International Food Research Journal 29(4): 900 - 908 (August 2022)
Journal homepage: *http://www.ifrj.upm.edu.my*

Mitigation of acrylamide by cations in Chinese fried bread, *youtiao*

[1,2]Qiu, C. K. and [1]*Liu, X. Y.

[1]*College of Food Science and Technology, Huazhong Agricultural University, No.1, Shizishan Street, Hongshan District, Wuhan, 430070, Hubei Province, China*
[2]*College of Food and Biology Science and Technology, Wuhan Institute of Design and Sciences, No.1, Yangqiao Lake Avenue, Jiangxia District, Wuhan, 430205, Hubei Province, China*

Article history

Received:
16 January 2021
Received in revised form:
20 September 2021
Accepted:
20 December 2021

Keywords

youtiao,
acrylamide,
mitigation,
cation

Abstract

Youtiao, which is a deep-fried bread consisting of two twisted sticks, is commonly consumed as breakfast in China. The present work aimed to investigate the effect of the addition of KCl, $CaCl_2$, and $MgCl_2$ into flour on the acrylamide (AA) contents and *youtiao* quality. Results indicated that the AA contents in *youtiao* decreased by 10.0 ~ 71.0% depending on cation type and amount. The AA contents were negatively correlated with the amount of cations (r = -0.704, p = 0.0230). However, cation addition caused up to 36.8% of increase (maximum) in the content of 5-hydroxymethylfurfural (HMF). Cations had little effect on the moisture content, oil uptake, or texture properties of *youtiao*, but it rendered colour of *youtiao* less attractive (albeit still within the acceptable range). However, adding 20 mmol or more cations led to *youtiao* tasting bitter. Therefore, 20 mmol was suggested as the maximum addition amount for mitigating AA in *youtiao*.

DOI
https://doi.org/10.47836/ifrj.29.4.17

© All Rights Reserved

Introduction

Acrylamide (AA) has been added to the list of food-borne toxicants since Swedish National Food Administration detected AA in several heat-treated, carbohydrate-rich foods in 2002. It was classified as Group 2A carcinogen by the International Agency for Research on Cancer (IARC).

Youtiao is a traditional Chinese breakfast snack that has been consumed for thousands of years. It is a deep-fried twisted dough stick which is rich in carbohydrates, and processed at high temperature. Therefore, AA in *youtiao* has raised the concern among Chinese researchers. In China, the average consumption of *youtiao* is 3.24 g/day/person, and the average consumption of *youtiao* in Northern and Southern China is 5.28 and 1.45 g/day/person, respectively (CFSA, 2012). The mean dietary exposure to AA for the Chinese population is estimated to be 0.319 μg/kg bw/day (Gao *et al.*, 2016), which is relatively lower than that at the international level (1 μg/kg bw/day) as assessed by JECFA (2011), but higher than that in China's neighbour, Japan (0.15 μg/kg bw/day for men and 0.19 μg/kg bw/day for women) (Kito *et al.*, 2020). The latest three Chinese Total Diet Study (TDS) showed a notable upward trend in AA exposure (Gao *et al.*, 2016).

Although strategies to reduce AA in foods have been studied extensively, most existing studies have focused on potato products (Rifai and Saleh, 2020), baked products (Sarion *et al.*, 2021), and coffee (Schouten *et al.*, 2020). There are few studies about how to decrease the content of AA in *youtiao*. Since *youtiao* is a popular and inexpensive breakfast snack, and often purchased from street vendors or snack shops, it is not a large-scale industrial product, and most of the industrial strategies to reduce AA are not applicable for *youtiao*. It has been reported that cations can reduce the content of AA in model system (Gökmen and Şenyuva, 2007). Therefore, adding cations to flour to prepare *youtiao*-specific powder will be a potential method to reduce AA in *youtiao*.

Nevertheless, reducing AA level in foods by adding cations to formulations may have some negative effects. For example, replacing ammonium salt with sodium salt as leavening agent will bring adverse changes in sensory quality, taste, softness,

*Corresponding author.
Email: liuxiaoyu@mail.hzau.edu.cn

suppleness, and porousness, which is unacceptable to consumers (Ciesarova *et al.*, 2009). High doses of $NaHCO_3$ will give biscuits an alkaline taste, accompanied by an unpleasant taste known as soda bite (Manley, 2011). However, not all foods become worse following cation addition. $CaCl_2$ can simultaneously improve sensory and functional properties of breads including porosity, elasticity, and loaf volume (Ciesarova *et al.*, 2011). Following the addition of 1% $CaCO_3$, the overall acceptability of cookies significantly increased (Chang *et al.*, 2014). Therefore, the aim of the present work was to explore the mitigation effect of cation addition on AA in *youtiao*, and its impact on the quality of *youtiao*.

Materials and methods

Chemicals and reagents

$^{13}C_3$-acrylamide (99% purity), acrylamide (analytical grade, ≥ 99% purity), and 5-hydroxymethylfurfural (HMF) (analytical grade, ≥ 98% purity) were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile (HPLC-grade), bromine (analytical grade), hydrobromic acid (analytical grade), and potassium bromide (analytical grade) were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Materials

Baking powder (consisting of 35% disodium dihydrogen pyrophosphate, 30% sodium bicarbonate, 1% citric acid, 10% monocalcium phosphate, 5% calcium carbonate, 19% starch) and baking soda were from Angel Yeast Co., Ltd. (Yichang, China); wheat flour (Jinshahe Fuqiang high-gluten wheat flour) (Xingtai, China) and soybean oil were from China Oil and Food Import and Export Corporation (Beijing, China); and lastly salt (Wuhan, China). All these were purchased from a local supermarket (Zhongbai Holdings Group Co., Ltd., Wuhan, China).

Preparation of youtiao

Youtiao was prepared using a basic recipe; 100 g of wheat flour, 1.2 g of baking powder, 0.4 g of baking soda, 8 g of soybean oil, 1 g of salt, and 50 g of water (purified drinking water, C'estbon). All ingredients were kneaded for 8 - 10 min until an elastic, smooth, and soft dough was formed. Then, the dough was rested at 28°C for 4 h. After resting, it was rolled out and cut into 55 g/piece with thickness of 0.5 cm and breadth of 2 cm. Then, two pieces were placed together with one piece on the top of the other to make a pair. The stacked pairs were fried at 200°C for 120 s. In order to study the effects of cations on acrylamide in *youtiao*, KCl, $CaCl_2$, and $MgCl_2 \cdot 6H_2O$ of 5, 10, and 20 mmol were separately added to each 100 g of flour in the formula.

Determination of acrylamide

Acrylamide contents were determined according to China National Standard (GB) 5009.204-2014. The experimental steps were as follows:

Extraction

Homogenised samples (2 g) were put into a 50 mL centrifuge tube, and 0.25 mL of $^{13}C_3$-acrylamide (10 mg/L) was added as an internal standard. Then, the solution was vortexed for 5 min. Next, 10 mL of *n*-hexane was added into the centrifuge tube, homogenised (FJ-200, Shanghai Specimen and Model Factory, Shanghai, China) at 18,000 rpm for 2 min, shaken (THZ-82, Changzhou Guohua Electric Appliance Co., Ltd, Changzhou, China) at 220 rpm for 10 min for degreasing, and centrifuged (TDL-80-2B, Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 4,000 rpm for 5 min. Subsequently, the supernatant was discarded, and 10 mL of *n*-hexane was added and re-degreased. Afterwards, 10 mL of methanol as extractant was added to centrifuge tube, shaken for 30 min at 220 rpm, and centrifuged (Neofuge 18 R, Heal Force Bio-Meditech Holdings Limited, Shanghai, China) at 10,000 rpm for 10 min to obtain the supernatant as the sample extract.

Purification

The extract was purified by C_{18} SPE column (150 mg/3 mL; Welch Materials, Inc., Shanghai, China).

Derivatisation

Purified sample (2 mL) was pipetted into a 10 mL centrifuge tube, the methanol was blow dried with nitrogen, and 1 mL of deionised water and 1 mL of bromine reagent (20 g of potassium bromide, 1 mL of hydrobromic acid, and 16 mL of saturated bromine dissolved in 100 mL of deionised water) were added. The mixture was vortexed for 1 min, and the centrifuge tube was placed in 4°C refrigerator for 1 h. Subsequently, 100 μL of sodium thiosulphate was added to remove the excessive bromine reagent, and 2 mL of ethyl acetate was added, vortexed for 1 min, and then centrifuged at 5,000 rpm for 5 min. The supernatant was transferred to another centrifuge tube containing 0.1 g of anhydrous sodium sulphate, and the remaining solution was added with 2 mL of ethyl acetate for repeated extraction. The supernatant obtained from two centrifugation were combined and stood for 30 min at room temperature. The obtained solution was transferred to another clean centrifuge tube, blow-dried with nitrogen, added with 1 mL of ethyl acetate, and vortexed for 1 min. Finally, the solution was passed through 0.22 μm microporous filtration membrane for subsequent analysis.

Determination

Brominated acrylamide (1 μL) was injected into an TSQ 8000 EVO GC-MS system (Thermo Fisher Scientific, Waltham, USA) in splitless mode at 240°C. Helium carrier gas flow rate was maintained at 1.5 mL/min. Thermo Fisher HP-5 MS (0.25 μm × 0.25 mm × 30 m) capillary column was employed. The initial temperature was maintained at 65°C for 1 min, then raised at 15°C/min to 200°C, further raised at 40°C/min to 240°C, and maintained for 2 min. The ion source was EI, and its energy was 70 eV. The temperature was 250°C. The mass spectrometer was operated in selected ion monitoring mode. Four ions were used to monitor acrylamide (*m/z* 106, 133, 150, 152), and another four ions were used to monitor $^{13}C_3$-acrylamide (*m/z* 108, 136, 153, 155). The ion *m/z* 150 and 155 were used to quantify acrylamide and $^{13}C_3$-acrylamide, respectively. The concentration of acrylamide was quantified based on the constructed calibration curve (ranging from 10 to 1,000 μg/L), with the internal standard concentration of 50 μg/L.

Determination of HMF

The HMF was determined according to Nguyen *et al.* (2016) with some modifications. Homogenised samples (5 g) were put into a 50 mL centrifuge tube, and 20 mL of *n*-hexane was added for degreasing. Then, *n*-hexane was removed by centrifugation for 5 min at 4,000 rpm, and 20 mL of deionised water was added, incubated for 1 h at 50°C, and centrifuged again for 10 min at 12,000 rpm. The supernatant was passed through 0.22 μm microporous filtration membrane for subsequent analysis. Next, 10 μL sample was injected into an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, USA) equipped with an Ultraviolet (UV) detector and an Agilent ZORBAX Eclipse XDB-C_{18} (4.6 mm × 250 mm × 5 μm) column. The mobile phase consisted of 5% acetonitrile in water at a flow rate of 1 mL/min. The wavelength of the UV detector was 284 nm. The concentration of HMF was quantified by an external standard procedure using a calibration curve within the concentration range of 1 ~ 100 μg/mL.

Determination of moisture content

The moisture content was determined according to China National Standard (GB) 5009.3-2016, and the samples were dried at 65°C in a vacuum drying oven (DZF-6020, Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China) at 50 kPa.

Determination of oil uptake

The oil uptake was determined according to China National Standard (GB) 5009.6-2016, and the oil was extracted from the sample by a Soxhlet extraction device.

Determination of pH

The pH was determined by placing 10 g of dough into a 250 mL beaker which contained 90 mL of deionised water, and then, the beaker was placed onto a magnetic stirring apparatus (79-179-1, Changzhou Guohua Electric Appliance Co., Ltd, Changzhou, China). The dough in beaker was stirred at 1,000 rpm for 30 min, and rested for 5 min. The pH value of supernatant was then determined by a pH meter (FE 20, Mettler Toledo, Zurich, Switzerland).

Determination of colour

The colour was determined by using the CIE $L^*a^*b^*$ colour system using a colorimeter (UltraScan VIS, Hunter Associates Laboratory, Inc., Reston, USA). The colour difference (△E) was determined by comparing the results with the control samples, and calculated by using Eq. 1:

$$\triangle E = [(\triangle L^*)^2 + (\triangle a^*)^2 + (\triangle b^*)^2]^{1/2} \qquad \text{(Eq. 1)}$$

where, L^* = brightness or lightness (100 = perfect white, 0 = black); a^* = greenness/redness (negative (green) to positive (red)); b^* = yellowness/blueness (negative (blue) to positive (yellow)); $\triangle L^*$, $\triangle a^*$, and $\triangle b^*$ = absolute values of the differences between the control and samples.

Determination of texture

The texture parameters were determined by a texture analyser (TA. XT plus, Stable Micro Systems, Surrey, UK). Each *youtiao* sample was cut to pieces with a length of 5.0 cm by a knife. The texture profile analysis (TPA) was performed with P36/R probe with a pre-test speed of 2.0 mm/s, test speed of 0.5 mm/s, post-test speed of 2.0 mm/s, compression ratio of 50%, and trigger force of 5.0 g.

Statistical analysis

All experiments were carried out in triplicate or in quintuplicate. The data were tested for multiple comparisons by ANOVA. Least Significant Difference was used to determine the differences between means at 5% confidence level. The data were represented by mean ± SD.

Results and discussion

Effect of cations on acrylamide contents in youtiao

The contents of acrylamide in *youtiao* are shown in Table 1.

Table 1. Effect of types and amounts of cations on acrylamide levels of *youtiao* (n = 3).

Cation	Amount		
	5 mmol	10 mmol	20 mmol
Control		156.4 ± 6.3^{a}	
KCl	140.1 ± 3.0^{b}	140.7 ± 9.0^{b}	101.6 ± 4.0^{c}
$CaCl_2$	140.2 ± 7.1^{b}	97.9 ± 1.8^{c}	77.0 ± 0.5^{d}
$MgCl_2$	103.7 ± 0.6^{c}	53.7 ± 1.8^{e}	45.4 ± 0.4^{f}

Means followed by different lowercase superscripts are significantly different ($p < 0.05$).

The content of acrylamide in the control group was 156.4 μg/kg, while those in the treatment group were 45.4 - 140.7 μg/kg. The addition of cations significantly ($p < 0.05$) reduced the contents of acrylamide in *youtiao*. When 5, 10, and 20 mmol of potassium chloride were separately added to 100 g of flour, the acrylamide contents in *youtiao* were reduced by 10.4, 10.0, and 35.0%, respectively, when compared with the control group. When 5, 10, and 20 mmol of calcium chloride was separately added to 100 g of flour, the acrylamide contents in *youtiao* were reduced by 10.4, 37.4, and 50.8%, respectively, When 5, 10, and 20 mmol of magnesium chloride was separately added to 100 g of flour, the acrylamide contents in *youtiao* were reduced by 33.7, 65.6, and 71.0%, respectively. The inhibition effect of divalent cations on acrylamide was better than that of monovalent cations. The contents of acrylamide decreased with the increase in cations, and there was significant negative correlation (r = -0.704, p = 0.0230) between acrylamide content and cation addition.

The inhibition effect of cations on acrylamide in model system was quite obvious. The addition of equimolar amounts of monovalent, divalent, and trivalent cations into the equimolar mixture of asparagine and glucose led to 97% or more reduction in the amount of formed acrylamide during heating at 150°C for 20 min (Gökmen and Şenyuva, 2007). In some food matrix, cations also showed inhibition effect on acrylamide. For example, calcium salt could reduce the contents of acrylamide in deep-fried puffed shrimp chips (Chen *et al.*, 2016), and the addition of sodium chloride could reduce the acrylamide contents in cookies at baking temperature of 180 - 190°C (Van Der Fels-Klerx *et al.*, 2014). Acrylamide formation has been reported to be inhibited by 30.3, 53.3, and 89.3% when the sliced potato strips were soaked in 0.001, 0.01, and 0.1 M vanadyl sulphate solutions, respectively, for 60 min before frying (Kalita and Jayanty, 2013). The presence of cations could reduce acrylamide formation mainly due to the fact that the cations could effectively prevent the formation of Schiff base, the key intermediate for acrylamide generation (Gökmen and Şenyuva, 2007).

Effect of cations on HMF contents in youtiao

HMF is an excellent indicator which reflects quality deterioration when carbohydrate-containing food is subjected to excessive heating or storage for a period of time (Ameur *et al.*, 2006). The contents of HMF in *youtiao* are shown in Table 2.

Table 2. Effect of types and amounts of cations on HMF levels of *youtiao* (n = 3).

Cation	Amount		
	5 mmol	10 mmol	20 mmol
Control		1.90 ± 0.06^{d}	
KCl	1.95 ± 0.04^{d}	2.11 ± 0.09^{c}	2.18 ± 0.04^{b}
$CaCl_2$	2.14 ± 0.07^{c}	2.47 ± 0.09^{b}	2.56 ± 0.09^{a}
$MgCl_2$	2.23 ± 0.02^{c}	2.53 ± 0.07^{b}	2.60 ± 0.09^{a}

Means followed by different lowercase superscripts are significantly different ($p < 0.05$).

When compared with the control group (1.9 mg/kg), the contents of HMF in *youtiao* exhibited a maximum increase of 36.8%, reaching up to 2.60 mg/kg after adding cations. The contents of HMF in *youtiao* increased with the increase in added cations, and there was a significant positive correlation (r = 0.686, p = 0.0287) between them. In general, the HMF contents in *youtiao* displayed no significant (p > 0.05) difference at the same dose of added Ca^{2+} and Mg^{2+}, but it appeared to be significantly (p < 0.05) lower than that in *youtiao* added with K^+.

HMF is formed from fructose and glucose through direct dehydration (caramelisation) or Maillard reaction, and from sucrose through thermal decomposition at very high temperature. HMF formation could be facilitated by cations. This may explain why HMF contents in *youtiao* in the present work were higher than that in control group. In the presence of cations, some carbohydrates that were supposed to produce acrylamide were converted to HMF, thus resulting in the decrease in acrylamide contents and the increase in HMF contents as well as a significant negative correlation (r = -0.928, p = 0.0001) between them. Our results were consistent with one previous report that the amount of formed HMF during heating the mixture of asparagine and glucose at 150°C for 20 min significantly (p < 0.05) increased with the increase in Ca^{2+}, Mg^{2+}, and Fe^{3+} contents in the reaction mixture (Gökmen and Şenyuva, 2007). Sugars can be caramelised directly to produce HMF under acidic conditions (Hamzalıoğlu and Gökmen, 2018). In the presence of cations, fructose can be converted into a very active fructofuranosyl cation, which can increase the HMF production rate (Dong *et al.*, 2018).

The increase in HMF is an undesirable result since HMF has been reported to be hepato- and nephro-toxic in animals (Cueva *et al.*, 2017), but other studies have suggested that HMF poses no serious health risk (Severin *et al.*, 2010). Therefore, the toxicity of HMF to human remains to be further investigated. Fortunately, even after the addition of cations, the HMF contents in *youtiao* were not high relative to most foods (Shapla *et al.*, 2018).

Effect of cations on pH in dough

The pH levels of dough are shown in Table 3.

Table 3. Dough pH, and moisture content and oil uptake of *youtiao* (n = 3).

Type and amount of cation		**Dough pH**	***Youtiao* moisture content**	***Youtiao* oil uptake**
Control		6.39 ± 0.02[a]	24.4 ± 0.35[a]	13.7 ± 1.04[a]
KCl	5 mmol	6.29 ± 0.01[b]	25.5 ± 1.54[a]	13.9 ± 0.52[a]
	10 mmol	6.20 ± 0.01[d]	25.4 ± 0.62[a]	14.0 ± 0.29[a]
	20 mmol	6.07 ± 0.02[f]	25.6 ± 0.23[a]	13.9 ± 0.41[a]
$CaCl_2$	5 mmol	6.24 ± 0.01[c]	24.7 ± 0.75[a]	13.8 ± 0.43[a]
	10 mmol	6.13 ± 0.02[e]	24.3 ± 0.10[a]	13.5 ± 0.14[a]
	20 mmol	5.91 ± 0.01[g]	24.2 ± 0.16[a]	13.6 ± 0.54[a]
$MgCl_2$	5 mmol	6.21 ± 0.01[d]	23.8 ± 1.38[a]	13.8 ± 0.81[a]
	10 mmol	6.07 ± 0.02[f]	24.1 ± 0.10[a]	13.8 ± 0.19[a]
	20 mmol	5.92 ± 0.01[g]	23.5 ± 0.02[a]	13.9 ± 0.35[a]

Means followed by different lowercase superscripts in a column are significantly different (p < 0.05).

Results indicated that the dough pH levels significantly (p < 0.05) decreased after the addition of cations. The pH levels of dough added with cations decreased by 0.1 - 0.48, as compared to that in the control group (pH = 6.39). An extremely negative significant correlation (r = -0.925, p = 0.0001) was observed between pH levels and the amount of cations.

Huang *et al.* (2008) reported that adjusting the pH level of dough added with citric acid to 6.0 reduced the acrylamide content in *youtiao*. Mestdagh *et al.* (2008) reported that adding sodium acid pyrophosphate, citric acid, acetic acid, and L-lactic acid into a potato powder model system could significantly reduce the final acrylamide content. The internal pH level of a food influences acrylamide formation, and low pH level in the system inhibits the formation of the Schiff base by protonation of the amine group of amino acid (Dastmalchi *et al.*, 2016). The decrease in dough pH level may be partially responsible for the decrease in acrylamide in *youtiao* assessed in the present work (r = 0.878, p = 0.0008).

Furthermore, dough pH level may also be related to the increase in *youtiao* HMF (r = -0.895, p = 0.0005). The degradation of the Amadori product is dependent on the pH level of the system. When pH level is 7 or lower, the Amadori product undergoes mainly 1,2-enolisation to generate furfural (in the presence of pentoses) or HMF (in the presence of hexoses) (Martins *et al.*, 2001). As earlier described, there was a slight decrease (0.1 - 0.48) in dough pH level, thus the decrease in pH might be a minor reason for the acrylamide decrease in *youtiao*, but not a major one. The major reason lied in that the addition of cations suppressed the formation of acrylamide by inhibiting the Maillard reaction, meanwhile it promoted part of carbohydrates to produce HMF during frying, and these carbohydrates were supposed to generate acrylamide.

Effect of cations on moisture content and oil uptake of youtiao

The moisture content and oil uptake (Table 3) are also important quality indicators of *youtiao*. No statistically significant (p > 0.05) differences in moisture content and oil uptake were found between *youtiao* with and without cation addition. The moisture content (r = -0.064, p = 0.8612) and oil uptake (r = -0.044, p = 0.9044) of *youtiao* exhibited no correlation with the cation type or cation amount. In short, cations had no effect on the moisture content and oil uptake of *youtiao*.

Effect of cations on colour of youtiao

The CIE $L^*a^*b^*$ and △E values of *youtiao* are shown in Table 4.

Table 4. CIE $L^*a^*b^*$ and △E values of *youtiao* (n = 3).

Type and amount of cation		L^*	a^*	b^*	△E
Control		59.76 ± 0.80^{d}	3.02 ± 0.14^{a}	38.32 ± 0.94^{a}	-
KCl	5 mmol	60.16 ± 0.38^{d}	2.56 ± 0.19^{bcd}	37.37 ± 0.24^{b}	1.13
	10 mmol	58.72 ± 0.61^{e}	2.62 ± 0.17^{bc}	36.31 ± 0.72^{cde}	2.30
	20 mmol	61.48 ± 0.49^{c}	2.33 ± 0.30^{cd}	35.39 ± 0.52^{efg}	3.46
$CaCl_2$	5 mmol	61.69 ± 0.87^{c}	2.77 ± 0.14^{ab}	36.63 ± 0.40^{bcd}	2.58
	10 mmol	63.50 ± 0.44^{b}	2.25 ± 0.09^{d}	35.70 ± 0.32^{def}	4.63
	20 mmol	64.72 ± 0.58^{a}	1.86 ± 0.20^{e}	34.82 ± 0.48^{fg}	6.18
$MgCl_2$	5 mmol	65.25 ± 0.26^{a}	1.86 ± 0.10^{e}	36.99 ± 0.61^{bc}	5.77
	10 mmol	64.70 ± 0.31^{a}	1.70 ± 0.15^{ef}	35.48 ± 0.15^{efg}	5.85
	20 mmol	64.59 ± 0.50^{a}	1.46 ± 0.06^{f}	34.67 ± 0.34^{g}	6.25

Means followed by different lowercase superscripts in a column are significantly different (p < 0.05).

Since yellow was the characteristic colour of *youtiao*, we mainly analysed b^* values which reflect the yellow level. When compared with that of the control group, the b^* values of all the youtiao samples added with cations significantly decreased (p < 0.05). With the increase in added cations, b^* value decreased (r = -0.919, p = 0.0002) with the minimum decrease of 2.48%, and the maximum decrease of 9.52%, thus indicating that cations had an effect on the colour of *youtiao*, but not drastic. When the same amount of cations were added, no significant (p > 0.05) difference in the b^* values of *youtiao* was observed among different cation types.

Browning, texture, and flavour development caused by the Maillard reaction simultaneously occur during frying, accompanied by acrylamide formation. Therefore, the formation of acrylamide is related to colour development since both are related to the Maillard reaction (Vinci *et al.*, 2012). During conventional heating, measuring the degree of browning on the food surface is a good method to estimate the formation of acrylamide. Our data indicated an extremely significant positive correlation (r = 0.839, p = 0.0024) between b^* values and acrylamide contents in *youtiao*, which is in agreement with one previous report on the significant correlation between acrylamide levels and L^*, a^*, and b^* values or HMF levels (p < 0.05) for French fries and potato chips (Michalak *et al.*, 2019). The decrease in b^* values in *youtiao* can be explained by the possibility that cations might inhibit the Maillard reaction responsible for the attractive colour generation of *youtiao*.

△E represents the difference in colour between the treatment group and control group, which could reflect the effect of cations on the colour of *youtiao*.

The changes in L^* and b^* values of *youtiao* added with cations showed that the colour of *youtiao* became less attractive. The larger the △E, the less attractive the colour of *youtiao*. After the addition of cations, *youtiao*'s colour change trend (less attractive) was consistent with the acrylamide content change trend (decreasing). We also found that the negative effect of divalent cations on *youtiao*'s colour was greater than that of monovalent cations. There was an extremely negative significant correlation ($r = -0.886$, $p = 0.0015$) between △E and acrylamide contents.

Effect of cations on texture of youtiao

The four texture parameters representing *youtiao* texture properties are listed in Table 5.

Table 5. Texture parameters of *youtiao* ($n = 5$).

Type and amount of cation		Hardness	Adhesiveness	Chewiness	Cohesiveness
Control		4208.3 ± 79.0^{a}	0.13 ± 0.06^{a}	1279.3 ± 121.1^{a}	0.45 ± 0.04^{b}
KCl	5 mmol	3947.7 ± 145.3^{bc}	0.13 ± 0.12^{a}	1123.1 ± 335.3^{a}	0.45 ± 0.06^{b}
	10 mmol	3841.9 ± 52.0^{bcd}	0.10 ± 0.17^{a}	1090.9 ± 140.8^{a}	0.56 ± 0.04^{ab}
	20 mmol	3831.4 ± 71.8^{cd}	0.20 ± 0.30^{a}	1389.8 ± 517.6^{a}	0.47 ± 0.13^{ab}
$CaCl_2$	5 mmol	4002.1 ± 88.1^{bc}	0.07 ± 0.06^{a}	1120.8 ± 258.9^{a}	0.45 ± 0.06^{b}
	10 mmol	3905.8 ± 171.0^{bcd}	0.17 ± 0.06^{a}	1284.9 ± 322.2^{a}	0.49 ± 0.02^{ab}
	20 mmol	3737.5 ± 46.4^{d}	0.07 ± 0.06^{a}	1271.2 ± 321.7^{a}	0.57 ± 0.05^{a}
$MgCl_2$	5 mmol	4035.5 ± 88.1^{b}	0.13 ± 0.12^{a}	1265.2 ± 114.0^{a}	0.52 ± 0.03^{ab}
	10 mmol	3998.5 ± 86.5^{bc}	0.10 ± 0.00^{a}	963.1 ± 144.0^{a}	0.49 ± 0.07^{ab}
	20 mmol	3886.0 ± 114.2^{bcd}	0.03 ± 0.06^{a}	869.0 ± 63.4^{a}	0.58 ± 0.01^{a}

Means followed by different lowercase superscripts in a column are significantly different ($p < 0.05$).

No statistically significant ($p > 0.05$) differences in the adhesiveness and chewiness were observed between *youtiao*, with and without additional cations. The cohesiveness of *youtiao* added with cations was slightly higher than that in the control group, but most data were not statistically significant ($p > 0.05$). The hardness of *youtiao* was more affected by cations than the other three parameters (cohesiveness, chewiness, and adhesiveness). When compared with that in the control group, the hardness of *youtiao* in the treatment group significantly decreased ($p < 0.05$), with the minimum decrease of 4.11%, and the maximum decrease of 11.2%. An extremely significant negative correlation was observed between cations addition and *youtiao* hardness ($r = -0.851$, $p = 0.0018$). Generally speaking, when cations were added, the hardness of *youtiao* slightly decreased, but the other texture properties were barely affected. The possible reason for this might be that the decreased dough pH level hindered the development of the dough protein network. Our speculation is supported by Amiri *et al.* (2019)'s report that pH augmentation promotes the aggregation of gluten, and increases gluten yield. Underdeveloped gluten network makes it difficult for the gas to enter the dough, thus resulting in the insufficient dough expansion during the frying process, and the decreased hardness of *youtiao*.

Due to only slight changes in colour and texture parameters in our samples, we did not conduct sensory evaluation experiment, but simply invited participants to taste the *youtiao*. The change in hardness of *youtiao* was undetected by the tasters, but when 20 mmol of the cation was added, *youtiao* was found to become bitter, thus indicating that 20 mmol might be the maximum cation addition amount.

Conclusion

Our experiment results indicated that cations could reduce the acrylamide contents in *youtiao* by up to 71.0% (maximum). Meanwhile, the HMF contents in *youtiao* added with cations was significantly ($p < 0.05$) higher than the control group. Cations had little effect on moisture content, oil uptake, and the texture properties of *youtiao*. Although the colour of *youtiao* became less attractive, it was still acceptable. It should be noted that adding too much cations (more than 20 mmol) could cause a bitter taste in *youtiao*. The addition of cations into flour is a very simple and effective method to reduce the acrylamide in *youtiao* because this method changes neither the recipe nor the processing technology of *youtiao*. In addition, it also enriches the contents of potassium, calcium, and magnesium in *youtiao*.

Acknowledgement

The authors would like to thank Hubei Provincial Outstanding Young and Middle-Aged Science and Technology Innovation Team Project (grant no.: T201635) for its financial support. We also acknowledge Huazhong Agricultural University and Wuhan Institute of Design and Sciences for their support.

References

Ameur, L. A., Trystram, G. and Birlouez-Aragon, I. 2006. Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method. Food Chemistry 98(4): 790-796.

Amiri, A., Farshi-Marandi, P. and Shahedi, M. 2019. Impact of sodium citrate on structural properties of gluten. Journal of Food Science and Technology 56(2): 1090-1093.

Chang, B. K. L., Wang, J. S. and Sung, W. C. 2014. Calcium salts reduce acrylamide formation and improve qualities of cookies. Journal of Food and Nutrition Research 2(11): 857-866.

Chen, T. Y., Luo, H. M., Hsu, P. H. and Sung, W. C. 2016. Effects of calcium supplements on the quality and acrylamide content of puffed shrimp chips. Journal of Food and Drug Analysis 24(1): 164-172.

China National Center for Food Safety Risk Assessment (CFSA). 2012. Risk assessment of dietary exposure to aluminium in Chinese population. In Technical Report of China National Expert Committee on Food Safety Risk Assessment, No. 2011-002. China: CFSA.

Ciesarova, Z., Kukurova, K. and Markova, L. 2011. Success and limitations in acrylamide mitigation efforts. Part 2: Impact of interventions in cereal food processing on exposure. Agro Food Industry Hi-Tech 22(4): 25-27.

Ciesarova, Z., Kukurova, K., Bednarikova, A., Markova, L. and Baxa, S. 2009. Influence of food processing on acrylamide level in gingerbreads and cookies. Aspects of Applied Biology 97(1): 87-92.

Cueva, S. P., Álvarez J., Végvári, A., Montilla-Gómez, J., Cruz-López, O., Delgado-Andrade, C. and Rufián-Henares, J. A. 2017. Relationship between HMF intake and SMF formation *in vivo*: An animal and human study. Molecular Nutrition Food Research 61(3): article ID 1600773.

Dastmalchi, F., Razavi, S. H., Faraji, M. and Labbafi, M. 2016. Effect of *Lactobacillus casei-casei* and *Lactobacillus reuteri* on acrylamide formation in flat bread and bread roll. Journal of Food Science and Technology (53): 1531-1539.

Dong, K., Zhang, J., Luo, W., Su, L. and Huang, Z. 2018. Catalytic conversion of carbohydrates into 5-hydroxymethyl furfural over sulfonated hyper-cross-linked polymer in DMSO. Chemical Engineering Journal (334): 1055-1064.

Gao, J., Zhao, Y. F., Zhu, F., Ma, Y. J., Li, X. W., Miao, H. and Wu, Y. N. 2016. Dietary exposure of acrylamide from the fifth Chinese Total Diet Study. Food and Chemical Toxicology (87): 97-102.

Gökmen, V. and Şenyuva, H. Z. 2007. Effects of some cations on the formation of acrylamide and furfurals in glucose-asparagine model system. European Food Research and Technology (225): 815-820.

Hamzalıoğlu, A. and Gökmen, V. 2018. Investigation and kinetic evaluation of the reactions of hydroxymethylfurfural with amino and thiol groups of amino acids. Food Chemistry (240): 354-360.

Huang, W. N., Yu, S. D., Zou, Q. B. and Tilley, M. 2008. Effects of frying conditions and yeast fermentation on the acrylamide content in you-tiao, a traditional Chinese, fried, twisted dough-roll. Food Research International 41(9): 918-923.

Kalita, D. and Jayanty, S. S. 2013. Reduction of acrylamide formation by vanadium salt in potato French fries and chips. Food Chemistry 138(1): 644-649.

Kito, K., Ishihara, J., Yamamoto, J., Hosoda, T., Kotemori, A., Takachi, R., … and Tsugane, S. 2020. Variations in the estimated intake of acrylamide from food in the Japanese population. Nutrition Journal 19(1): 1-9.

Manley, D. 2011. Additives as biscuit ingredients. In Manley, D. (ed). Manley's Technology of

Biscuits, Crackers and Cookies, p. 223-234. Cambridge, United Kingdom: Woodhead Publishing.

Martins, S. I. F. S., Jongen, W. M. F. and Van Boekel, M. A. J. S. 2001. A review of Maillard reaction in food and implications to kinetic modelling. Trends in Food Science and Technology 11(9-10): 364-373.

Mestdagh, F., Maertens, J., Cucu, T., Delporte, K., Van Peteghem, C. and Meulenaer, B. D. 2008. Impact of additives to lower the formation of acrylamide in a potato model system through pH reduction and other mechanisms. Food Chemistry 107(1): 26-31.

Michalak, J., Czarnowska-Kujawska, M. and Gujska, E. 2019. Acrylamide and thermal-processing indexes in market-purchased food. International Journal of Environmental Research and Public Health 16(23): article no. 4724.

Nguyen, H. T., Van Der Fels-Klerx, H. J., Peters, R. J. B. and Van Boekel, M. A. J. S. 2016. Acrylamide and 5-hydroxy-methylfurfural formation during baking of biscuits. Part I: Effects of sugar type. Food Chemistry 192(1): 575-585.

Rifai, L. and Saleh, F. A. 2020. A review on acrylamide in food: Occurrence, toxicity, and mitigation strategies. International Journal of Toxicology 39(2): 93-102.

Sarion, C., Codina, G. G. and Dabija, A. 2021. Acrylamide in bakery products: A review on health risks, legal regulations and strategies to reduce its formation. International Journal of Environmental Research and Public Health 18(8): article no. 4332.

Schouten, M. A., Tappi, S. and Romani, S. 2020. Acrylamide in coffee: Formation and possible mitigation strategies - a review. Critical Reviews in Food Science and Nutrition 60(22): 3807-3821.

Severin, I., Dumont, C., Jondeau-Cabaton, A., Graillot, V. and Chagnon, M. C. 2010. Genotoxic activities of the food contaminant 5-hydroxymethylfurfural using different *in vitro* bioassays. Toxicology Letters 192(2): 189-194.

Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I. and Gan, S. H. 2018. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. Chemistry Central Journal 12(1): 1-18.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2011. Evaluation of certain contaminants in food: Seventy-second report of the joint FAO/WHO expert committee on food additives. Geneva: WHO.

Van Der Fels-Klerx, H. J., Capuano, E., Nguyen, H. T., Mogol, B. A., Kocadağlı, T., Taş, N. G., … and Gökmen, V. 2014. Acrylamide and 5-hydroxymethylfurfural formation during baking of biscuits: NaCl and temperature-time profile effects and kinetics. Food Research International (57): 210-217.

Vinci, R. M., Mestdagh, F. and Meulenaer, B. D. 2012. Acrylamide formation in fried potato products - present and future, a critical review on mitigation strategies. Food Chemistry 133(4): 1138-1154.