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# Potassium bicarbonate improves dough and cookie characteristics through influencing physicochemical and conformation properties of wheat gluten

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ARTICLE INFO	A B S T R A C T
Keywords:	Baking soda (NaHCO <sub>3</sub> ) has critical technological functions in cookie products. Health concern on excessive
Potassium bicarbonate	sodium consumption is increasing; therefore, it is necessary to explore NaHCO <sub>3</sub> alternatives, such as KHCO <sub>3</sub> , for
Sodium reduction	bakery products. This study investigated the impact of KHCO <sub>3</sub> on the technological behaviors of cookie dough
Cookie Gluten aggregation	and end-uses in comparison with control samples prepared with $NaHCO_3$ and explore the changes of physico- chemical and conformation properties of soft wheat gluten during the process. Dough theological measurements
Gliadin to glutenin ratio Secondary structure	demonstrated that addition of KHCO <sub>3</sub> reduced dough stickiness, and adding KHCO <sub>3</sub> achieved similar dough and
	baking performances as using NaHCO <sub>3</sub> , which were partially attributed to the decrease of gliadin to glutenin
	ratio, changes of secondary structure, and intensive aggregation of gluten by introducing KHCO <sub>3</sub> . Cookie sensory
	attributes were also not adversely affected by using KHCO $_3$ . Therefore, partially replacing NaHCO $_3$ with KHCO $_3$

in cookie products can be an effective approach for sodium reduction.

# 1. Introduction

Sodium (Na) plays an essential role in maintaining normal body function and health. However, excessive sodium consumption can significantly increase blood pressure (Stamler et al., 2018), which has been associated with cardiovascular diseases. Currently, the average intake of sodium is 3,440 mg per day in the U.S., which largely exceeds the amount of 2,300 mg per day recommended by the 2015 Dietary Guidelines for Americans (HSS, 2015). Table salt (NaCl) and baking soda (NaHCO<sub>3</sub>) in processed foods are the two largest contributors of dietary sodium (Mattes & Donnelly, 1991). Reducing their amount in foods, especially in bakery and other cereal products, accounting for over 30% of dietary sodium sources, could be a crucial step to mitigate the excessive dietary sodium exposure (Angus, 2007). However, baking soda provides critical technological functions, which can impact dough processability and product quality attributes, including their wellknown sensory and leavening functions. Therefore, desirable baking soda replacement should provide similar technological functions without negatively impacting dough processing and cookie qualities.

Potassium (K) is another important mineral and one of the key electrolytes for the proper functioning of human body. Diets rich in potassium lower blood pressure and have many health benefits (Geleijnse, Kok, & Grobbee, 2003). Vegetables and fruits have been the major sources of dietary potassium. The minimal daily intake of potassium is recommended to be 4,700 mg (HSS, 2015). However, the current diet of most population contains much more sodium but much less potassium (2,800 mg/day) than the recommended level (HSS, 2015). Therefore, (partially) substitution of sodium salts (e.g., NaCl, NaHCO<sub>3</sub>) with potassium salts in some food products could bring dual benefits: sodium reduction and potassium supplementation. In a previous research on low sodium cookie, application of KCl and K<sub>2</sub>CO<sub>3</sub> combination (1:1) in three flours resulted in higher texture scores compared with cookies prepared with NaCl only (Bala, Kaur, & Bakshi, 2004). Potassium bicarbonate (KHCO<sub>3</sub>) has a higher water solubility than NaHCO<sub>3</sub> (i.e., 33% vs. 9% at 20 °C), which could be an advantage in formulating some food products. It also has FDA approved Generally Recognized as Safe (GRAS) status and is appropriate for use in foods (FDA, 2016). Therefore, KHCO<sub>3</sub> could be a promising chemical leavening agent, especially in reduced-sodium formulas.

Wheat flour dough is regarded as a composite material of a gluten network embedded in other ingredients such as starch. A three-dimensional network of gluten can form via different inter- and intramolecular linkages, and wheat gluten network are partially responsible for the dough behavior and end-product quality. Gluten structure evolution during dough mixing and development is critical in manipulating dough physical and rheological properties, which determines final product quality. Pareyt, Brijs, and Delcour (2009) observed that spread behavior of cookie was related to the gluten cross-linking. Singh

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and MacRitchie (2014) reported that the polymerization of gluten network may involve oxidation of sulfhydryl (SH) groups. The alkaline reagent was found to influence the structure and interaction of wheat gluten (Wu, Beta, & Corke, 2006). For example, NaHCO3 was reported to induce gluten aggregation via disulfide (SS) group formation (Shiau & Yeh, 2001). However, to the best of our knowledge, there has been no mechanistic investigation into the impact of KHCO<sub>3</sub> on gluten structure, dough texture, and cookies properties. We hypothesized that addition of KHCO3 could induce gluten polymerization and strengthen the structure of gluten through both physical and chemical interactions. Therefore, the objective of this study was to explore the effect of different levels of KHCO<sub>3</sub> on the physical and rheological properties and conformational changes of gluten in wheat dough, in comparison with NaHCO<sub>3</sub>. A typical bakery product, sugar-snap cookie, was produced using KHCO<sub>3</sub>, and the quality was characterized as well. This study provides the critical knowledge towards fundamentally understanding the functions of KHCO<sub>3</sub> in wheat products and benefits the development of working strategies to reduce sodium in bakery products.

#### 2. Materials and methods

#### 2.1. Materials

Soft wheat flour (pastry flour, protein level: 8.05%, moisture level: 13.05%, flour weight basis) was supplied by Mennel Milling Company (Fostoria, OH, USA). Salt, sucrose, dextrose and shortening were purchased from local supermarkets. Chemicals and solvents used were at least analytical grade. Sodium bicarbonate, potassium bicarbonate, Tris hydrochloride (Tris-HCl), urea, sodium sulfite, trifluoroacetic acid (TFA), and acetonitrile were purchased from Fisher Scientific (Fairlawn, NJ, USA). Tetrasodium ethylene diamine tetraacetate (EDTA), wheat gliadin standard (> 95%), sodium dodecyl sulfate (SDS), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), and 2-nitro-5-thio-sulfobenzoate (NTSB) were obtained from Sigma Aldrich (St. Louis, MO, USA).

#### 2.2. Dough mixing properties

Mixograph (AACCI Method 54–40.02) and farinograph (AACCI Method 54–21.02) (National Manufacturing Co, Lincoln, NE, USA) were used to assess the mixing characteristics of doughs added with different levels of KHCO<sub>3</sub>. Doughs were prepared by mixing flour, water, and KHCO<sub>3</sub> added at 0.5, 1.0, 1.5 and 2.0%, or 2.0% NaHCO<sub>3</sub> on flour weight basis (coded as K1, K2, K3, K4 and S1, respectively), as well as a blank control sample without KHCO<sub>3</sub> or NaHCO<sub>3</sub> (coded as C1). The mixing parameters were collected for each treatment only containing the flour, leavening agent (NaHCO<sub>3</sub> or KHCO<sub>3</sub>), and water.

#### 2.3. Cookie dough physical and rheological properties

All measurements were performed using the same TA-XTplus Texture Analyzer. Dough strength and extensibility were analyzed using an SMS/Keiffer rig, and resistance to extension (force) and extensibility (distance) of dough samples were recorded (Chen, Hu, & Li, 2018). Dough elongational viscosity was tested with a TA25 cylinder probe via a lubricated uniaxial compression study (Miller & Hoseney, 2008). Elongational viscosity (η) was calculated as:  $\eta = (2FH)/(R^2V_z)$ , where F is the peak force for deformation, H is the dough height, and R is the dough radius, and  $V_z$  is the cross-arm speed. Dough stickiness was measured according to the previous method with an SMS/Chen-Hoseney rig (Chen et al., 2018).

#### 2.4. Protein extractability characterizations

Gluten was extracted from dough for all the treatments according to AACCI Approved Method 38-10.01 (AACC, 2000). Lyophilized gluten

samples were ground into fine powder, sealed, and stored in -20 °C freezer until further analysis. Extraction of gliadin and glutenin from lyophilized samples was performed according to Chen et al. (2018). The extracted gluten protein profiles were obtained using reversed phase high performance liquid chromatography (RP-HPLC), using an Aeris XB-C18 column (3.6  $\mu$ m, 150  $\times$  4.6 mm, Phenomenex, Torrance, CA, USA) and HP1100 equipment, with a diode array detector (DAD) operating at 210 nm (Agilent Technologies, Santa Clara, CA, USA). All gliadin or glutenin fractions were filtered with a 0.45 µm membrane (PhenexTM filter membranes, Phenomenex, Torrance, CA, USA) and loaded to the column, and gradient elution was conducted at a flow rate of 0.6 mL/min using two mobile phases composed of deionized water and acetonitrile, respectively, both containing 0.1% trifluoroacetic acid. Acetonitrile content was increased linearly from 24 to 56% between 0 and 30 min. The retention time and spectra of gliadin and glutenin fractions were compared with standards for qualitative analysis, while the quantitative analysis was performed from the respective calibration curves and the linearity ranged from 0.07 to 1.10 mg/ml, in which the correlation coefficient indicated a good linearity  $(R^2 > 0.98).$ 

#### 2.5. Disulfide-sulfhydryl analysis

Disulfide (S-S) and sulfhydryl (SH) contents were measured using a previous method with some modifications (Rombouts, Jansens, Lagrain, Delcour, & Zhu, 2014). Each Sample (30 mg) was mixed with 3 mL Tris-HCl buffer (0.2 M, pH 8.0) containing 8 M urea, 3 mM tetrasodium ethylene diamine tetraacetate (EDTA), and 1% (v/v) SDS for 1 h, and then 0.3 mL Tris-HCl (0.2 M, pH 8.0) buffer containing 10 mM 5,5dithio-bis-(2-nitrobenzoic acid) (DTNB) was added into the suspension and mixed for one hour. The solution was then centrifuged at 13,600 xg at room temperature for 15 min, followed by measuring the absorbance at 412 nm using a VWR spectrophotometer (UV-6300PC, Radnor, PA, USA). The amount of total SH group was determined according to the report from Thannhauser, Konishi, and Scheraga (1987) with minor modifications. Each sample (10 mg) was mixed with 1 mL Tris-HCl buffer (0.2 M, pH 9.5) containing 0.1 M sodium sulfite, 3 mM EDTA, 1% SDS, and 0.5 mM 2-nitro-5-thiosulfobenzoate (NTSB) for 1 h in dark. followed by centrifuging at 13,600 xg for 15 min. The supernatant was collected and diluted (1:10) by adding Tris-HCl buffer without NTSB, and the absorbance was determined at 412 nm. The concentration of total SH group (Ct) and free SH group (Cf) was obtained according to the equation:  $C = \frac{A}{\varepsilon b}$ , where A is the respective absorbance,  $\varepsilon$  is the extinction coefficient of 13,600  $M^{-1}cm^{-1}$ , and b is the cell path length. The concentration of total SS group (Css) was calculated using the equation:  $Css = \frac{Ct - Cf}{2}$ .

## 2.6. FTIR measurements

Conformation of gluten (secondary structure) was analyzed via collecting Fourier-transform infrared (FTIR) spectra by means of an ATR module on a 400 FTIR Spectrometer (PerkinElmer, Inc., Waltham, MA, US) under the following conditions: 4000–400 cm<sup>-1</sup> spectral range, 4 cm<sup>-1</sup> resolution, and total of 64 scans. The amide I band (1600–1700 cm<sup>-1</sup>) components were further interpreted by deconvolution using OriginPro 2016 software (OriginLab, Inc. Northampton, MA, US) and calculated according to the previous method (Georget & Belton, 2006).

#### 2.7. Cookie preparation and characterization

The formula of cookie contained: flour (222.7 g), water (18.3 g), sugar (130.0 g), shortening (64.0 g), sodium chloride (2.1 g), dextrose solution (33.0 g), and leavening agent (KHCO<sub>3</sub> of 0, 0.5, 1.0, 1.5, or 2.0%, or 2.0% NaHCO<sub>3</sub>, fwb). Cookie was baked according to AACCI

Approved Method 10–50.05 (AACC, 2000). Cookie weight and diameter were measured using a digital caliper, and spread ratio was calculated. Cookie color was measured by a CIE-LAB color system (XITIAN machine equipment Co., Ltd., Huizhou, China), which provides lightness (L\*), redness-greenness (a\*) and yellowness-blueness (b\*) values. The pH of cookies was tested following AACCI Approved Method 02–52.01 (AACC, 2000). Cookie firmness was measured using the TA-XTPlus Texture Analyzer with a three-point bending probe at test speed of 1.0 mm/*sec*, strain of 50%, and trigger force of 20 g.

## 2.8. Sensory test

Sensory analysis of cookie was performed by a panel consisting of 21 members. Participants evaluated a set of selected cookies (i.e., control sample, 1.0% KHCO<sub>3</sub>, 2.0% KHCO<sub>3</sub>, and 2.0% NaHCO<sub>3</sub>) presented in blind condition and random order across subjects (Torri et al., 2016). Consumers rated their liking for appearance, odor, taste, texture, and overall acceptance on a nine-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like). They were required to assess the samples avoiding social interaction under white light, and rinse their mouths with water between sample evaluations.

#### 2.9. Statistical analysis

Data were subjected to the analysis of variance (ANOVA) and the mean values and standard deviations were calculated. The dough physical and rheological properties, cookie characterization, gluten physicochemical and structural characterizations (protein extractabilities, disulfide-sulfhydryl, and FTIR analysis) were conducted in at least three replications. A SAS Institute software version 9.4 (Cary, NC, US) was used to perform the statistical analysis via Tukey's test at a significance being p < 0.05 in all cases.

## 3. Results and discussion

#### 3.1. Impact of KHCO<sub>3</sub> on dough mixing properties

Dough mixing characteristics are summarized in Table 1. The recorded mixograph parameters include optimal water absorption and dough development time, whereas farinograph provides the water absorption, mixing time, and dough stability (Supplementary document, Fig. S1 & S2). In the mixographs, water absorption and mixing time for control dough were 62% and 5.5 mins, respectively. With the increasing levels of KHCO<sub>3</sub> in the dough to 2.0%, both water absorption and mixing time gradually increased, and similar effect was observed for the sample added with 2.0% NaHCO<sub>3</sub>. Salt (i.e., ionic compound) competes with flour macromolecules (e.g., gluten, starch) for water, and therefore, delaying gluten hydration and lengthening mixing time to reach the peak. Similar results were found for doughs added with other types of ionic compounds, such as sodium chloride, sodium dodecyl sulfate, and sodium sulfate (Danno & Hoseney, 1982; He, Roach, & Hoseney, 1992). In the farinographs, the peak time was 8.7 min for the dough with 2.0% KHCO<sub>3</sub> compared to the control sample (1.2 min), where the dough stability also increased from 11.6 min to 15.7 min. The dough strengthening mechanism was partially attributed to the effect of ionic compound, where more KHCO<sub>3</sub> in the systems may induce protein aggregation and interaction, yielding the stronger gluten network. Overall, KHCO<sub>3</sub> exhibited similar effect on dough mixing properties as NaHCO<sub>3</sub>.

# 3.2. Impact of KHCO<sub>3</sub> on dough rheological and physical properties

It is well known that dough rheological behavior is correlated with wheat gluten microstructures (McCann & Day, 2013). Kieffer extensibility test showed that the forces to break the doughs increased significantly with high level of KHCO3 addition (2.0% fwb), in comparison with the sample without  $KHCO_3$  (p < 0.05), whereas the elongation was not significantly different from the control one (p > 0.05). When it comes to the elongational viscosity, the cookie dough treated with KHCO<sub>3</sub> had significantly increased viscosity values (p < 0.05), being similar to the influence of 2.0% NaHCO<sub>3</sub>. As illustrated in Table 1, adding KHCO<sub>3</sub> could mitigate dough stickiness, adhesion and cohesiveness in all the samples containing KHCO<sub>3</sub> and 2.0% NaHCO<sub>3</sub>. The degree of stickiness was significantly reduced (p < 0.05) from 60.7 g (control) to 44.1 g (2.0% KHCO<sub>3</sub>), which may be due to an obstruction effect of alkaline salt on gluten hydration by interacting with water molecules (Caramanico et al., 2017). Hence, the presence of KHCO<sub>3</sub> caused the dough less sticky with higher viscosity, and it was in agreement with the Kieffer test results of the formation of stiffer and less elastic dough compared with the control. This may be caused by more gluten aggregation during dough development and dough formation (Chen et al., 2018).

# 3.3. Effect of $KHCO_3$ on the structure, physico-chemical and conformation properties of gluten

To evaluate the degree of protein polymerization, gluten extractability was determined. The profiles of gluten subunits are presented in Fig. 1. In a recovery test, the gluten samples without NaHCO<sub>3</sub> or KHCO<sub>3</sub> were added with 1% gliadin standard to obtain an average recovery rate of 88% (n = 3). It is reported that gliadin to glutenin ratio (GTG) contributes to the rheological properties of doughs and the bakery end-use quality (Ukai, Matsumura, & Urade, 2008; Wieser, 2007). As illustrated in Table 2, although there was no significant difference for the GTG ratio between the control and 2.0% NaHCO<sub>3</sub> treatment (p > 0.05), the GTG in the treatment of 2.0% KHCO<sub>3</sub> addition was 1.50, in comparison with the value of 1.96 for the control, indicating that high level of KHCO<sub>3</sub> significantly reduced the ratio (p < 0.05).

Table 1

Cookie d	lough 1	nixing c	haracteristics,	and rhe	ological	and	physical	properties v	with di	ifferent l	levels	of KHCO <sub>3</sub>	and 2%	δ NaHCO <sub>3</sub> .
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Sample*	Mixograp	oh	Farinogra	aph		Compression Test		Stickiness test		Kieffer Test	
	Water Abs (% fwb)	Mix Time (min)	Water Abs (% fwb)	Mix Time (min)	Stability (min)	Elongational viscosity (Pa·s)	Stickiness (g)	Work of adhesion (g·sec)	Cohesiveness (mm)	Resistance to extension (g)	Extensibility (mm)
C1	60	E E	E0 7	1.0	11.6	$49041 \pm 250.0^{d}$	$607 \pm 46^{a}$	$220 \pm 51^{a}$		$77 \pm 0.2^{\circ}$	$620 \pm 60^{ab}$
CI	02	5.5	39.7	1.2	11.0	4094.1 ± 239.0	00.7 ± 4.0	23.0 ± 5.1	8.0 ± 0.5	7.7 ± 0.2	02.9 ± 0.9
K1	62	7.0	59.8	4.9	14.8	$8061.0 \pm 172.4^{b}$	$37.9 \pm 3.2^{b}$	$3.4 \pm 1.2^{b}$	$2.7 \pm 0.8^{b}$	$9.1 \pm 0.7^{bc}$	$66.7 \pm 6.5^{a}$
K2	63	7.9	59.4	7.7	13.6	$8384.3 \pm 152.5^{ab}$	$42.3 \pm 3.7^{b}$	$6.0 \pm 1.9^{b}$	$3.3 \pm 1.0^{b}$	$10.9 \pm 1.2^{ab}$	$42.8 \pm 7.2^{\circ}$
K3	63	8.5	59.8	9.5	14.3	$9076.5 \pm 408.8^{a}$	$45.4 \pm 0.9^{b}$	$6.1 \pm 1.3^{b}$	$3.4 \pm 0.3^{b}$	$9.6 \pm 1.3^{abc}$	$62.0 \pm 4.4^{ab}$
K4	64	9.2	59.8	8.7	15.7	$7672.3 \pm 323.2^{b}$	$44.1 \pm 1.3^{b}$	$4.6 \pm 1.3^{b}$	$2.8 \pm 1.0^{b}$	$12.3 \pm 2.1^{a}$	$50.4 \pm 3.9^{bc}$
S1	64	11.9	60.1	10.3	16.9	$6732.0 \pm 261.5^{c}$	44.6 $\pm$ 5.1 <sup>b</sup>	$5.1 \pm 1.9^{b}$	$3.0 \pm 0.9^{\mathrm{b}}$	$12.0 \pm 1.5^{a}$	$45.4 \pm 8.1^{\circ}$

Means with different superscripts within the same column are significantly different at p < 0.05. Statistics were not run on mixograph and farinograph data as there was only one replicate after achieving optimized water absorbance.

\* Sample identification: C1: control, K1: 0.5% KHCO<sub>3</sub>, K2: 1.0% KHCO<sub>3</sub>, K3: 1.5% KHCO<sub>3</sub>, K4: 2.0% KHCO<sub>3</sub>, and S1: 2.0% NaHCO<sub>3</sub>.



Fig. 1. Typical RP-HPLC chromatographs of gluten extract separated from soft wheat flour dough as: (A) gliadin in control; (B) glutenin in control; (C) gliadin in sample added with 2% KHCO<sub>3</sub>; (D) glutenin in sample added with 2% KHCO<sub>3</sub> via the Aeris WIDEPORE XB-C18 column (3.6  $\mu$ m, 150  $\times$  4.6 mm) with UV detection at a wavelength of 210 nm.

The addition of KHCO<sub>3</sub> possibly enhanced the polymeric interactions between gliadin and glutenin through oxidation of SH groups or SH-SS exchanges (Lagrain, Thewissen, Brijs, & Delcour, 2007). Besides, the change in gluten extractability in the presence of KHCO<sub>3</sub> agrees with Wu et al. (2006), who reported that alkali induced gluten aggregation, and this may be ascribed to covalent SS formation and/or hydrogen bonding (Shiau & Yeh, 2001; Tuhumury, Small, & Day, 2014). Therefore, adding KHCO<sub>3</sub> could enhance gluten polymerization through gliadin-glutenin cross-linking, which may contribute to the mechanical and rheological properties of dough and the texture characteristic of cookies.

As presented in Table 2, the free SH content obviously increased with increasing alkaline concentrations, and the content in 2.0% KHCO<sub>3</sub> treatment (1.02 nmoles/mg) was significantly different from the control one (0.83 nmoles/mg) (p < 0.05); however, there were no significant differences of total SH and SS concentration among all the samples (p > 0.05). This observation was not in accordance with the results of Shiau and Yeh (2001) who found the loss of the SH groups since alkali enhanced SH oxidation/SS formation. Nevertheless, Friedman (1999) indicated that free SH groups could also be induced by  $\beta$ -elimination, which corresponded with our result of increase of the SH contents for the sample added with 2.0% KHCO<sub>3</sub>. Therefore, non-redox reaction (e.g.,  $\beta$ -elimination) may induce an observable increase of the free SH when adding 2.0% KHCO<sub>3</sub> and account for the aggregation of wheat gluten during dough development, to some extent. It is

noteworthy that a significant change of the free SH concentration was not observed in 2.0% NaHCO<sub>3</sub> treatment (p > 0.05). The discrepant results might be due to the different ion bonds effect in the system, and other interaction patterns deserve to be further investigated.

To understand the relationship between macroscopic strength of dough and aggregation behavior of wheat gluten, FTIR spectroscopy was used to investigate the differences in their secondary structures that was influenced by different levels of KHCO<sub>3</sub> and NaHCO<sub>3</sub>. In the five sections of amides I bands in FTIR spectra, the peak at 1650–1660  $\text{cm}^{-1}$ is assigned to  $\alpha$ -helix, and 1624–1640 and 1681 cm<sup>-1</sup> are associated with  $\beta$ -sheet conformation. The band in the region of 1640–1650 cm<sup>-1</sup> is assigned to random coil, and 1660–1670 and 1694  $\rm cm^{-1}$  region is assigned to  $\beta$ -turn conformation, respectively. As shown in Table 2, the proportions of extended chains, a-helix and β-turn structures were not significantly different among all the treatments (p > 0.05), suggesting little impact on the three gluten conformations exerted by KHCO<sub>3</sub> and NaHCO<sub>3</sub>. On the other hand,  $\beta$ -sheet structure was 19.48% in the control and 24.08% in the sample added with 1.0% KHCO<sub>3</sub> (significant difference, p < 0.05), which may be ascribed to the decrease of random coil structure (Table 2). The conformation of  $\beta$ -sheet is referred as the characteristic of gluten aggregation (Surewicz & Mantsch, 1988); therefore, higher proportions of  $\beta$ -sheet may demonstrate stronger protein interaction and/or polymerization in the samples added with 1.0 and 1.5% KHCO<sub>3</sub> than the control. The increased  $\beta$ -sheet conformation in the samples could explain the more strengthened gluten

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Sample	Secondary structure*					Gluten extractability	y		Concentration of SH and	l SS groups	
	Extended chains (%)	β-Sheet (%)	Random coil (%)	α-helix (%)	ß-turn (%)	Gliadin (µg/mg)	Glutenin (µg/mg)	Gliadin to Glutenin	Total SH (nmoles/mg)	Free SH (nmoles/mg)	SS (nmoles/mg)
IJ	$4.74 \pm 0.47^{a}$	$19.48 \pm 0.90^{\rm b}$	$27.16 \pm 2.10^{a}$	$14.30 \pm 1.04^{a}$	$14.60 \pm 1.02^{a}$	$234.19 \pm 21.15^{a}$	$119.86 \pm 7.74^{a}$	$1.96 \pm 0.14^{a}$	$7.18 \pm 0.52^{a}$	$0.83 \pm 0.11^{a}$	$3.17 \pm 0.26^{a}$
KI	$4.64 \pm 0.53^{a}$	$21.19 \pm 0.05^{ab}$	$18.17 \pm 0.72^{ab}$	$24.55 \pm 2.52^{a}$	$20.49 \pm 2.99^{a}$	$230.80 \pm 26.40^{a}$	$130.50 \pm 12.92^{a}$	$1.78 \pm 0.21^{ab}$	$7.13 \pm 0.32^{a}$	$0.92 \pm 0.04^{ab}$	$3.10 \pm 0.14^{a}$
K2	$5.18 \pm 0.62^{a}$	$24.08 \pm 1.13^{a}$	$16.33 \pm 1.36^{b}$	$19.94 \pm 3.84^{a}$	$18.26 \pm 6.85^{a}$	$201.83 \pm 6.11^{a}$	$133.56 \pm 6.41^{a}$	$1.51 \pm 0.03^{\rm b}$	$7.38 \pm 0.30^{a}$	$1.00 \pm 0.15^{ab}$	$3.19 \pm 0.10^{a}$
K3	$4.68 \pm 0.70^{a}$	$23.59 \pm 0.42^{a}$	$13.43 \pm 2.88^{\rm b}$	$18.12 \pm 2.43^{a}$	$17.09 \pm 1.46^{a}$	$216.37 \pm 19.61^{a}$	$115.73 \pm 6.05^{a}$	$1.87 \pm 0.08^{ab}$	$8.26 \pm 0.85^{a}$	$0.92 \pm 0.06^{ab}$	$3.67 \pm 0.42^{a}$
K4	$3.12 \pm 0.92^{a}$	$18.09 \pm 0.53^{\rm b}$	$17.82 \pm 4.11^{ab}$	$24.20 \pm 0.22^{a}$	$12.50 \pm 4.28^{a}$	$197.66 \pm 26.19^{a}$	$131.58 \pm 11.03^{a}$	$1.50 \pm 0.08^{\rm b}$	$8.30 \pm 0.85^{a}$	$1.02 \pm 0.02^{\rm b}$	$3.54 \pm 0.22^{a}$
S1	$4.57 \pm 0.82^{a}$	$20.46 \pm 0.23^{ab}$	$22.04 \pm 0.83^{ab}$	$20.11 \pm 2.09^{a}$	$18.01 \pm 1.12^{a}$	$216.34 \pm 18.35^{a}$	$132.56 \pm 14.43^{a}$	$1.64 \pm 0.05^{ab}$	$7.62 \pm 0.04^{a}$	$0.89 \pm 0.04^{ab}$	$3.37 \pm 0.03^{a}$

<sup>ab</sup> Means with different superscripts within the same column are significantly different at p < 0.05.

cm<sup>-1</sup>), random coil (1640–1650 cm<sup>-1</sup>),  $\alpha$ -helix as extended chains (1600–1615 cm<sup>-1</sup>),  $\beta$ -Sheet (1624–1640, 1681 \* Gluten secondary structures deconvolved from amide bands: I. Full assignment is given See Table 1 for sample identification.  $(1650-1660 \text{ cm}^{-1})$ ,  $\beta$ -turn (1660-1670,  $1694 \text{ cm}^{-1}$  microstructure in the dough (Table 1); moreover, in comparison with the control sample, the results were also supported by farinograph parameters and dough physical properties with higher dough stability and strength and less stickiness.

# 3.4. Influence of KHCO<sub>3</sub> on macroscopic cookie properties

Table 3 summarizes the characteristics of cookies containing the control sample, KHCO<sub>3</sub> under different concentrations, and 2.0% NaHCO<sub>3</sub>. The pH value, W/T spread, hardness, and flexibility of cookie were significantly influenced when adding 0.5–2.0% KHCO<sub>3</sub> or 2.0% NaHCO<sub>2</sub>. The hardness and flexibility of cookie were decreased with the increase of KHCO<sub>3</sub> concentration, and adding 2.0% NaHCO<sub>3</sub> also resulted in significantly lower hardness and flexibility values compared with the control (p < 0.05). The W/T spread ratio increased to 0.94 with 1.5% KHCO3 addition compared with 0.71 for the control (p < 0.05), and higher spread ratio was considered as a more desirable quality attribute for cookies (Barak, Mudgil, & Khatka, 2014). As shown in Table 3 & Fig. 2, cookies added with KHCO<sub>3</sub> were significantly brighter than the control one (p < 0.05); however, all levels of KHCO<sub>3</sub> also led to high degree of redness (a\*). Moreover, cookies containing KHCO<sub>3</sub> or NaHCO<sub>3</sub> presented greater yellowness (b\*) than the control, where the value of 1.5% KHCO<sub>3</sub> treatment (31.60) was significantly (p < 0.05) higher than that of the control (15.87). The strong yellow coloration in the cookie samples may be caused by the formation of flavone-c-diglycosides at alkaline pH (Asenstorfer, Wang, & Mares, 2006). Based on the aforementioned information, in addition to improving the cookie coloration, it can be postulated that the high amount of KHCO<sub>3</sub> may induce gluten interaction that contributed to the higher W/T spread ratio and fluffy texture of cookies.

Taken together, remarkable differences of rheological and physicochemical properties in dough and cookies were found in the treatments added with higher concentrations of KHCO<sub>3</sub>. Hydration and mixing of flour with water result in an 'elastic backbone' of gluten network that is stabilized by covalent and/or non-covalent interactions. The continuous viscoelastic structure warrants the wide utilization of wheats for various products (Domenek, Morel, Redl, & Guilbert, 2003). When adding high amount of KHCO3 into the system, it competes for water molecules and delays flour and gluten hydration, and hydrogen bonds may confer the alternation of the secondary conformation in forming more  $\beta$ -sheet structure, thus it can generally increase the molecular flexibility of samples. In the meantime, the gliadin-glutenin crossinglinking is pronounced via covalent interactions (e.g.,  $\beta$ -elimination) in the microstructure. Consequently, gluten aggregation promotes a compact network which may prevent excessive starch swelling or leaching from the structure to such extent that it results in the fluffy texture of cookie after baking.

#### 3.5. Cookie sensory test

Consumers' acceptability tests were conducted to compare the organoleptic characteristics of cookies with or without KHCO<sub>3</sub> and NaHCO<sub>3</sub>. Cookie sensory attributes were affected by both the addition of KHCO<sub>3</sub> and NaHCO<sub>3</sub> (Table 4). The sample added with 1.0% KHCO<sub>3</sub> showed the highest overall acceptance compared with other treatments, although the samples containing 2.0% NaHCO<sub>3</sub> or 2.0% KHCO<sub>3</sub> did have significantly higher texture score than the control (p < 0.05), in agreement with the other cookie macroscopic parameters described previously. The sensory attribute scores for cookies with KHCO<sub>3</sub> were similar as that with NaHCO<sub>3</sub>. Texture perception from consumers confirmed the instrumental results that the blank control sample has the lowest score. Our results demonstrated that KHCO<sub>3</sub> was comparable with NaHCO<sub>3</sub> as an effective leaving agent for cookie production, without adversely influencing the organoleptic characteristics of cookies.

#### Table 3

Sample	рН	W/T spread ratio	Hardness (g)	Flexibility (mm)	Color		
					L*	a*	<i>b</i> *
C1 K1 K2 K3 K4 S1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrr} 11258 \ \pm \ 457^{a} \\ 9234 \ \pm \ 519^{b} \\ 7523 \ \pm \ 772^{b} \\ 4699 \ \pm \ 148^{c} \\ 5384 \ \pm \ 441^{c} \\ 7638 \ \pm \ 573^{b} \end{array}$	$\begin{array}{rrrr} 1.80 \ \pm \ 0.22^a \\ 1.39 \ \pm \ 0.32^{ab} \\ 0.85 \ \pm \ 0.15^{bc} \\ 0.79 \ \pm \ 0.02^c \\ 0.66 \ \pm \ 0.02^c \\ 0.71 \ \pm \ 0.09^c \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 0.02 \ \pm \ 0.01^d \\ 3.53 \ \pm \ 0.43^c \\ 6.32 \ \pm \ 0.51^b \\ 8.01 \ \pm \ 0.69^a \\ 6.34 \ \pm \ 0.29^b \\ 2.39 \ \pm \ 0.54^c \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

 $^{abcdef}$ Means with different superscripts within the same column are significantly different at p < 0.05. See Table 1 for sample identification.



Fig. 2. Images of cookies of: (C1) control, (K1) 0.5% KHCO<sub>3</sub>, (K2) 1% KHCO<sub>3</sub>, (K3) 1.5% KHCO<sub>3</sub>, (K4) 2.0% KHCO<sub>3</sub>, and (S1) 2.0% NaHCO<sub>3</sub>.

Table 4Sensory liking attributes of cookies.

Sample	Appearance	Odor	Taste	Texture	Overall acceptance
C1 K2 K4 S1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 4.9 \ \pm \ 0.9^{a} \\ 6.1 \ \pm \ 0.8^{ab} \\ 6.7 \ \pm \ 0.7^{b} \\ 6.6 \ \pm \ 1.1^{b} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

<sup>ab</sup> Different superscript letters indicate a significant difference (p < 0.05) among attributes in the same column. See Table 1 for sample identification.

#### 4. Conclusion

In the present study, cookie dough systems were obtained by adding KCHO3 or NaHCO3, and their corresponding dough and end-use qualities were compared. Overall, the inclusion of high level of KHCO3 aggravated the macromolecular aggregation via non-redox reactions rather than SH oxidation. In addition, it altered gluten secondary structure, which contributed to the changes of the dough viscoelastic properties and cookie qualities. The elongational viscosity and dough strength were improved, and the stickiness degree was mitigated with KHCO3 addition; a proper addition level (e.g., 1.0% fwb) of KHCO3 did improve cookie texture and positively impact its coloration. Based on the organoleptic evaluation, replacement of NaHCO<sub>3</sub> with KHCO<sub>3</sub> is a feasible approach aiming for sodium reduced diet without compromising the acceptance of the cookie product. Although more studies are still needed to address the mechanism of gluten aggregation, these results have indicated that adding KHCO3 in dough system could be a proposing alternative method of developing high quality cookies and achieving sodium reduction in cereal-based products.

#### CRediT authorship contribution statement

Gengjun Chen: Methodology, Formal analysis, Investigation. Ruijia Hu: Investigation. Yonghui Li: Conceptualization, Methodology, Formal analysis, Resources, Supervision, Project administration, Funding acquisition.

#### **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.

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#### Appendix A. Supplementary data

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