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Aggregation behavior of semolina gluten during dough production and fresh pasta cooking upon kansui treatment

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ABSTRACT

Sodium salt reduction in cereal products has been one of the top health trends. During pasta-making, kansui (an alkaline salt with reduced sodium) was added at 0, 0.5, 1.0, 1.5, and 2.0% (total flour weight basis) to modify semolina gluten aggregation reactions in dough production and pasta cooking. Adding 1.0% kansui enhanced pasta dough elasticity and strength, but cooking quality was changed barely. These consequences may be attributed to more polymeric glutenin incorporated in the network through thiol (SH)/disulfide (SS) exchange or other non-redox reactions/interactions by introducing kansui, which was confirmed by SDS-PAGE, FTIR, and HPLC results. The protein cross-linking induced by kansui (1.0%) improved the texture properties of pasta without compromising the cooking and coloration characteristics. Considering the process convenience and food safety of reducing sodium chloride with natural alkaline salt reagent in industrial pasta production, this could be a potential approach for sodium reduction.

1. Introduction

Pasta, a traditional Italian cuisine, has been a staple food worldwide with a market size of $23 billion in 2016. Salt (i.e., NaCl), an indispensable additive for many cereal food products, is generally added during pasta production to improve dough characteristics and pasta quality. However, an average sodium intake of 3.6 g day^{-1} (much higher than the recommended amount of 2.3 g per day^{-1} by the 2015–2020 Dietary Guidelines for Americans) of the American adults enlarges the risk of many chronic diseases. The American Heart Association and Academy of Nutrition and Dietetics both concluded that excessive sodium intake may result in cardiovascular disease, kidney disease, and other chronic diseases (AHA, 2016). In addition, excessive consumption of chloride dependent has been found to influence the cardiovascular disease and blood pressure regulation in dietary and animal studies on renal chloride balance and transporters in vascular tissues (McCallum, Lip, & Padmanabhan, 2015). There is no doubt that reduction of sodium chloride in pasta could benefit health. However, due to the critical technological functions of salt, reduction or removal of salt can cause some deleterious effects on dough properties and end-use quality, such as reduced dough strength, less desirable texture, and shorter shelf-life. It is in need of salt alternatives to achieve similar technological functions in pasta without causing any deleterious effects on pasta quality.

Kansui, also known as alkaline salt solution, contains a mixture of sodium carbonate and potassium carbonate and is widely applied during Asian noodle preparation to enhance the cooking quality (Bellido & Hatcher, 2009). It was reported that noodles with alkali (i.e., sodium hydrogen phosphate) were more elastic and firmer like NaCl did, with bright and yellow appearance (Wang, Hou, Hsu & Zhou, 2009). Alkali (calcium hydroxide) was added to other cereal noodles to improve the texture (Han, Lu, Hao, Cheng & Li, 2012). Ong, Ross and Engle (2010) reported that the inclusion of alkali correlated with increased dough stiffness. The improvement of rheological and textural properties of dough and noodle may be attributed to the microstructure change of the gluten (Rombouts, Jansens, Lagrain, Delcour & Zhu, 2014). In spite of the above, the mechanisms explaining the impact of kansui on the dough and end-use qualities are not fully clear.

Gluten aggregation is considered to have a key role in pasta quality (Marti & Pagani, 2013). During pasta production, durum wheat semolina and water are mixed to form crumbly dough that are extruded or sheeted to form continuous strands. In the process, gluten subunits of semolina can form inter- and intra-molecular linkages via various reactions, which play an essential role in the formation of a three-dimensional polymeric network. Covalent reactions and some non-covalent interactions such as hydrogen bonding and hydrophobic interactions are important for the aggregation of gliadin and glutenin (Wieser, 2007). With semolina gluten responsible for network

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development, alkali could alter or induce hydrophobic and/or electrostatic interactions, which may take a principal effect on aggregation of gluten proteins (Wu, Beta & Corke, 2006). Shiau and Yeh (2001) found more disulfide (SS) cross-linking for gluten network formation in noodles added with kansui. Moreover, alkali in the recipe of wheat noodle dough was found to influence the level of extractable glutenin (Ong et al., 2010). It is therefore hypothesized that kansui can cause semolina gluten to polymerize via the covalent reactions and/or non-covalent interactions during the pasta dough development and impact the end-use quality. However, to what extent the proteins is altered and involved in the network and how it is induced by kansui are not well understood. Also, the impact of kansui concentration on macromolecular interaction remains to be investigated. Thus, the objective of this study was to understand the impact of kansui on semolina gluten aggregation during laboratory scale pasta dough mixing and cooking. More specifically, our objectives were to: 1) evaluate the influence of kansui on the dough rheological and texture properties and pasta qualities; 2) study the change of the gluten cross-linking reactions; and 3) explore physico-chemical properties and conformational changes of semolina gluten components.

2. Material and methods

2.1. Materials

Durum wheat semolina (*Triticum durum Desf.*, protein content: 11.2%, moisture content: 12.1%, dry weight basis, db) was purchased from local supermarket. All chemicals, solvents, and reagents used in the experiments were at least of analytical grade unless otherwise specified and purchased from Fisher Scientific (Fairlawn, NJ, USA) or Sigma-Aldrich (St. Louis, MO, USA). Coomassie stain solution was purchased from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Preparation of fresh pasta

The basic formulation of fresh pasta consisted of wheat semolina flour (100 g) and distilled water (40 g). Kansui (a 4:3 mixture of sodium carbonate to potassium carbonate) was dissolved in the water and then added to the flour at 0% as control (C), 0.5% (K1), 1.0% (K2), 1.5% (K3), and 2.0% (K4) based on total semolina flour weight basis (fwb), respectively. The ingredients were mixed in a pin mixer for 8 min to obtain a crumbly dough. After a resting time of 30 min, pasta strand was prepared using a pasta maker (Model 150, Imperia, Italy) by passing the dough through 4 decreasing roll gaps (2.0, 1.6, 1.2, and 0.8 mm, respectively), three times each. A portion of the final dough sheet was reserved for color measurement. The remainder of the flatted dough sheet was immediately cut into fresh strands (thickness: 0.8 mm, width: 5 mm) using a sheet cutter. The samples were then freeze-dried, ground, and stored in sealed plastic bags at −20 °C for further physico-chemical analysis.

2.3. Oscillational rheology

Oscillational rheological properties of dough were measured using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA, USA) with a PP 20 parallel plate. The dough was freshly prepared as previously described. After mixing, the dough was rested for 30 min in a closed container before loading on the rheometer. Excessive sample outside the plate edge was trimmed, and paraffin oil was applied on the lateral surface to prevent moisture evaporating or drying. The gap size was set at 1200 μm, and a frequency sweep was performed from 0.1 to 100 Hz at 25 °C in a linear viscoelastic range with the strain amplitude set at 0.1%. Storage modulus (G′), loss modulus (G″), and tan δ (= G″/G′) were recorded to evaluate the viscoelastic properties of dough. Two replicates were conducted for each treatment.

2.4. Elongational viscosity

Lubricated uniaxial compression was conducted using a TA-XTPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA; Stable Micro System, Godalming, Surrey, UK) with a 5.1 cm diameter cylinder probe. Doughs were rested for 30 min, sheeted to 7.9 mm thick, covered and rested for another 10 min. A disk (2.5 cm diameter) was cut and coated with mineral oil, followed by compressing with 5.0 mm s^{-1} pre-test speed, 0.4 mm s^{-1} test speed, 10.0 mm s^{-1} post-test speed under 50% strain, and 5 g trigger force. The elongational viscosity was calculated as: 2 F H/R^2 Vz, where Vz was the cross-arm speed, F was the peak force, H was height and R was the radius of dough (Miller & Hoseney, 1997). Three disks per dough were analyzed.

2.5. Stickiness analysis

Dough stickiness analysis was performed using the same TA-XTPlus Texture Analyzer with an SMS/Chen-Hoseney dough stickiness rig, a dough stickiness cell, and a 25 mm Perspex cylinder probe (P/25P). Each dough was prepared following the same formulation of pasta, and then 10 g of sample was placed into a chamber, and an extruder lid was screwed on, followed by resting the sample for 30 s before releasing the pressure from extrusion. The test parameters were 0.5 mm s^{-1} pre-test speed, 0.5 mm s^{-1} test speed, 10.0 mm s^{-1} post-test speed, 4 mm distance, 40 g force, 0.1 s contact time, and 5 g trigger force. Three replications per dough were performed.

2.6. Keiffer extensibility test

Extensibility analysis was tested using the same TA-XTPlus Texture Analyzer as previously mentioned with SMS/Keiffer rig. After mixing and resting as the same procedure of pasta-making, each prepared dough (10 g) was molded into rectangle and put on a grooved section of the Teflon former. A lametta strip was placed in each groove to aid the dough strip removal. The cover block was placed on top of the former and a clamp compressed the blocks together. Excess dough forced out the sides of the former was trimmed off. After following a 30 min rest, the lametta strips were then removed without deformation for testing. The testing parameters were 5.0 mm s^{-1} pre-test speed, 3.3 mm s^{-1} test speed, 10.0 mm s^{-1} post-test speed, 4 mm distance, 5 g trigger force, and 75 mm total distance. Five strips per dough were tested, and the output quantities were the resistance to extension (g) and the extensibility (mm).

2.7. Pasta color

Fresh pasta color was measured using a Color Analyzer Digital Precise Colorimeter CIELAB (XITIAN machine equipment Co., Ltd., Huizhou, China). The system provides the CIE L^* (− black to +white), a^* (− green to +red), b^* (− blue to +yellow). Between each measurement, the pasta sheets were stored in sealed plastic bags. Three replications per treatment were performed.

2.8. Cooking properties

Pasta cooking properties (cooking loss and water absorption) were determined following AACCI approved method 66–50 (AACC International, 2000). Pasta sample (25 g) was boiled in 450 mL of boiling distilled water for 5 min. Then, the water was collected into a volumetric flask and adjusted to 500 mL with distilled water. An aliquot of 100 mL was transferred into a beaker, which was then pre-dried and evaporated in an air oven at 105 °C until completely dry. The residue was weighed and expressed as a percentage of the raw pasta. The water absorption was reported as the mass ratio after and before cooking.

2.9. Reverse-phase high performance liquid chromatography

The lyophilized fresh or cooked pasta (100 mg) was extracted twice with 1.0 mL of a salt solution (0.4 M NaCl with 0.067 M Na_2HPO4/NaH_2PO4 solution, pH 7.6) for 12 min at room temperature (albumin/globulin fraction); twice with 1.0 mL of 60% (v/v) aqueous ethanol for 12 min at room temperature (gliadin fraction); and twice with 1.0 mL of a solution containing 50% (v/v) propan-1-ol, 2 M urea, 1% (w/v) dithioerythritol and 0.05 M Tris-HCl (glutenin fraction) for 45 min at 60 °C. Each extraction step started with vortex mixing for 1 min. The suspensions were centrifuged (15,000 × g, 10 min) at 20 °C, and the corresponding extracts were combined and filtered (Phenex™ filter membranes, Phenomenex, Torrance, CA, USA) before analyzing. The previous RP-HPLC method (Wieser, Antes & Seilmeier, 1998) was followed to analyze the extractable fractions of gliadin and glutenin using a HP1050 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a diode array detector (DAD); where a 3.6 µm Aeris WIDEPORE XB-C18 column (150 X 4.6 mm, Phenomenex, Torrance, CA, USA) was used to separate protein fractions. The elution solvents were water containing 0.1% trifluoroacetic acid (A), and acetonitrile containing 0.1% trifluoroacetic acid (B), with flow rate of 0.6 mL min^{-1} at 60 °C. Injection volume was 10 µL, and the linear gradient was 0 min 24% B, 30 min 56% B with detection at 210 nm. A qualitative analysis was performed by comparing retention times of gliadins or glutenin with the previous report (Wieser et al., 1998), where quantitative analysis was conducted to analyze their peak areas from the chromatograms compared with gliadin standard. Three replications were conducted for each treatment.

2.10. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to a literature method (Li, Guo, Zhu & Zhou, 2018). Freeze-dried pasta sample (10 mg) was dissolved in 1.0 mL buffer (pH 6.8) which included 0.01 M Tris–HCl (pH 6.8), 10% (w/v) SDS, 10% (v/v) glycerol, 0.1% (w/v) bromphenol blue. Each sample was stirred in the extraction buffer for 30 min and left for 1 h at room temperature, and then centrifuged (6000 × g, 10 min) after boiling in water for 5 min. The 10 µL of supernatant was loaded on the gel slab that contained 12% separating gel (pH 8.8) and 4% stacking gel (pH 6.8) and electrophoresis was run in a PROTEAN II vertical electrophoresis apparatus (Bio-Rad, Hercules, CA, USA). The gel was washed three times with deionized water, and water was removed before adding 50 mL Bio-Safe Coomassie stain solution to cover the gel completely in a staining container. The gel was gently shacked (50 rpm) for 2 h, and rinsed in 200 mL deionized water for 30 min.

2.11. Sulfhydryl/disulfide groups determination

The levels of free sulfhydryl (SH) were tested according to the method reported by Rombouts et al. (2014). Each sample (30 mg) was suspended in 3.0 mL reaction buffer A (8 M urea, 3 mM EDTA, 1% SDS, and 0.2 M Tris-HCl, pH 8.0), and then vortexed and mixed for 60 min at room temperature. Next, buffer B of 0.3 mL (10 mM DTNB in 0.2 M Tris-HCl, pH 8.0) was added, and the sample was shaken for another 60 min and centrifuged (13,600 × g) for 15 min at room temperature. Total SH levels were determined by the previous methods (Thannhauser, Konishi & Scheraga, 1987). Reaction buffer containing 3 mM EDTA, 1% SDS, 0.2 M Tris-HCl, 0.1 M sodium sulfite, pH 9.5, and 0.5 mM 2-nitro-5-thiosulfobenzoate (NTSB) was prepared. Sample (10.0 mg) was added into 1.0 mL reaction buffer and shaken for 60 min in dark. After centrifuging (13,600 × g) for 15 min, the supernatant (0.3 mL) was diluted in 2.7 mL reaction buffer without NTSB. The absorbance of samples was measured at 412 nm using a double beam spectrophotometer (VWR UV-6300PC, Radnor, PA, USA). It was calculated as: free or total SH content $(C_{SH}) = \frac{A}{\varepsilon b}$ (where A is the absorbance, ε is the extinction coefficient of 13,600 M^{-1} cm^{-1}, b is the cell path length); and the disulfide (SS) content $(C_{SS}) = \frac{C_{TSH} - C_{FSH}}{2}$ (C_{TSH} is the total SH content, and C_{FSH} is the free SH content). Three replications per treatment were conducted.

2.12. Surface hydrophobicity determination

Sodium dodecyl sulfate (SDS) binding methodology was used to measure surface hydrophobicity of gluten proteins according to Kato, Matsuda, Matsudomi and Kobayashi (1984) with some modifications. More specifically, gluten fractions were isolated and collected following AACCI Approved Method 38-10.01 (AACC International, 2000). The lyophilized and ground gluten sample (10 mg) was dissolved in 40 mL SDS (0.1 mmol L^{-1}) solution and then mixed for 60 min. After dialyzing in deionized water for 48 h, the inner dialyzate was added to 20 mL $CHCl_3$ with 5 mL of methylene blue (0.024 g L^{-1}), followed by centrifuging (2500 × g, 15 min). The mixture in the lower layer was detected at 655 nm (VWR UV-6300PC, Radnor, PA, USA). The SDS solution of a range of 0.01–0.1 mmol L^{-1} was prepared as the standard. The hydrophobicity (H) is calculated with the following equation: H = C × 20 × 288.38/10; where C is the concentration of SDS represented by the standard curve. Three replications per sample were performed.

2.13. Secondary structure analyses

Secondary structure of gluten in pasta was analyzed using a 400 Fourier-transform infrared (FTIR) Spectrometer (PerkinElmer, Inc., Waltham, MA, USA) with a diamond attenuated total reflectance (ATR) cell. For all spectra, 64 scans were recorded at a 4 cm^{-1} resolution in the range of 400–4000 cm^{-1}. The subtracted spectra were fitted into a Gaussian shape (Georget & Belton, 2006) and then deconvoluted using OriginPro 2016 (OriginLab, Inc., Northampton, MA, USA) to quantify the conformation of gluten within the amide I region (1600–1700 cm^{-1}). Each treatment was conducted in duplicate.

2.14. Statistical analysis

All statistical analyses were conducted using a SAS software (v.9.4, SAS Institute, Cary, NC, USA). The mean values and standard deviations were calculated. Analysis of variance (ANOVA) was performed with comparison of mean values using of significance being $p < 0.05$.

3. Results and discussion

3.1. Pasta dough rheological and texture properties

The effect of kansui on pasta dough dynamic rheological properties is presented in Fig. 1, containing the variation of storage modules (G′), loss modulus (G″) and tangent delta (tan δ = G″/G′). All the values of tan δ, referred to as a measure of viscoelasticity, were less than 0.6. The observation suggested that the pasta dough can be classified as a viscoelastic solid (Yiannopoulos, Kontogiorgi, Poulli & Krokida, 2014). The dough containing kansui exhibited higher Ǵ and G" than the control; furthermore, the increase of G' with higher amount of kansui indicated that kansui increased the elasticity of dough, which was in agreement with a previous conclusion (Wu et al., 2006). The change of rheological properties might be due to gluten polymerization via intermolecular cross-links induced by alkaline salt addition (Larsson, 2002).

It is well known that sticky dough could lead to the disruption of production process and lower yield of final product (Sayar, Erdogdu, Eydemir & Nayman, 2016). As shown in Table 1, influence of kansui on mitigating dough stickiness and adhesion was observed in the groups containing 1.0, 1.5, and 2.0% kansui. The stickiness was significantly

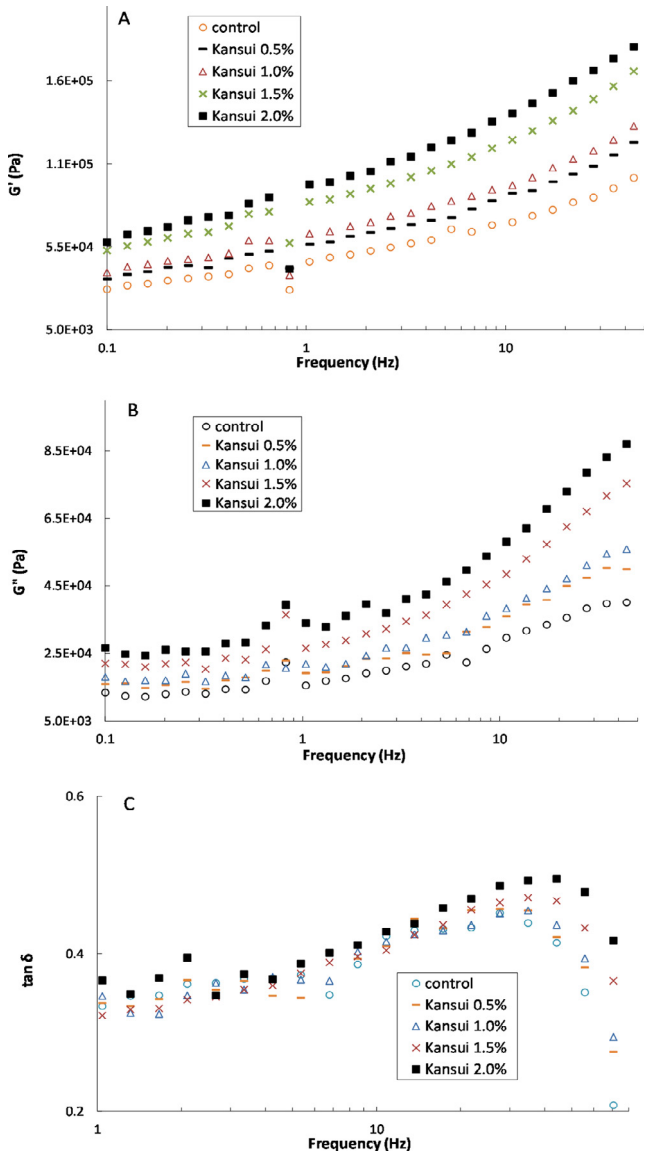

Fig. 1. Effect of kansui addition on dynamic rheological properties of pasta doughs as a function of frequency at a strain of 0.1%: (A) Storage modulus (G′); (B) Loss modulus (G″); and (C) tan δ = (G″/G′).

decreased from 14.53 g (control) to 9.26 g (2.0% kansui). The reduced stickiness may be attributed to the delay of gluten hydration by alkaline salt via competing interaction with water molecules. Previous study suggested that dough strain hardening behavior was correlated with the gluten network (McCann & Day, 2013). Table 1 shows that dough strength (force) increased significantly at higher levels of kansui additions (1.0, 1.5, and 2.0% fwb), in comparison with the control group ($p < 0.05$). This result was consistent with the previous finding from Ong et al. (2010) who observed that alkali could strengthen the gluten structure in the wheat noodle dough; whereas the extensibility (distance) of treatment groups with kansui did not significantly differ from the control ($p > 0.05$). As for the elongational viscosity, the pasta dough containing 1.0, 1.5, and 2.0% kansui resulted in significantly higher value of viscosity than the control group ($p < 0.05$). Overall, the presence of alkali in semolina pasta doughs enhanced the elasticity, strength and elongational viscosity, and it may be due to the stronger polymeric interaction of gluten in these systems, which will be further discussed in the next sections.

Table 1
Texture and rheology properties of pasta dough and pasta qualities with kansui additions.

Sample	Keiffer extensibility		Elongational Viscosity (10^5 × Pa.s)	SMS/Chen-Hoseney Stickiness			Cooking qualities		Color		
	Force (g)	Distance (mm)		Stickiness (g)	Adhesion (g.s)	Cohesiveness (mm)	Water Abs (%)	Cooking loss (%)	L*	a*	b*
C1	34.04 ± 5.94^{c}	17.04 ± 0.42^{a}	0.9 ± 0.1^{a}	14.53 ± 0.25^{a}	0.28 ± 0.01^{a}	0.33 ± 0.01^{a}	115.6 ± 8.1^{a}	6.3 ± 1.7^{a}	73.69 ± 0.54^{a}	0.11 ± 0.05^{a}	22.74 ± 0.46^{a}
K1	37.85 ± 6.19^{c}	17.24 ± 1.54^{a}	1.0 ± 0.2^{a}	13.19 ± 0.41^{ab}	0.24 ± 0.01^{b}	0.26 ± 0.01^{b}	114.4 ± 1.3^{a}	6.2 ± 1.2^{a}	71.57 ± 0.06^{a}	0.12 ± 0.06^{a}	24.96 ± 1.98^{a}
K2	61.90 ± 9.21^{b}	13.70 ± 2.56^{a}	2.2 ± 0.2^{b}	12.17 ± 0.59^{b}	0.21 ± 0.01^{bc}	0.25 ± 0.02^{b}	122.3 ± 7.1^{ab}	7.1 ± 0.9^{ab}	71.96 ± 1.07^{a}	0.25 ± 0.05^{a}	29.74 ± 1.20^{b}
K3	86.54 ± 6.16^{a}	15.95 ± 1.02^{a}	2.4 ± 0.1^{b}	10.50 ± 0.41^{c}	0.18 ± 0.01^{c}	0.25 ± 0.01^{b}	131.4 ± 8.2^{b}	8.3 ± 1.2^{b}	71.37 ± 0.26^{a}	0.32 ± 0.05^{b}	30.74 ± 0.95^{b}
K4	89.60 ± 5.61^{a}	14.66 ± 2.34^{a}	2.5 ± 0.3^{b}	9.26 ± 0.43^{c}	0.18 ± 0.02^{c}	0.26 ± 0.01^{b}	118.4 ± 7.5^{a}	9.2 ± 1.8^{b}	71.48 ± 1.15^{a}	0.45 ± 0.09^{b}	30.15 ± 1.20^{b}

^{abc} Means with different superscripts within the same column are significantly different at $p < 0.05$. Value is represented as the mean ± standard deviation.

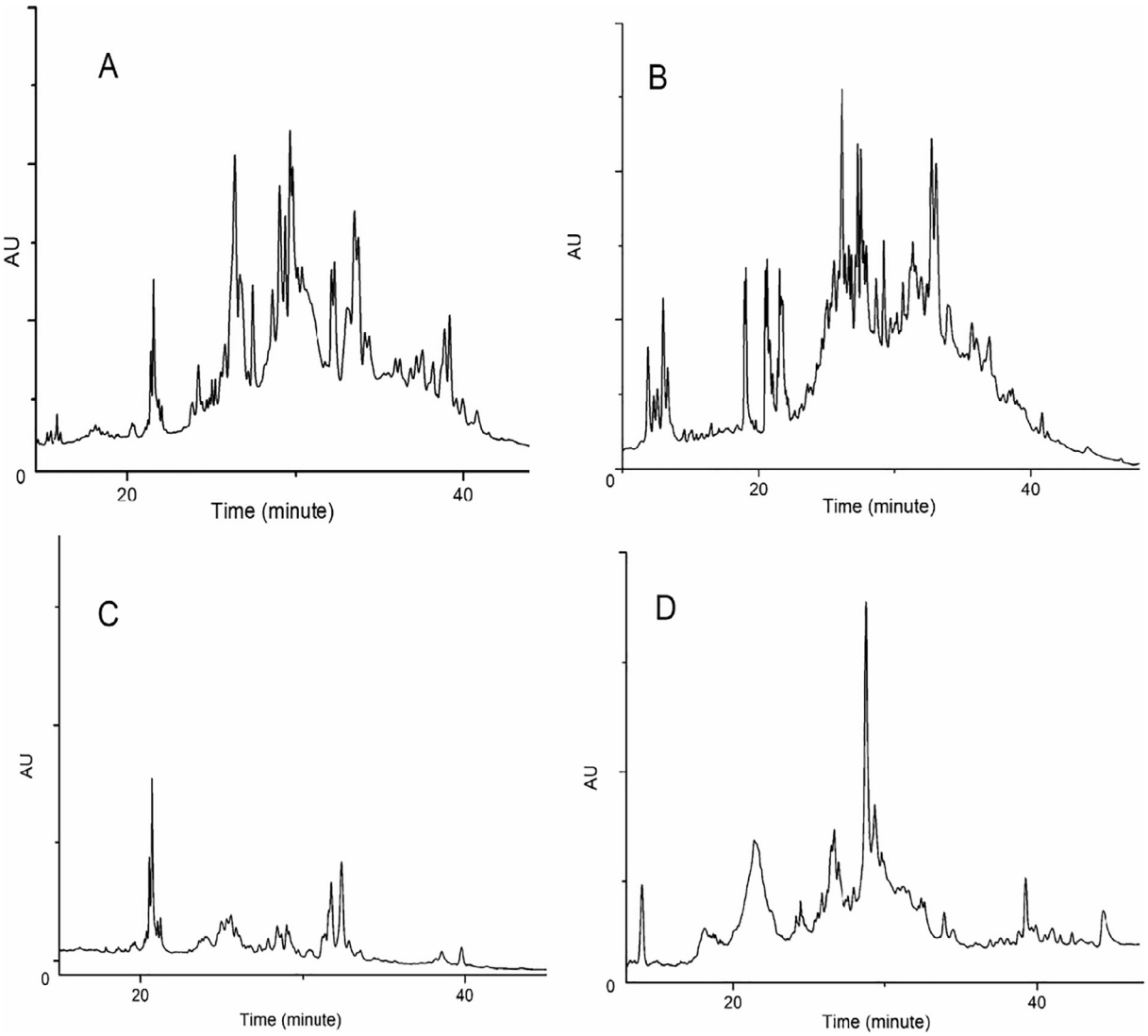

Fig. 2. Typical RP-HPLC chromatographs of semolina gluten separated from pasta samples of (A) gliadin in fresh pasta; (B) glutenin in fresh pasta; (C) gliadin in cooked pasta; (D) glutenin in cooked pasta.

3.2. Pasta color and cooking properties

In premium pasta markets, consumers prefer a bright, clean and yellow pasta. As shown in Table 1, the pasta groups prepared with kansui were not significantly brighter (L^*) than the control sample ($p > 0.05$); whereas adding high levels of kansui (1.5 and 2.0%) to dough systems led to higher redness (a^*) than the control. Moreover, pasta treated with kansui (1.0, 1.5 and 2.0%) exhibited much greater yellowness (b^*). For example, the yellowness value of 1.5% kansui pasta treatment (30.74) is significantly ($p < 0.05$) higher than control one (22.74). The strong yellow coloration of pasta with high levels of kansui may derive its color from flavone-c-diglycosides, which undergo a chromophoric shift at alkaline pH (Asenstorfer, Wang & Mares, 2006).

Cooking properties are key quality parameters for pasta products. Table 1 showed that adding 2.0% kansui significantly increased the cooking loss of the pasta, where the cooking loss was 9.2%, in comparison to only 6.3% for the control. That may be because the addition of alkali could promote the gelatinization of starch, which may allow an easier leaching of starch from the gluten micro-network of the pasta (Lai, Karim, Norziah, & Seow, 2002). On the other hand, when adding 1.5% kansui, we found that the water absorption significantly increased to 131.4% ($p < 0.05$); whereas it was down to 118.4% when 2.0% kansui was added to the system, which may be attributed to the excessive cooking loss. Therefore, adding 1.0% kansui may improve the coloration quality of pasta without compromising the cooking qualities.

3.3. Protein extractabilities by RP-HPLC

To evaluate protein aggregation during pasta production and cooking, gluten extractability of fresh and cooked pasta was determined. The gliadin and glutenin fractions were sequentially extracted and further analyzed using the Aeris WIDEPORE XB-C18 column (Fig. 2). The method was validated by a standard calibration curve ranged from 0.065 to 1.05 mg with a good linearity ($R^2 > 0.98$). The limit of detection and the limit of quantification were 0.016 and 0.053 mg mL^{-1}, respectively. An average recovery rate was calculated as 88% by spiking the control samples with 1% gliadin standard ($n = 3$). As illustrated in Fig. 3A, the average amount of extractable gliadin with the addition of 2.0% kansui was 23.61 μg mg^{-1} while the value was 26.79 μg mg^{-1} in the control sample before cooking. The extractable glutenin amount significantly increased ($p < 0.05$) by 22% when adding 2.0% kansui (43.99 μg mg^{-1}) compared to the control (36.15 μg mg^{-1}). At the meantime, the total gluten extractability remained almost constant (Fig. 3A). These results indicated that polymeric interaction between gliadin and glutenin was enhanced via cross-linking induced by kansui during the pasta production. Alkali could

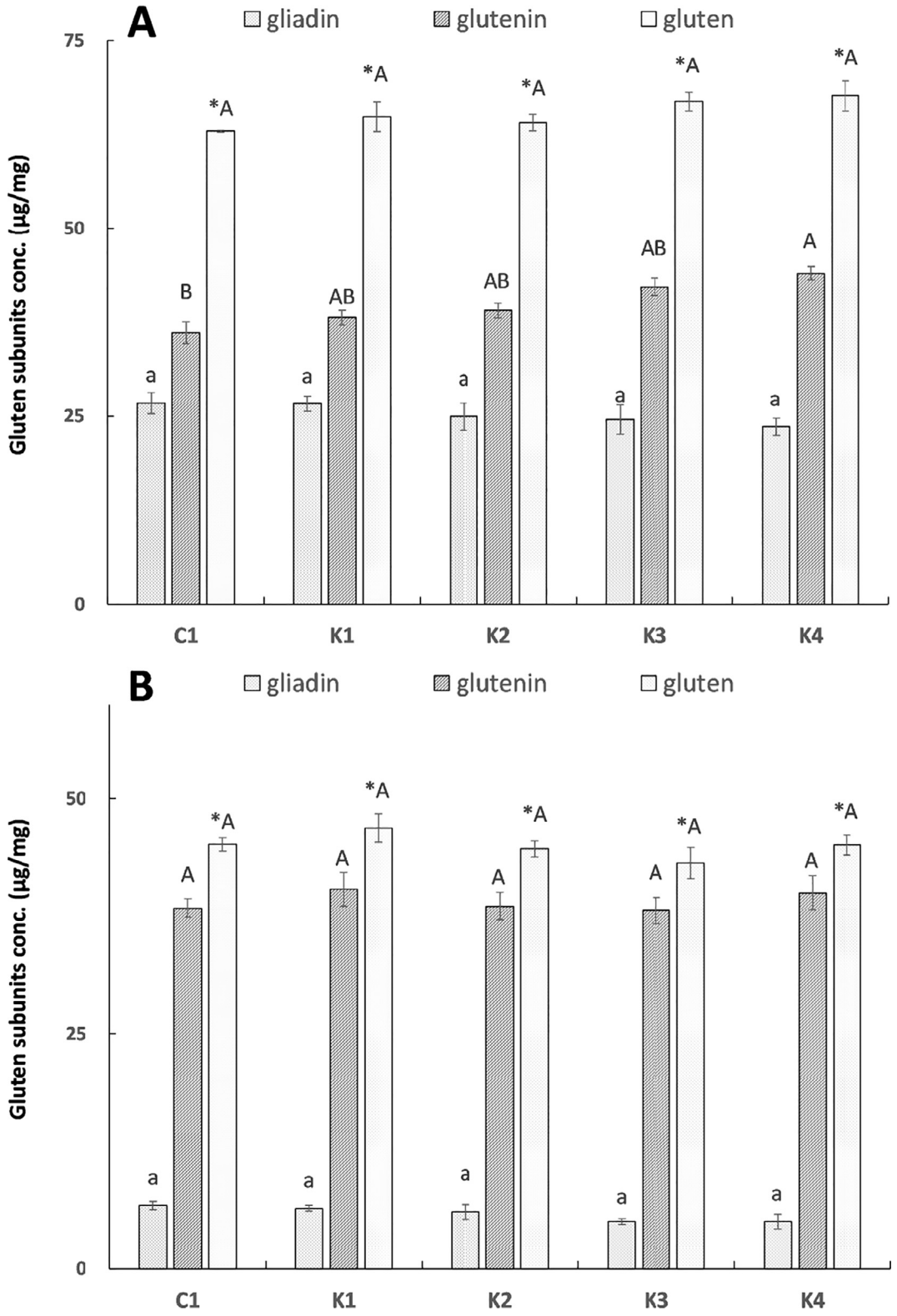

Fig. 3. Semolina gliadin, glutenin, and gluten extractabilities of fresh (A) and cooked pasta (B) when adding different levels of kansui. Bars of the same protein type with different letters differ significantly ($p < 0.05$).

cause covalent (SH/SS exchange or SS bonding) and/or non-covalent (hydrophobic or electrostatic) interactions for gluten aggregation based on the previous reports (Shiau & Yeh, 2001; Tuhumury, Small & Day, 2014). These interactions possibly contributed to the accelerated glutenin polymerization in the fresh pasta with high levels of kansui.

The data of semolina gluten extractability of cooked pasta is presented in Fig. 3B. The contents of total extractable gliadin fraction were decreased in all the treatments compared to the uncooked pasta (Fig. 3A). The result was consistent with Lamacchia et al. (2007) who indicated that increasing of gliadins polymerization occurred at cooking temperature more than 90 °C. The loss of total gluten extractability during cooking was attributed to heat-induced formation of cross-

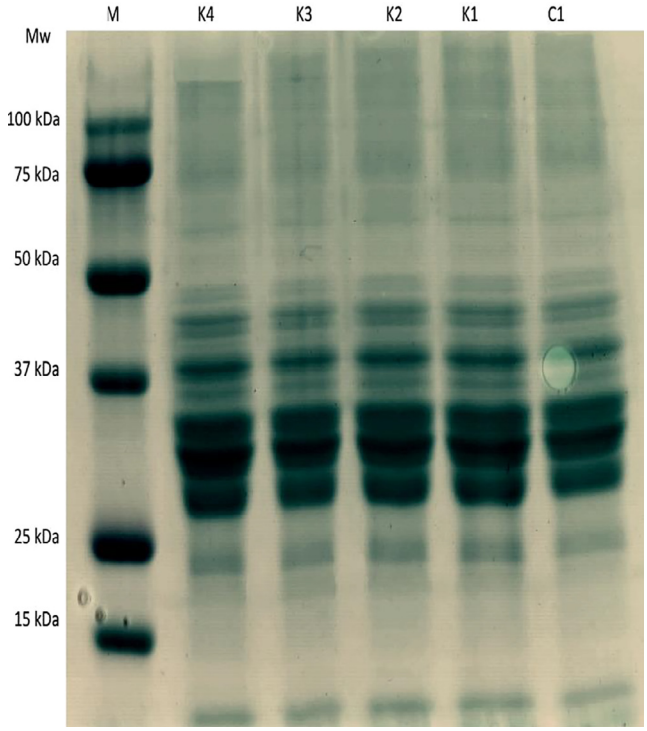

Fig. 4. SDS-PAGE analysis of pasta protein. M: standard marker; the line C1, K1, K2, K3, and K4 represent the pasta with various levels of kansui (0%, 0.5%, 1.0%, 1.5% and 2.0%).

linking. However, among the treatment groups with kansui, neither gliadin nor glutenin extractabilities were significantly different ($p > 0.05$). It is worthy noticed that the average ratio of gliadin/glutenin in the control, 0.5, 1.0, 1.5, and 2.0% kansui treated pasta (uncooked) were 0.74, 0.70, 0.64, 0.58, and 0.54, respectively, where decreasing of the ratio may lead to desirable viscoelastic properties of pasta like elasticity. After cooking, the values of samples were around 0.15. In conclusion, adding kansui into the pasta dough system could gradually enhance gliadin-glutenin cross-linking and decrease the ratio of gliadin/glutenin in fresh pasta, which might contribute to the positive changes of the rheological/texture properties of samples. During cooking, heat caused the significant structural damage of gliadins, which might mitigate the effect of kansui.

3.4. SDS-PAGE pattern of pasta protein

SDS-PAGE of pasta protein was collected to analyze the molecular weight (Mw) distribution and investigate the extent of protein aggregation. As shown in Fig. 4, distinct decrease in the intensity of protein bands (Mw > 100 kDa) of pasta samples with increasing kansui concentration was observed on the top part of the separation gel; especially, some parts of band on the very top vanished with 1.5 and 2% kansui treatments. These changes indicated the formation of larger aggregates that could not be extracted by SDS buffer solution, which was consistent with the increase of the glutenins extractability by RP-HPLC. It was possibly due to the formation of intermolecular cross-linking (oxidation of SH), as also found in the previous study (Rao, Vatsala & Rao, 2002). In addition, Netto et al. (2007) believed that the SS cross-linking was the major covalent bonding force for the gluten polymerization. Interactions occurred in the processing of pasta with the addition of kansui will be further discussed in the next section.

3.5. Determination of sulfhydryl (SH) and disulfide (SS) contents

Sulfhydryl group (SH) and disulfide bonds (SS) may greatly influence the rigid structures and functional properties of wheat gluten (Netto et al., 2007). The effect of kansui addition on the SH and SS of the semolina gluten is presented in Table 2. It shows that the free SH content in the dough was significantly decreased from 1.00 nmol mg^{-1} (control) to 0.70 nmol mg^{-1} when 2% kansui was added ($p < 0.05$). Meanwhile, the SS content of the 2% kansui sample was 3.42 nmol mg^{-1} compared to 3.29 nmol mg^{-1} in control, but the SS content was not significantly changed ($p > 0.05$). Overall, the high level of kansui in our system did not seem to induce significant SH oxidation, which is different from Netto et al. (2007) who observed significant SH-SS interchange reactions in the production of noodles. The discrepant results may be caused by different types and concentrations of alkaline solution. On the other hand, the loss of free SH in the samples treated with kansui may be ascribed to other non-SS cross-linking that might occur in the dough development. For instance, Rombouts et al. (2014) found that dehydroalanine residue could lead to the formation of lanthionine cross-linking at alkaline pH. Moreover, other non-SS interaction patterns like hydrophobic interaction deserve to be investigated because of their possible contribution to the macromolecular unfolding or aggregation (Wieser, 2007).

3.6. Determination of surface hydrophobicity

The tertiary and quaternary conformations of gluten may be influenced by interactions between hydrophobic side chains of the amino acid residues. By determining SDS binding capacity, surface hydrophobicity of semolina gluten was obtained and presented in Table 2. No significant differences were found among all the treatments ($p > 0.05$), which indicated that kansui inducing conformation changes of gluten microstructure might not be mainly via altering the changing of surface hydrophobicity.

Table 2
Physico-chemical and secondary structure of semolina protein in pasta induced by kansui.

	Sample				
	C1	K1	K2	K3	K4
Total SH (nmol/mg)	7.58 ± 0.27[a]	7.32 ± 0.24[a]	7.34 ± 0.23[a]	7.40 ± 0.41[a]	7.57 ± 0.75[a]
Free SH (nmol/mg)	1.00 ± 0.05[a]	0.97 ± 0.12[ab]	0.94 ± 0.11[ab]	0.70 ± 0.02[b]	0.72 ± 0.08[b]
SS (nmol/mg)	3.29 ± 0.11[a]	3.17 ± 0.18[a]	3.20 ± 0.16[a]	3.35 ± 0.21[a]	3.42 ± 0.41[a]
Extended Chains (%)[a]	5.80 ± 1.20[a]	4.81 ± 1.15[a]	4.95 ± 0.26[a]	4.92 ± 0.40[a]	3.63 ± 1.33[a]
β-Sheet (%)[A]	18.35 ± 0.74[b]	19.36 ± 1.30[ab]	24.14 ± 2.03[ab]	27.14 ± 1.88[ab]	27.80 ± 2.28[a]
Random coil (%)[A]	30.54 ± 0.54[a]	24.43 ± 3.90[ab]	20.94 ± 1.39[ab]	16.51 ± 1.32[b]	18.36 ± 0.23[b]
α-Helix (%)[A]	17.13 ± 1.27[ab]	20.57 ± 2.45[ab]	12.11 ± 2.23[b]	24.26 ± 0.26[a]	21.42 ± 1.43[ab]
β-Turn (%)[A]	10.24 ± 1.39[a]	14.94 ± 2.70[a]	18.67 ± 5.55[a]	15.74 ± 2.62[a]	21.10 ± 1.84[a]
Hydrophobicity	20.53 ± 1.30[a]	19.54 ± 1.08[a]	19.56 ± 0.87[a]	19.94 ± 1.03[a]	20.22 ± 1.52[a]

[ab] Means with different superscripts within the same row are significantly different at $p < 0.05$. Value is represented as the mean ± standard deviation.
[A] Secondary structure of semolina protein deconvolved from amide bands I (Georget & Belton, 2006).

3.7. Secondary structures of semolina gluten

The secondary structures of gluten proteins can be derived from the amide I region (1600–1700 cm^{-1}), where gluten exhibits a strong absorption band which could provide a biochemical basis for macromolecular interactions and hence may contribute to the pasta quality (Wang et al., 2015). Accomplished via secondary derivative spectra deconvoluted from the amide I region (Georget & Belton, 2006), the secondary structure of semolina gluten backbone is regarded as the sum of extended chains (1600–1615 cm^{-1}), β-Sheet (1624–1640, 1681 cm^{-1}), random coil (1640–1650 cm^{-1}), α-helix (1650–1660 cm^{-1}), and β-turn (1660–1670, 1694 cm^{-1}). As shown in Table 2, it is noticed that the content of β-sheet structures was 18.35% in the control and 27.80% in the group added with 2% kansui, and the changes were remarkably significant ($p < 0.05$). Another significant change of random coil structure was also observed, which might contribute to the increase of β-sheet. Wang et al. (2015) also found more β-sheet structure in alkaline noodle samples. The increasing hydrogen or SS bonding between the gluten induced by alkali was liable to form β-sheet, which is considered as a relative stable secondary structure (Wellner, Bianchini, Mills & Belton, 2003). According to the previous proposed mechanisms (Shewry, 2009; Tuhumury et al., 2014), when hydration occurs, the hydrogen bonds may promote the formation of gluten loop structure with changing to β-sheet conformation; consequently, the gluten aggregation is more pronounced. Therefore, adding kansui to pasta dough system may influence the secondary structure of semolina gluten to cause development of a network appearing as large aggregate.

Taken together, our data showed that significant differences of dough rheology and texture properties and pasta qualities were found in the treatments with kansui, which could be attributed to gluten polymerization aggravated by kansui, to some extent. To explain physico-chemical, rheological and macromolecules conformational changes in alkaline dough and pasta, a single proposed mechanism perhaps cannot be thoroughly enough. Nevertheless, it is postulated that adding appropriate amount of kansui (e.g., ≤1.0% fwb) could enhance pasta dough strength and elasticity via gliadin-glutenin crossing-linking, and rarely bring cooked pasta of low quality since gluten aggregation confers pasta particles a microstructure which can prevent excessive starch swelling or leaching from the network during cooking. However, when adding up to 2% kansui, too much large aggregate of glutenin incorporated in this network could tightens it to such extent that it lacks sufficient flexibility to cope with starch and hence reduce pasta qualities, such as resulting in the higher cooking loss. More in-depth studies are needed to address the influence of kansui on other interaction patterns like non-redox cross-linking during the dough formation and pasta cooking.

4. Conclusion

In the study, adding kansui (Na_2CO_3/K_2CO_3) in pasta dough system aggravated gluten polymerization via covalent reactions (SH/SS exchange or other non-redox reactions) and non-covalent interactions (hydrogen bonding). Thus, it greatly influenced the dough properties and end-use qualities. Based on the extensibility, rheology, and viscoelasticity measurements, both the elasticity, elongational viscosity, and strength of the pasta dough were enhanced and stickiness was decreased with the addition of kansui, which is desirable for pasta processors and preferred by consumers. Kansui affected the secondary structure and free SH content of semolina; however, it did not change the surface hydrophobicity of pasta protein. The extractable ratio of gliadins/glutenins reduced when adding kansui in the pasta doughs. Moreover, a moderate level (1.0% fwb) of kansui did not sacrifice the cooking qualities (i.e. water absorption and cooking loss) and positively impact on the pasta coloration. These results provide useful guidance in proposing alternative ways of achieving salt technological functions in pasta-making and ultimately reducing sodium in pasta products.

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