Food Chemistry 221 (2017) 1178–1185

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The impact of protein cross-linking induced by alkali on the quality of buckwheat noodles

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 4 November 2016
Accepted 7 November 2016
Available online 9 November 2016

Keywords:
Protein cross-linking
Alkali
Quality
Buckwheat noodle

ABSTRACT

The impact of alkali addition on the dough rheological properties and quality of buckwheat noodles was investigated. Farinograph measurements showed that the addition of alkali increased the water absorption and development time of the dough. Dynamic rheological properties analysis showed that alkali addition enhanced G′ and G″ of dough. In addition, the texture properties of buckwheat noodles improved by the increase of the hardness and tensile force. Furthermore, an obvious decrease in the intensity of the protein bands with lower molecular weights was observed in SDS–PAGE patterns and the extractability of protein in sodium dodecyl sulfate containing medium (SDSEP) decreased, which demonstrated that alkali addition promoted the degree of protein polymerization in the buckwheat noodles. CLSM analysis showed alkali addition produced a tight and continuous protein network in buckwheat noodles. The protein cross-linking induced by alkali improved rheological properties of dough and texture properties of buckwheat noodles.

1. Introduction

Noodle, as a traditional food in many Asian countries, has been widely consumed since ancient time. The noodle ingredients are diverse, such as wheat, rice, buckwheat and starches separated from potato and pulses (Fu, 2008). As to processing and quality of noodles, gluten is considered to play an essential role (Marti & Pagani, 2013). In addition, the quantity and quality of protein also made a significant contribution to noodle qualities (Hu, Wei, & Wang, 2007). However, due to the increasing population of individuals with celiac disease (Kim, Kee, Lee, & Yoo, 2014b), wheat flour, as single raw material, no longer met people's needs for health.

Buckwheat, which contains common buckwheat and *tartary* buckwheat, grows mainly in China, Russia, Japan, and Indian. It is abundant in several flavonoids, i.e. rutin, quercitin and so on (Cai, Corke, & Li, 2004). The flavonoids have been proved to be effective in reducing the risk of cardiovascular diseases (Cai et al., 2004). Meanwhile, the protein in buckwheat proved to have the highest content of Lys in pseudocereals (Mota et al., 2016), and hence buckwheat can complement well with other vegetable proteins (Li & Zhang, 2001). Based on the nutritional value, buckwheat is very suitable for making cereal food, especially used for patients with gluten sensitivity (Cai et al., 2004). However, the composition of buckwheat protein is very different from the wheat protein which is high in glutenin and glidian (70–75%), it has been reported that albumin is the predominant protein fraction (Cai et al., 2004). Therefore, compared with wheat protein containing gluten, it is difficult for other cereal protein to form optimal network and thus the noodles present poor processing and texture properties. In order to solve the problem, one way is facilitating starch itself to form a starch network replacing protein network under the extrusion condition (Giménez-Bastida, Piskuła, & Zieliński, 2015), another way is choosing suitable formulations and recipes to substitute the role of gluten in the noodles (Giménez-Bastida et al., 2015). Yalcin and Basman (2008) took use of xanthan gum to improve the quality of corn noodles, it turned out that noodle samples supplemented with xanthan gum had low cooking loss and high sensory scores. Besides, the third way is promoting the cross-linking of protein. It is reported that TG could improve the quality of oat noodles by cross-linking of oat protein (Wang, Huang, Kim, Liu, & Tilley, 2011). Meanwhile, high temperature drying can also improve the quality of gluten-free pasta by inducing polymerization of proteins (D'Amico et al., 2015). Furthermore, alkali (calcium hydroxide) has been added in the buckwheat noodles to modify the texture of buckwheat noodles (Han, Lu, Hao, Cheng, & Li, 2012), which explained the impact of calcium hydroxide on the texture of buckwheat noodles just

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http://dx.doi.org/10.1016/j.foodchem.2016.11.041
0308-8146/

from the perspective of starch. Accordingly, the impact of protein cross-linking induced by alkali on the quality of cereal food like buckwheat noodles remains to be investigated.

Alkali reagents are widely applied for making yellow alkaline noodles in the Canton and Hokkien provinces of China (Fu, 2008). The alkali called kansui always contains a mixture of sodium and potassium carbonates at the ratio of 9:1 (Moss, Miskelly, & Moss, 1986). And low level (0.1–0.3% carbonates) kansui was added to improve the quality of products, high level (0.5–1.0%) kansui was used for introducing alkaline flavor (Fu, 2008). It was reported that the noodles added with alkali were more elastic and firmer than those made with NaCl and the addition of alkali could give the noodles bright and yellow appearance (Moss et al., 1986; Wang, Hou, Hsu, & Zhou, 2011).

The alkali addition could improve the cooking quality of noodles, but mechanism of how the alkali affect the cooking quality was not clear. The effect of alkali addition on the protein polymerisation of buckwheat noodles during cooking remains to be researched. Against the background, the objectives of the present study were to: investigate the impact of alkali (kansui) addition on the dough rheological properties; determine the quality (cooking quality and texture quality) of buckwheat noodles; determine the behavior of protein cross-linking during cooking by SDS-PAGE and SE-HPLC; observe the microstructure of cooked noodles by confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

Wheat flour (moisture content 13.10% and protein 11.65%, respectively; manufactured by Yihai Kerry Grain and Oil Industry Co., Ltd., Kunshan, China) was obtained from the local market. Buckwheat flour (moisture content 12.87% and protein content 13.54%, respectively) was obtained by grinding the grains using a cyclone mill (Chuangli Corp., Hangzhou, China) and passed by an 80 mesh sieve. All chemicals, solvents, and reagents used in the experiments were at least of analytical grade.

2.2. Dough rheological properties

Farinograph properties were determined by the AACC method 54–21 (AACC, 2000) in a Brabender farinograph, using a 300-g mixing bowl. A 300 g of buckwheat flour mixture (wheat to buckwheat flour is 7:3(w/w)) sample supplemented with different levels of alkali (0.0%, 0.2%, 0.5%, 1.0%, 1.5%) were loaded in the farinograph. The addition of water was determined by yielding a dough consistency of 500 BU. Parameters obtained from farinograph were dough water absorption, dough development time (DDT), dough stability time (DST) and degree of softening (DS).

The dynamic rheological tests were performed according to the method reported by Kim, Kee, Lee, and Yoo (2014a). Frequency sweep tests were performed from 0.01 to 10 Hz to determine the storage modulus (G'), loss modulus (G''), and tangent delta (tanδ) as functions of frequency.

2.3. Preparation of buckwheat noodles

The formulation of the buckwheat noodles consisted of 75 g buckwheat flour, 175 g wheat flour and 80 g of distilled water. On total flour weigh basis, the kansui (a 9:1 mixture of sodium to potassium carbonate) was added at 0.0% (as control), 0.2%, 0.5%, 1.0% and 1.5%. Alkaline salt was dissolved in water prior to addition. Wheat flour was mixed with buckwheat flour in a KitchenAid mixer (Kitchen Aid, St. Joseph, USA). The mixing speed and time were as follows: first at 71 rpm for 20 s(low-speed), then turned to 135 rpm for 180 s (high-speed), and at last 89 rpm for 360 s (medium-speed). Then, the prepared dough was placed in a plastic bag to rest for 30 min. After that, the dough was sheeted through the semi-automatic sheeting machine (JMTD-168/140, Beijing Dongfu Jiuheng Instrument Technology Co., Ltd., Beijing, China) at the roll gap of 2.0, 1.6, 1.2, 1.0 mm for 5 times, respectively. Then, the sheet was cut into fresh noodles (width 2.0 mm, thickness 1.0 mm) and all noodles were dried at room temperature.

2.4. Cooking properties and texture analysis of buckwheat noodles

The cooking loss and water absorption of buckwheat noodles were determined by the AACC method 66–50 (AACC, 2000). For cooking loss, around 25 g sample of noodles was cooked in 400 mL deionized water at the optimal cooking time. Then, the cooking water was collected into the volumetric flask and adjusted to 500 mL with deionized water. An aliquot of 100 mL was added to the beaker and evaporated in an air oven at 105 °C until dryness. The residue was weighed and the cooking loss was reported as the percentage of the raw noodle. The water absorption was expressed as the mass ratio after and before cooking.

Texture quality of cooking noodles was measured by using TA-XT2i Texture Analyzer (Stable Micro Systems, London, England) according to the method described by Han et al. (2012). Buckwheat noodles were cooked at the optimal time and immediately cooled with running tap water. The test was carried out at room temperature exactly 15 min after cooking. For TPA analysis, two cooked noodle strands were placed on a metal platform and compressed by a HDP/PFS probe at the speed of 0.8 mm/s and 75% of the strain. For tensile test, the A/SPR probe was used at the test speed of 1.5 mm/s and distance of 60 mm. Analyses were repeated at seven times for each set of noodles.

2.5. Pasting properties of buckwheat noodles

Pasting properties of buckwheat noodles were determined by a Rapid Viscosity Analyzer (RVA4500, Perten Instruments, NSW, Australia) according to the AACC Method 76-21 (AACC, 2000). The program STD1 was executed in the tests. In addition, the noodles were ground to pass through 100-μm sieve.

2.6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was porformed by the method described by Wang, Huang et al. (2011). Freeze-dried cooked noodle samples (containing 6.0 mg protein) were dissolved in the 1.0 mL sample buffer (pH 6.8) which included 0.01 M Tris-HCl, 10% (w/v) SDS, 10% (v/v) glycerol, and 0.1% (w/v) bromphenol blue. The sample solution was centrifuged at 5220*g* for 10 min after boiling in water for 5 min. Then the 10 μL supernatants were loaded on the gel slab that contained 12% separating gel (pH 8.8) and 5% stacking gel (pH 6.8). The voltage of electrophoresis was stable at 100 V in the whole procedure.

2.7. Size-exclusion high performance liquid chromatography (SE-HPLC) analysis

The protein was extracted according to the method of Wagner, Morel, Bonicel, and Cuq (2011) with some modification. Freeze-dried cooked noodles samples (containing 1.0 mg protein) were dissolved in 1.0 mL of sodium phosphate buffer (0.05 M, pH 7.0) containing 1.0% sodium dodecyl sulfate (SDS) under non-reducing conditions, and for reducing conditions, 1.0% (w/v) dithiothreitol (DTT) was added into the sodium phosphate buffer. After centrifugation (10 min, 5220g) and filtration over polyethersulfone (Millex-HP, 0.45 μm, polyethersulfone), extracted samples were separated on a column using a LC-2010 system (Shimadzu, Kyoto, Japan). 20 μL supernatants were loaded on a TSK G4000-SWXL analytical column (Tosoh Biosep, Shanghai). The elution solvent was 0.05 M sodium phosphate buffer (1% SDS, pH 7.0) at the flow rate of 0.6 mL/min and column temperature 30 °C. The elution curve was monitored at 214 nm. To calculate the SDSEP content, the peak area under the SE profile is integrated with the software. The SDSEP content of cooked buckwheat noodles (under nonreducing or reducing conditions) is the ratio of the peak area under the SE profile of samples compared to the peak area under the SE profile of the reference sample. The reference is unheated buckwheat noodle which is extracted under reducing conditions.

2.8. Free thiol group (SH) determination

The levels of free SH was determined according to the method reported by Rombouts, Jansens, Lagrain, Delcour, and Zhu (2014) with some modifications. Two buffer solutions were used in this test. Buffer solution 1 (BS_1): contained Na-phosphate (0.05 M; pH 6.5) with 2.0% SDS, 3 M urea and 1 mM tetrasodium ethylenediamine tetraacetate (EDTA); Buffer solution 2 (BS_2): contained 0.1% (w/v) 5,50-dithio-bis (2-nitrobenzoic acid) (DTNB) in BS_1. Samples containing (5.0–10.0 mg) protein were added into the 5.0 mL BS_1 and shaken for 60 min. Then 500 μL BS_2 was added into the BS_1 and shaken for another 10 min. Next, the reaction tubes were centrifuged at 11000g for 3 min, the absorbance value of samples was measured exactly 45 min after adding the DTNB at 412 nm. The content of free SH was calculated using a calibration curve made with reduced glutathione and controls were without the DTNB reagent or samples.

2.9. Confocal laser scanning microscopy (CLSM) of cooked noodles

The images were filmed according to the method described by Silva, Birkenhake, Scholten, Sagis, and van der Linden (2013). The cooked noodles were cut in pieces of 5 mm length and sliced by freezing microtome (CM1850 UV, Leica, German), then post-stained with a solution of 0.25% (w/w) Fluorescein 5-isothiocyanate (FITC) and 0.025% Rhodamin B in water.

2.10. Statistical analysis

All experiments were performed at least in triplicates. One-way analysis of variance (ANOAN) and Duncan's tests were used to evaluate the significant differences ($P < 0.05$) among the data by the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Dough rheological measurements

3.1.1. Farinograph measurements

The effect of alkali on farinograph measurements was summarized in Table 1. For the buckwheat dough added with alkali, the water absorption of dough had a significant increase (P < 0.05), which agreed with the results found by Moss et al. (1986) when they added alkali to the wheat flour. Furthermore, with the increase of addition of alkali, the dough development time (DDT) and degree of softening (DS) significantly decreased ($P < 0.05$) while dough stability time (DST) had a significant increase ($P < 0.05$). The DDT had an effect on the productivity (Ding & Yang, 2013). Therefore, short DDT indicated improvement of output. The DST and DS indicated the flour strength and the rate of breakdown, with higher dough stability and lower degree of softening suggesting stronger dough (Alaunyte, Stojceska, Plunkett, Ainsworth, & Derbyshire, 2012; Rosell, Rojas, & Benedito de Barber, 2001). The increase of DST and decrease of DS both suggested the dough added with alkali had more stability and was more difficult to breakdown during the mixing. It also suggested that protein network in the buckwheat dough was strengthened, which probably due to the increase of degree of protein cross-linking induced by alkali.

3.1.2. Dynamic rheological measurements

The variation of storage modulus (G'), loss modulus (G'') and tangent delta (tanδ) for buckwheat dough added with alkali is shown Fig. 1. The dough with alkali exhibited higher G' and G" than that control and tanδ decreased with the increase of addition of alkali. The increase of G' indicated that addition of alkali increased the elasticity of dough, and strengthened the protein network in the buckwheat dough (Shiau & Yeh, 2001). The increase of G'' might be due to the increase of water absorption shown in Table 1. Since tanδ is the ratio of G" and G', the results indicated that the increase of G' was more rapid than the increase of G'', suggesting that alkali made the dough more solid-like material. The changes of dynamic rheological characteristics also suggested that addition of alkali contributed the improvement of the properties of dough processing, probably resulting from the changes of chemical bonding (such as oxidization of free sulfhydryl groups) induced by alkali in the protein (Shiau & Yeh, 2001).

3.2. Cooking properties of buckwheat noodles

Cooking loss, water absorption, hardness and tension force were key parameters to evaluate the quality of noodles. As shown in Table 2, the addition of alkali significantly ($P < 0.05$) increased the cooking loss of the noodles, the results were in agreement with Shiau and Yeh (2001). Cooking loss revealed the extent of the solids leaching to cooking water. In this experiment, alkali might impact

Table 1
Effects of addition of alkali on farinograph properties and pasting properties of buckwheat flour mixture (control: no additive).

Sample	Farinograph properties				Pasting properties		
	Water absorption (%)	DDT (min)	DST (min)	DS (BU)	PV (mPa.s)	BD (mPa.s)	FV (mPa.s)
Control	57.4 ± 0.4^{d}	3.30 ± 0.00^{bc}	3.35 ± 0.07^{d}	62.00 ± 0.00^{a}	1654 ± 18^{c}	393 ± 37^{c}	2281 ± 66^{d}
0.2% alkali	58.5 ± 0.4^{c}	4.10 ± 0.14^{a}	4.25 ± 0.21^{c}	58.50 ± 2.12^{a}	1950 ± 25^{b}	440 ± 16^{bc}	2833 ± 2^{c}
0.5% alkali	58.8 ± 0.1^{bc}	3.65 ± 0.49^{ab}	5.05 ± 0.07^{b}	47.00 ± 1.41^{b}	2024 ± 11^{a}	553 ± 107^{ab}	2963 ± 40^{b}
1.0% alkali	59.2 ± 0.1^{ab}	2.60 ± 0.28^{cd}	5.40 ± 0.28^{b}	37.00 ± 5.66^{c}	1927 ± 16^{b}	562 ± 35^{ab}	3281 ± 37^{a}
1.5% alkali	59.7 ± 0.1^{a}	2.55 ± 0.21^{d}	6.65 ± 0.21^{a}	28.00 ± 4.24^{d}	1997 ± 2^{a}	661 ± 11^{a}	3317 ± 2^{a}

Means in the same column with different small superscript letters indicate significant difference at $P < 0.05$.
DDT, dough development time; DST, dough stability time; DS, degree of softening; PV, peak viscosity; BD, breakdown; FV, final viscosity.

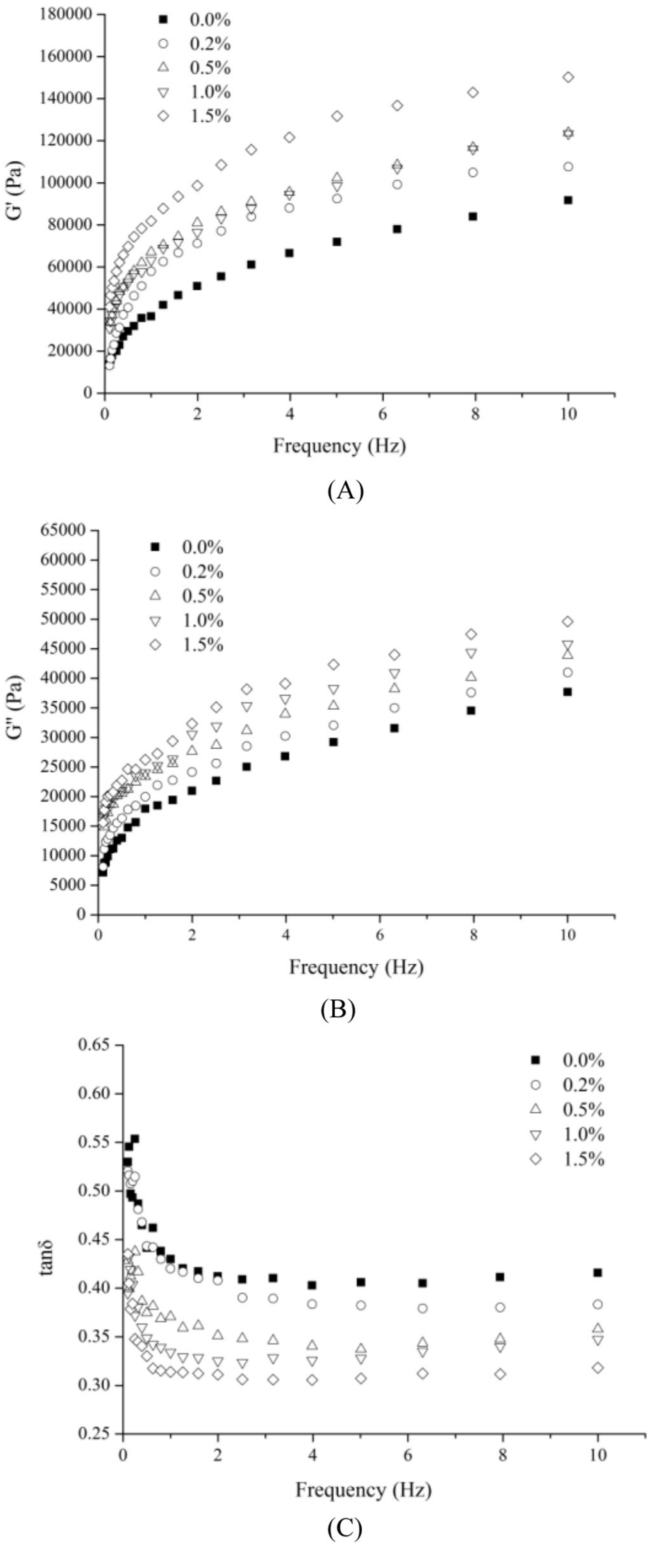

Fig. 1. Effects of alkali addition on dynamic rheological properties of buckwheat dough. G'; (B) G''; (C) tanδ.

the gelatinization properties of starch. It was reported that the addition of alkalis could depolymerize starch and also promoted the starch granules to swell larger extent than the control (Zhou, Wang, Li, Fang, & Sun, 2011). After gelatinization, starch was swelled and became larger which made it could not be well embedded in protein network on the surface of the noodles. So the cooking loss increased (Rombouts et al., 2014). As for water absorption, in Table 2, the data showed that the water absorption obviously increased when adding 0.5% alkali, this might be due to the impact of alkali on the pasting properties of starch. when adding alkali, more starch gelatinized at the surface of noodles, more water was absorbed (Nobile, Buonocore, Panizza, & Gambacorta, 2003). Meanwhile, the water absorption decreased from the addition of 1.0% alkali, which might be caused by the higher cooking loss.

Texture is one of the key parameter that imitates the sensory of consumers. The results of texture properties are shown in Table 2. The hardness and tensile force of noodles were higher than the control, which corresponded to the reports of Fu (2008), Wu and Corke (2005). With the increase of alkali, the values of hardness and tensile force significantly ($P < 0.05$) increased. As mentioned in the above, alkali might promote protein cross-linking and the formation of a tight protein network. The increase of hardness and tensile force could be attributed to a tight and rigid protein network. Meanwhile, it was reported that the tight protein network limited the swelling and disintegration of starch granules,

Table 2
Cooking and texture properties of cooked noodles with different levels of alkali (control: no additive).

Sample	Cooking properties		Texture properties	
	Water absorption (%)	Cooking loss (%)	Tension Force (g)	Hardness (g)
Control	145.9 ± 2.4^{c}	6.23 ± 0.02^{c}	18.80 ± 0.42^{d}	3317 ± 35^{d}
0.2% alkali	147.9 ± 0.1^{c}	6.49 ± 0.13^{c}	20.65 ± 1.63^{cd}	3632 ± 20^{c}
0.5% alkali	163.3 ± 0.3^{a}	7.35 ± 0.36^{b}	21.70 ± 0.02^{c}	3692 ± 45^{c}
1.0% alkali	153.4 ± 3.4^{b}	8.63 ± 0.31^{a}	26.40 ± 0.17^{b}	4359 ± 19^{b}
1.5% alkali	146.9 ± 0.9^{c}	9.11 ± 0.47^{a}	29.00 ± 0.07^{a}	4581 ± 52^{a}

Means in the same column with different small superscript letters indicate significant difference at $P < 0.05$.

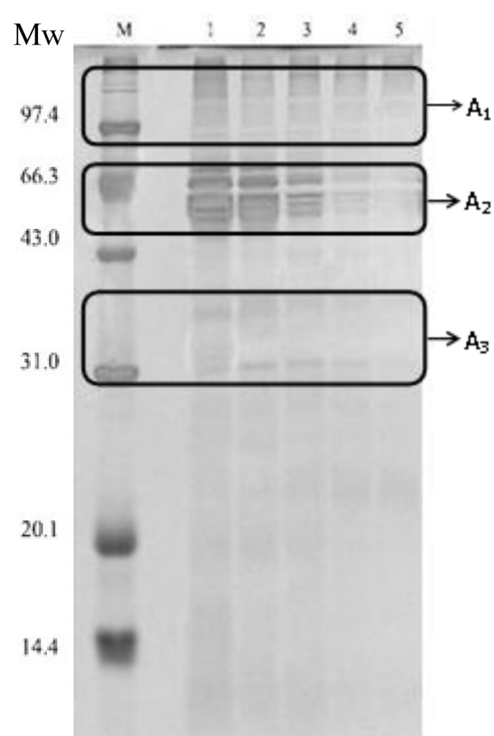

Fig. 2. SDS-PAGE analysis of cooked buckwheat noodles. M: Standard marker; The line 1, 2, 3, 4 and 5 represent cooked buckwheat noodles with various levels of alkali (0.0%, 0.2%, 0.5%, 1.0% and 1.5%).

leading to the increase of hardness (Zweifel, Handschin, Escher, & Conde-Petit, 2003).

3.3. Pasting properties of buckwheat noodles

The pasting properties of buckwheat noodles are present in Table 1. The results revealed that the peak viscosity (PV), breakdown (BD), and final viscosity (FV) of noodles with the addition of alkali were significantly ($P < 0.05$) higher than the control. As for PV, it was reported that the addition of Na_2CO_3 increased the peak viscosity via electrostatic interactions between Na^+ ions and starch hydroxyl groups (Lai, Karim, Norziah, & Seow, 2002). Simultaneously, PV of starch increased with large average granule size, which meant the starch on the surface of noodles absorbed more water and well proved the increase of water absorption (Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005). With regard to BD, the protein cross-linking might affect the BD of flour. It was reported that protein network would protect the starch granule resulting in the most shear resistant particles and thus led to the increase of BD (Bruneel, Pareyt, Brijs, & Delcour, 2010), which meant the loss and subsequent disruption of starch granule, leading to a release of more solubilized starch (Banchathanakij & Suphantharika, 2009). In addition, the increase of BD well illustrated the speculation about cooking loss that mentioned in Section 3.2. Meanwhile, leached starch molecules could be responsible for the increased FV (Banchathanakij & Suphantharika, 2009; Konik, Mikkelsen, Moss, & Gore, 1994). It was reported that the FV of flour was significantly ($P < 0.001$) correlated with noodle smooth and firmness (Konik et al., 1994), which revealed that high final viscosity accounted for rough and stiff noodles.

3.4. Non-reducing electrophoretic pattern of buckwheat noodle protein

Non-reducing SDS-PAGE was performed to illustrate the changes of protein molecular weight of cooked noodles and used to visually observe the extent of protein cross-linking in cooked noodles. In Fig. 2, an obvious decrease in the intensity of the protein bands was found (part A_1) on the top of the separation gel,

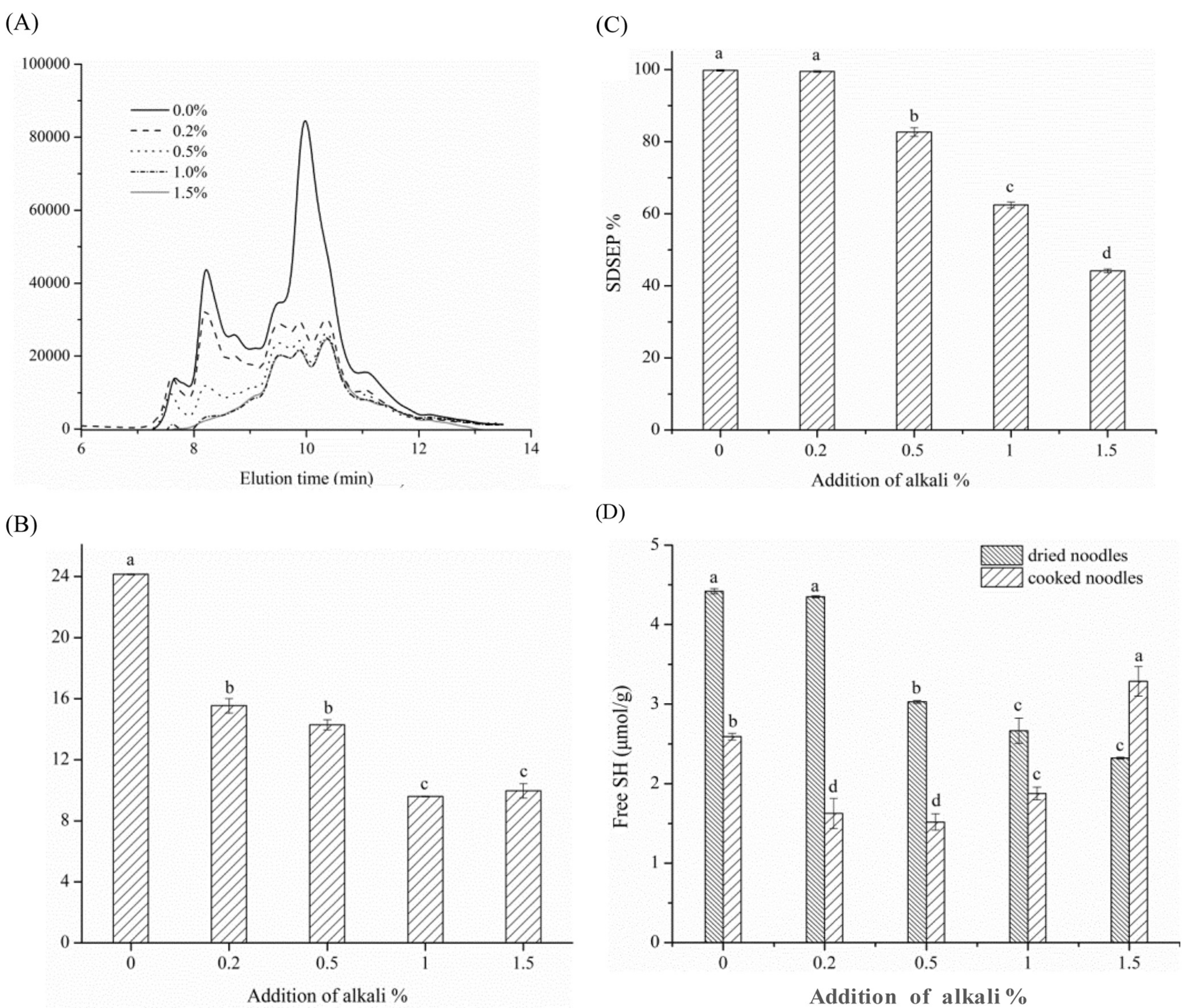

Fig. 3. Changes in levels of extractability of proteins in SDS buffer (SDSEP,%) and free SH groups (μmol/g protein) of cooked buckwheat noodles with various levels of alkali (0.0%, 0.2%, 0.5%, 1.0% and 1.5%). Different letters within each color bar denote significant difference ($P < 0.05$). (A) size exclusion distribution profile of SDS-extractable protein from cooked buckwheat noodles; (B) SDSEP under non-reducing condition; (C) SDSEP under reducing condition; (D) free SH groups (μmol/g protein).

with regard to lower molecular weight, vanish of some bands was observed compared to the control (part A_2 and part A_3). These changes indicated that low molecular protein aggregated and polymerized into larger aggregates that could not be extracted by the SDS buffer solution, which proved the conjecture mentioned in Section 3.1. In addition, the appearance of the dark bands is HMW proteins that appears at the very tops of gels in the alkaline samples. For proteins in noodle flours, alkali addition caused protein aggregation, and these proteins with higher molecular weight could not come into separating gel. It was reported that alkali made the protein had a negative charge leading to a strong disulfide bond, and thus induced protein aggregation (Seguchi, Hayashi, & Matsumoto, 1997). Meanwhile, it was reported that some non-disulfide bond reactions occurred when food treated with alkali (Friedman, 1999). Therefore, whether disulfide bond and non-disulfide bond reaction occurred in the processing of noodle cooking with the addition of alkali and the degree of them would be discussed in the next section.

3.5. Protein extractability

SE-HPLC was used to evaluate the extractability of proteins in SDS containing medium (SDSEP) under reducing and non-reducing conditions.

The data of protein extractability of cooked noodles under non-reducing condition is shown in Fig. 3B and the size exclusion distribution profile of protein molecular weight is shown in Fig. 3A. It could be observed that SDSEP had an obvious decrease with the increase of alkali level up to 1.0%. Besides, 1.0% and 1.5% alkali addition had no obviously different effect on the aggregation of protein. This indicated that cooking step induced the cross-linking of protein in noodles when alkali was added. Combined with the data mentioned in the Section 3.2, the correlation between the SDSEPs and quality of noodles was analyzed. It was found that the SDSEPs had a significant negative correlation with cooking loss, hardness and tensile force ($P < 0.05$), which demonstrated the speculation that protein cross-linking induced by alkali affected the quality of buckwheat noodles. It was reported that alkali (kansui) caused non-covalent interactions and β-sheet structure of protein (Tuhumury, Small, & Day, 2014), and it might induce disulfide bond reaction such as SH oxidation and SH-SS interchange reactions (Shiau & Yeh, 2001). In addition, alkaline also might induce the non-disulfide bond reaction (Friedman, 1999). These reasons were possible to account for SDS-extractability protein losses.

Therefore, in order to evaluate whether the SS cross-links occurred between proteins and the contribution of SS cross-links to the SDSEP losses, SDSEP was also determined under reducing conditions (Fig. 3C). SDSEP of the control (0% alkali) represented that the percentage of peak area of the control under reducing condition to the peak area of buckwheat flour under reducing condition. The SDSEP was about 100% with the addition of 0.2% alkali, which meant that there was almost SS cross-links during noodle cooking with low level addition. However, from addition of 0.5% alkali, the SDSEP presented an obvious decreasing tendency, especially when adding 1.5% alkali, the SDSEP was less than 50%. It could indicate that non-SS cross-links also occurred except for disulfide bond reaction during noodle cooking from the addition of 0.5% alkali to the recipe and formed high molecular weight proteins which were not extractable in SDS containing reduced agent

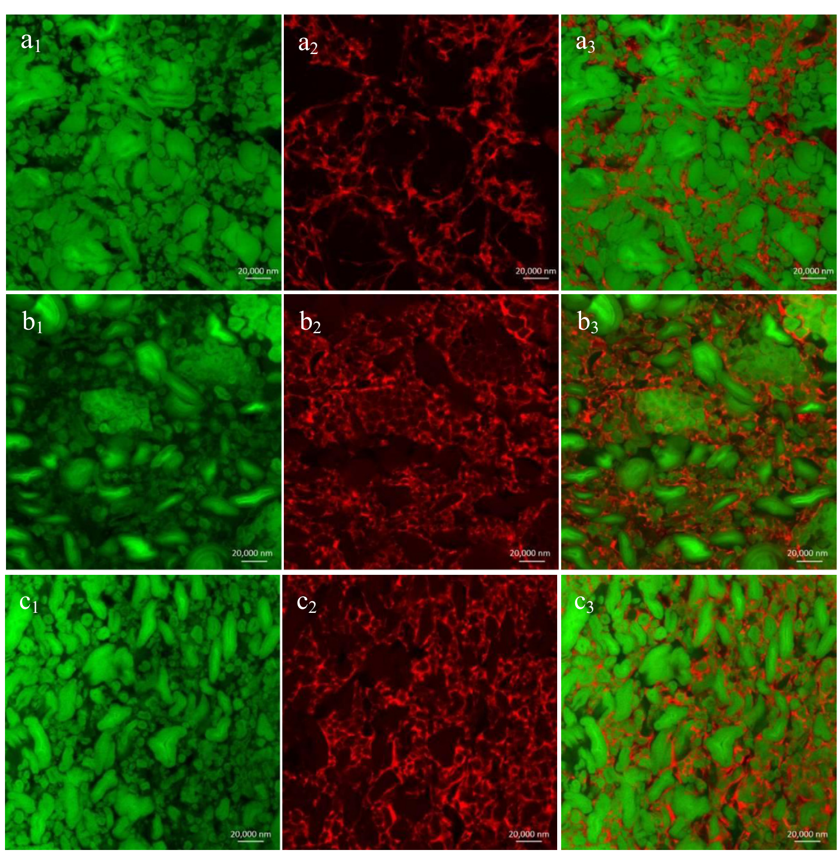

Fig. 4. CLSM analyses of cooked buckwheat noodles with different levels of alkali. Subscript 1–3 represents starch structure, protein structure and section structure of cooked noodles. (a) cooked buckwheat noodles with 0.0% alkali; (b) cooked buckwheat noodles with 0.2% alkali; (c) cooked buckwheat noodles with 1.5% alkali.

(Rombouts et al., 2014). Overall, low alkali level could only increase the SS cross-links, while high level alkali also induced the non-SS cross-links besides SS cross-links and the higher amount of alkali added, the higher degree of non-SS cross-links occurred.

3.6. Determination of free sulfhydryl (SH)

Free SH plays an extremely important role in protein polymerization in hydrothermal process (Lagrain, Thewissen, Brijs, & Delcour, 2008; Wagner et al., 2011), which is correlative with SH oxidation and SH-SS interchange reactions. The results were shown in Fig. 3D. For dried noodles, it was observed that the free SH of dried noodles decreased significantly ($P < 0.05$) from 0.5% alkali, this suggested that the addition of high level alkali increased the SH oxidation in the production of noodles resulting in the loss of free SH (Netto et al., 2007). For cooked noodles, when the addition of alkali was less than 0.5%, free SH significantly ($P < 0.05$) decreased, which indicated that alkali facilitated the SH oxidation and SH-SS interchange reactions during noodle cooking (Rombouts et al., 2014). In contrast, the free SH significantly ($P < 0.05$) increased from the addition of 0.5% alkali, which well demonstrated that the β-elimination of cystine actually occurred during heating and alkaline treatments of food (Friedman, 1999). Therefore, in this experiment, both SS cross-links (SH oxidation) and non-SS cross-links (β-elimination reaction) could impact the content of free SH. When adding 0.5% alkali, more SH oxidation (which consume free SH) reacted than the β-elimination reactions (which release free SH) so that the decrease of free SH was observed, on the contrary, when adding 1.0% or 1.5% alkali, β-elimination reactions were quantitatively more important than oxidation resulting in the increase of free SH. In conclusion, β-elimination reaction might occur when existing high level alkali and the more alkali added, the more strongly the β-elimination reacted.

3.7. Confocal laser scanning microscopy (CLSM) of cooked noodles

CLSM is a popular optical tool to observe the morphology of the starch and protein of cereal food (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001). In this test, the starch and protein were labeled by FITC and Rhodamin B, respectively. The green color represented starch and the red color represented protein. For starch, it could be observed that most of starch swelled and partly fused together in Fig. 4a_1, while there were more intact and not completely swollen starch granules in Fig. 4b_1, c_1. It was reported that the xanthan gum added in the sweet potato starch prevented the starch granules swelling, which made the granules still intact (Silva, Sagis, van der Linden, & Scholten, 2013). In addition, compared with the protein network in Fig. 4a_2, more and more protein cross-linking could be observed in Fig. 4b_2, c_2 and the protein network seemed to be tighter and continuous with the increase of alkali. Therefore, like xanthan gum, the protein network observed in Fig. 4b_2 also prevented the starch from swelling and amount of intact starch granules were well embedded in the protein network, the intact granules led to a higher entire volume fraction of solid particles and thus resulted in the increase of the stiffness of noodles (Walstra, 2002). In addition, a more obvious illustration could be seen in Fig. 4a_3, b_3 and c_3, which totally presented the interaction of starch and protein. In Fig. 4b_3, c_3, it could be observed that more starch granules were embedded in the protein network than that of Fig. 4a_3, which was consistent with the result (mentioned in Section 3.2) that protein cross-linking led to the increase of hardness and tensile force of cooked buckwheat noodles.

4. Conclusions

The inclusion of alkali (kansui) in the recipe of buckwheat noodles obviously improved the dough rheological properties. Furthermore, it increased the hardness and tensile force of buckwheat noodles. Based on the research of protein cross-linking behavior in the noodles, it was found that alkali addition obviously decreased the relative intensities of some protein bands with lower molecular weights in SDS–PAGE and the SDSEP under non-reducing condition, which illustrated that alkali promoted protein cross-linking during cooking. It was worth noting that low level alkali (0–0.2%) only induced SS cross-links, while high level alkali (0.5–1.5%) also induced non-SS cross-links. Micromorphology in buckwheat noodles was observed by CLSM which showed that protein network became tight and continuous. This also indicated that alkali addition increased the degree of protein cross-linking. The protein cross-linking induced by alkali could improve the rheological properties of dough and texture properties of buckwheat noodles.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31571871 and No.31371849), the Key R & D Programs of Jiangsu Province (BE2015327), National High Technology Research and Development Program of China (NO. 2013AA102201), Qing Lan Project and the Jiangsu province "Collaborative Innovation Center for Modern Grain Circulation and Safety" industry development program.

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