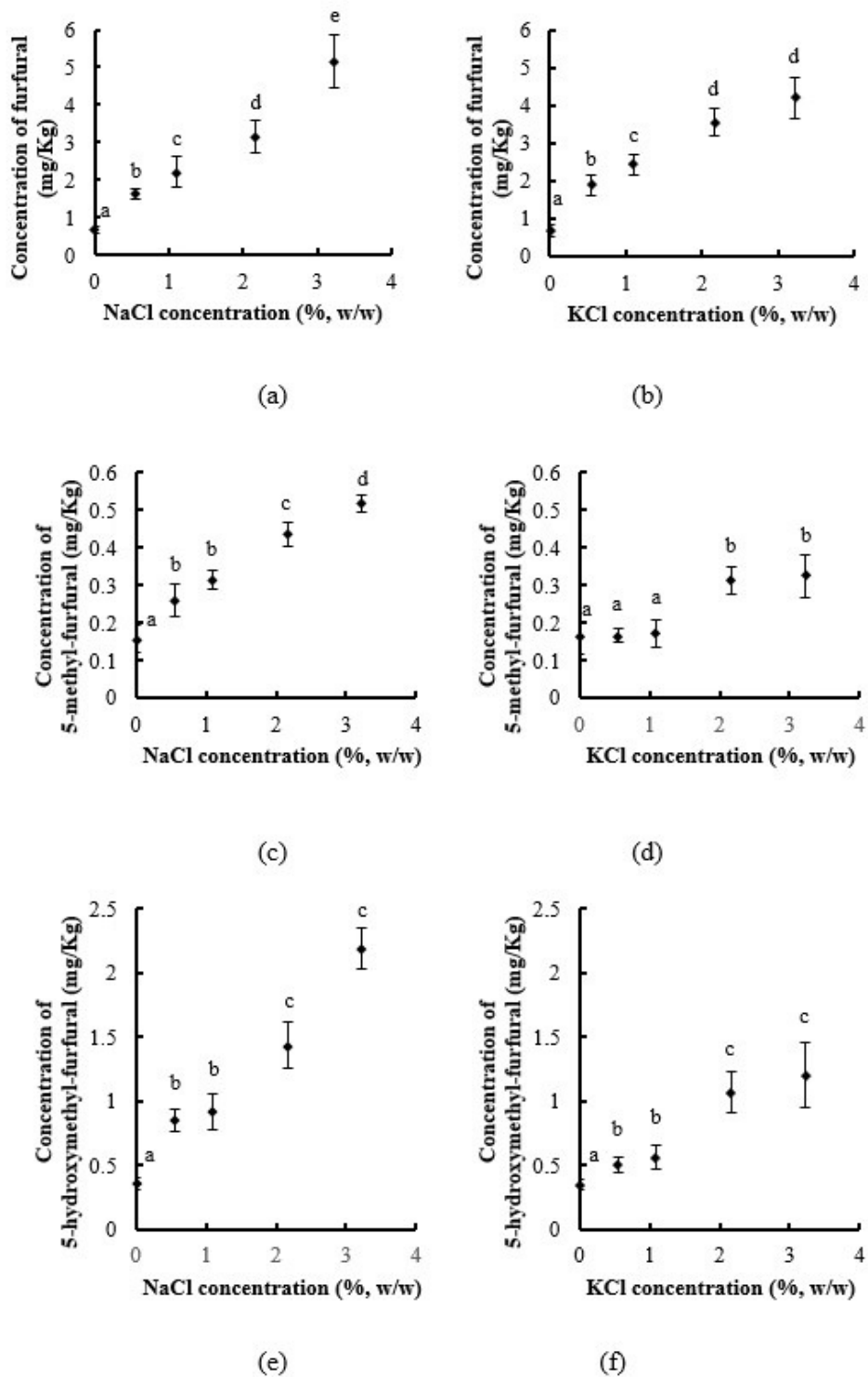
Figure 4. Impact of NaCl and KCl concentrations (%, w/w) on the generation of furfural, 5-methylfurfural and 5-hydroxymethyl-furfural (HMF) during caramelization. [abcde] Samples with the same letter code in any groups are not significantly different (p < 0.05) Mean ± standard deviation.

Impact of salt contents on the generation of propionic acid in the caramelization

Comparing with the samples which contained no salts, the amounts of propionic acid in all samples with salts (NaCl or KCl) increased significantly (P<0.05). However, there were

no significant differences (P<0.05) between the samples with different amounts of salt (NaCl or KCl) (Figure 6).

During caramelization of sucrose, the first step was the hydrolyzation of sucrose. In this stage, the sucrose molecule combined with the hydrogen ion (H+) from water and was
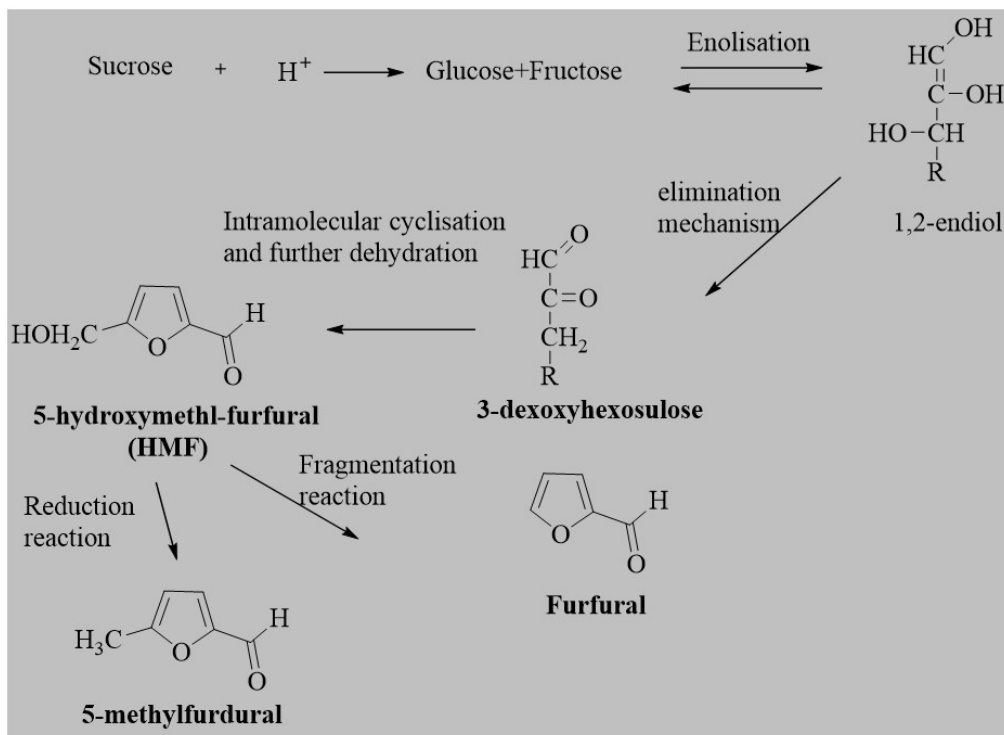
Figure 5. The schematic of reaction mechanisms from the sucrose to the furfural, 5-methylfurfural and 5-hydroxymethyl-furfural (HMF).
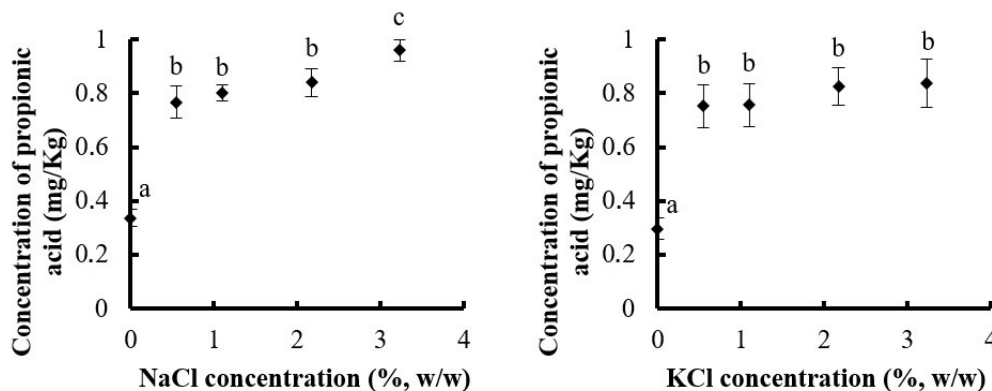

Figure 6. Impact of NaCl and KCl concentrations (%, w/w) on the generation of propionic acid during caramelization. [abc] Samples with the same letter code in any groups are not significantly different ($p < 0.05$) Mean ± standard deviation.

hydrolyzed to glucose and fructose (Clarke et al., 1997). After that, the glucose or fructose could generate 1-deoxy-D-erythro-hexo-2,3-diulose by enolization and the β-elimination mechanism of water (Kroh, 1994). The 1-deoxy-D-erythro-hexo-2,3-diulose could further be transformed to 1-deoxy-D-erythro-hexo-3,4-diulose (Davídek et al., 2006b). The 1-deoxy-D-erythro-hexo-3,4-diulose could be decomposed to D-glyceric acid and L-lactic acid by oxidative β-dicarbonyl cleavage (Davídek et al., 2006a). After that, the lactic acid might undergo dehydration followed by the acceptance of two hydrogen atoms (by reaction with some hydride donors such as glycolaldehyde), which led to the increasing of propionic acid. Theoretically, the propionic

acid might be also formed by the recombination of free radicals (Brands and van Boekel, 2001).

According to the proposed reaction mechanism (Figure 7), same reasoning could be applied to explain why the amount of propionic acid increased with the rising of salt content. For generating more propionic acid, the first step was the hydrolyzation of sucrose. And in this stage, water as a reactant played an essential role. The more water the system provided, the more sucrose was hydrolyzed after the temperature arrived at the caramelization temperature of sucrose (160 °C). Thus, more propionic acid could be obtained. With the increasing of salt contents, more water was combined with the salt and

locked in the system. When temperature arrived at 100 °C, less water was evaporated to air and more water was kept in the system. Therefore, the increasing of salt contents improved the production of propionic acid.

Impact of salt content on the generation of butyric acid in the caramelization

Comparing with the samples without salts, the amounts of butyric acid in all samples with salts (NaCl or KCl) increased significantly ($P<0.05$). However, there were no significant differences ($P<0.05$) between the samples with different amounts of salt (NaCl or KCl) (Figure 8).

The reaction mechanism for generating butyric acid was not clear. Some previous studies demonstrated that sugar could be degraded to the acids by α- and β-dicarbonyl splitting (Brands & van Boekel, 2001; Shah et al., 2018; Cirlini et al., 2019). Theoretically, the butyric acid might be also formed by recombination of free radicals (Rössner et al., 2001). However, for all possible reaction mechanisms, the first step was the hydrolysis of disaccharide and even polysaccharide. Only when disaccharide and polysaccharide
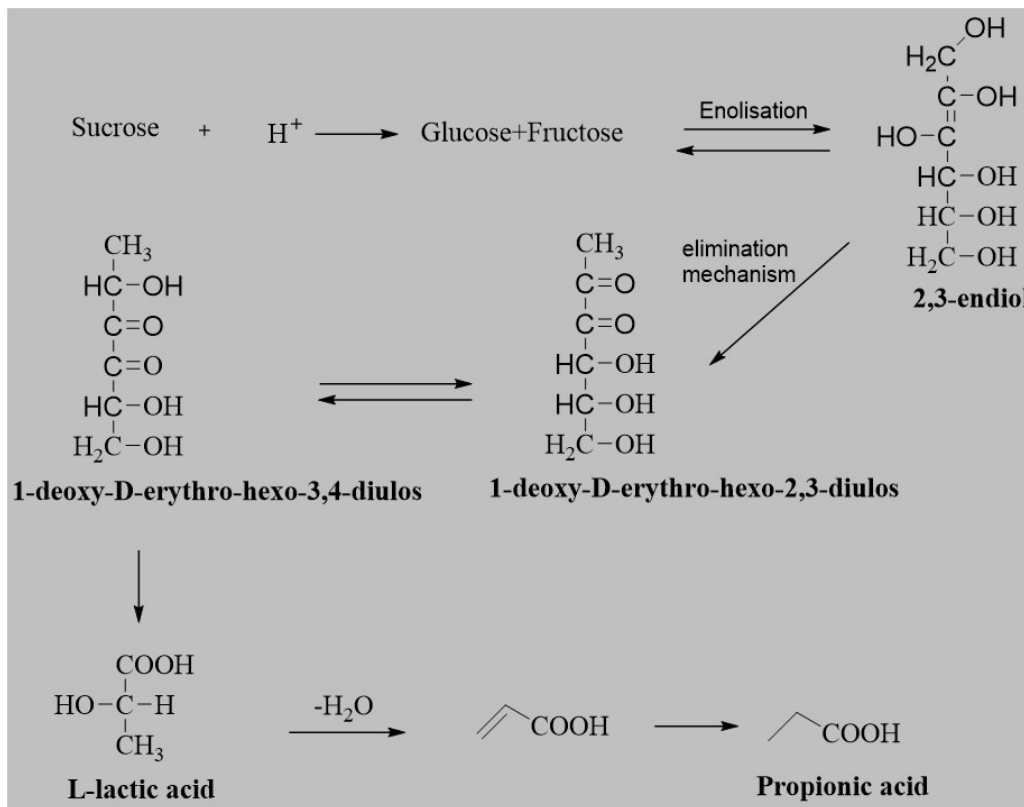

Figure 7. The schematic of reaction mechanisms from the sucrose to the propionic acid
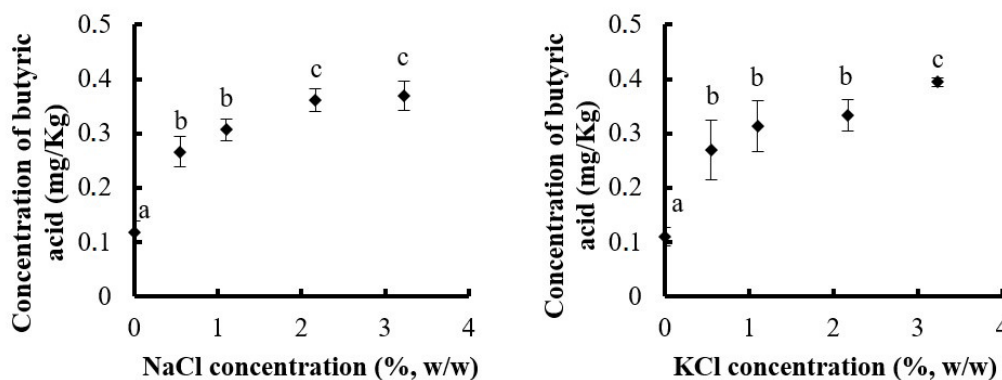

Figure 8. Impact of NaCl and KCl concentrations (%, w/w) on the generation of butyric acid during caramelization. [abc] Samples with the same letter code in any groups are not significantly different (p < 0.05) Mean ± standard deviation.

were decomposed to monosaccharides, the following reactions (enolisation, β-elimination and α-and β-dicarbonyl splitting) could continue to occur. Therefore, water as the reactant in the first reaction was very important and it had an essential influence on the properties of final caramel. Still, rise of salt contents in the starting syrup can result in the generation of more butyric acid from caramelization.

Our results suggested that the addition of salt increased the actual amounts of these volatile compounds during caramelization. However, there is a possibility that the increase of volatiles detected by GC-MS in salted caramels was mainly due to the improved release of these volatile compounds from the matrix. To exclude the possibility, we set out to compare the amount of 5-Hydroxymethyl-furfural (HMF) generated in Group 1 and Group 5. The caramel solids were dissolved in equal amount of water and salt was added in Group 1 solutions to assure these two groups contain the same amount of salt. HMF was then measured by HPLC.

As shown in Figure 9, the amount of HMF in the caramel solutions was significantly higher in Group 5 (10.32 ± 0.51 mg/mL, green peak) than in Group 1 (1.42 ± 0.11 mg/mL, red peak), which demonstrated that the increasing of NaCl content actually promoted the generation of HMF in caramelization, which contributed to the higher amount of volatiles in salted caramels detected by GC-MS. Besides HMF, differences in other peaks had been found in the caramel solutions between Group 1 and Group 5 by HPLC analysis, though they were not visualized in Figure 9.

A simple model was built in this research for investigating the effect of salt on the five volatile compounds generated in caramelization. The mechanism found in the study could be used for instructing the food production such as candy, biscuit et al.. These five aromas could be either positive or negative for customers. However, the actual odor or taste of a food product is a combined impact of all odorous and flavoring molecules and depends on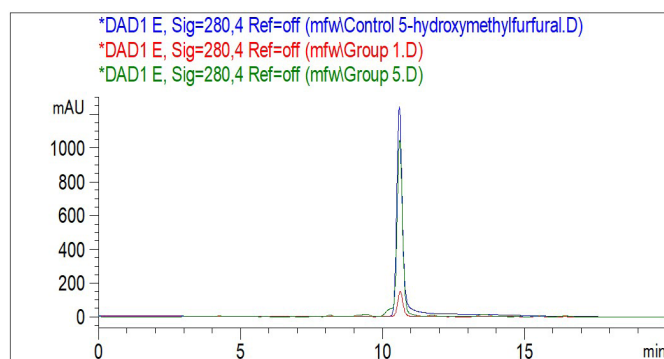 the relative amount of the positives and the negatives. The producers have to consider all possible factors impacting the food flavor in the actual production procedure so as to meet the requirements of customers.

4 Conclusion

In this study, we showed that addition of NaCl or KCl in syrup could lower the initial water activity of the syrup comparing with syrup without salt. While after heating to 185 °C, Aw in the resulted caramel solid was higher when salt was added although the color did not differ significantly. Besides, addition of salt also promoted the production of several aroma molecules in the caramel. We propose that it was the added salt in the sucrose syrup that attracted more water to the caramelization stage so that more water was available for hydrolyzation of sucrose to monosaccharides from which more aroma molecules could be generated.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

SX and IF conceived the experiments. SX, YL and FM drafted the manuscript. SX, XM and ZS conducted all the experiments. NY, HC and GT helped to discuss the results and perfected the language assisted with the structure elucidation and manuscript revision. IF designed and supervised the research and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figure 9. Liquid chromatogram of 5-Hydroxymethyl-furfural (HMF) generated in Group 1 and 5. Group 1 represented that 3.00 g of NaCl was added after dissolution. Group 5 represented that 3.00 g of NaCl was added before heating. The blue line represented the standard of HMF. The red line represented the Group 1. The green line represented the Group 5.

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