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The impact of salt and alkali on gluten polymerization and quality of fresh wheat noodles

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Key words

Chinese noodles; kansui; cooking loss; β-elimination

Abstract

We investigated the impact of table (NaCl) and alkaline (kansui) salts on changes to the gluten network during fresh wheat noodle production and cooking. Noodle production did not markedly change the gluten structure. In contrast, cooking increased gluten's average molecular weight by disulfide bond formation or reshuffling as evidenced by the decrease of protein extractability. Addition of NaCl (0.5 up to 3.0 weight % on flour basis) to the recipe reduced the extent of gluten polymerization during cooking. Kansui (0.2 up to 1.5 weight %) increased intermolecular disulfide bond formation. Furthermore, amino acid analysis revealed that kansui induced the formation of dehydroalanine-derived cross-links lanthionine and lysinoalanine. Optimal firmness was observed for noodles containing either 0.2 to 1.5% kansui or 2.0% NaCl. However, the addition of kansui reduced noodle nutritional quality, and high levels of table (2.0-3.0%) or alkaline (1.0-1.5%) salt increased cooking losses.

45 **1. Introduction**

46 For several thousands of years, wheat-based noodles have been an important part
47 of human diet in oriental countries. With the growing interest in ready-to-eat products,
48 their popularity increases outside Asia (Fu, 2008). Noodles account for more than
49 12% of global (Choy et al., 2012) and up to 50% of Asian (Cai, 1998) wheat
50 production. Based on the salts in the formula, two types of Asian noodles are
51 distinguished: (i) regular salted noodles containing added sodium chloride (NaCl), and
52 (ii) yellow alkaline noodles containing added kansui, a mixture of sodium and
53 potassium carbonate (Fu, 2008). Most noodles are dried or steamed and deep-fried to
54 increase their shelf life (Fu, 2008) even if fresh noodles have unique flavor and taste
55 (Cai, 1998).

56 Noodle quality is typically evaluated on the basis of color, surface appearance,
57 texture, taste and cooking loss, with noodle firmness, cohesiveness, tensile strength,
58 and sensory appreciation as the main discriminating factors (Zhou et al., 2013). Many
59 studies showed that wheat proteins (Hou et al., 2013), starch (Noda et al., 2001),
60 lipids (Lu et al., 2009), and enzymes (Fu, 2008) impact dough and noodle quality.
61 With respect to proteins, both content and quality of gluten, the wheat storage proteins,
62 are important. Gluten protein content is negatively correlated with cooking loss, and
63 positively with noodle tensile strength and firmness (Hou et al., 2013). Gluten quality,
64 evaluated by its sedimentation volume, protein composition, and/or dough rheology is
65 also positively correlated with noodle texture (Hou et al., 2013). Besides wheat flour
66 components, additives impact dough characteristics and noodle quality. Table salt

67 decreases water absorption but increases optimal dough development time and fresh
68 noodle elasticity (Wu et al., 2006). Furthermore, it improves flavor, color and textural
69 properties of cooked noodles (Fu, 2008). The addition of kansui increases water
70 absorption (Fu, 2008) and dough development time (Chu, 2004), and yields firm and
71 little extensible noodle dough (Fu, 2008). Moreover, cooked noodles containing
72 kansui have a firm texture and a distinct yellow color, due to the natural flour
73 flavonoid pigments at alkaline pH (Fu, 2008).

74 In spite of the above, the phenomena explaining the impact of (alkaline) salts on
75 the protein in dough and noodles are not entirely understood. With gluten as the major
76 component in dough responsible for network development, changed hydrophobic and
77 electrostatic interactions due to the addition of salt and alkaline reagents may very
78 well have a principal effect on association and dissociation of gluten proteins (Wu et
79 al., 2006). However, while the impacts of table salt and kansui on starch gelatinization
80 temperature and paste viscosity have been studied relatively well (Shiau and Yeh,
81 2001; Wu et al., 2006), their impact on gluten network formation has received only
82 little attention. Ong et al. (2010) reported that the inclusion of table or alkaline salts in
83 the recipe decreases the level of glutenin macropolymer extractable from noodle
84 dough after mixing, sheeting and compounding. The decrease in extractable glutenin
85 macropolymer level is more pronounced when alkaline salts are used and correlates
86 with increasing dough stiffness (Ong et al., 2010). Shiau and Yeh (2001) found that
87 the addition of kansui decreases the level of free SH groups and increases the level of
88 SS bonds in extruded noodles, suggesting the importance of disulfide (SS)

89 cross-linking for gluten network formation either by oxidation of free thiol (SH)
90 groups or by SH-SS interchange reactions. However, the impact of (alkaline) salt
91 addition on SS bond rearrangements during fresh noodle cooking remains to be
92 investigated.

93 In addition, it has been suggested that during the production of alkaline noodles
94 β-elimination of cystine occurs. In such reaction, the hydrogen atom of the chiral
95 carbon of an intra- or intermolecular cystine bond is abstracted and a persulfide in
96 β-position of the chiral carbon atom is eliminated (Friedman, 1999). The formed
97 intermediate dehydroalanine then reacts further with cysteine and/or lysine to form the
98 non-reducible cross-links lanthionine or lysinoalanine respectively. In hard pretzels,
99 cereal-based snacks dipped in an alkaline solution at high temperature prior to baking,
100 β-elimination and subsequent formation of dehydroalanine-derived cross-links
101 contribute to network formation (Rombouts et al., 2012). In noodles prepared using
102 kansui, lysinoalanine has been detected (Hasegawa et al., 1987), but literature reports
103 neither on formation of other dehydroalanine-derived cross-links in noodles, nor on
104 their importance for network formation. Also, the impact of alkali concentration
105 (kansui levels) on protein reactions remains to be studied. Finally, little if any
106 attention has been given to the potential nutritional consequences of the protein
107 chemistry during noodle making.

108 Against this background, the objective of the present study is to investigate the
109 impact of table salt and kansui levels on different gluten polymerization reactions
110 during noodle dough production and cooking. The obtained information will be

111 related to the quality of cooked noodles, evaluated on the basis of cooking losses,
112 firmness and nutritional value.
113

114 **2. Experimental**

115 *2.1. Materials*

116 Commercial wheat flour (13.9% moisture content, 13.5% protein content on dry
117 matter basis), well-suited for production of fresh (alkaline) salted noodles, was from
118 Dossche Mills (Deinze, Belgium). All chemicals, solvents, and reagents were of
119 analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany) or VWR
120 International (Leuven, Belgium), unless specified otherwise.

121 *2.2. Protein and moisture contents*

122 Protein contents were determined in triplicate using an automated Dumas
123 combustion protein analysis system (EAS VarioMax N/CN, Elt, Gouda, The
124 Netherlands). A conversion factor of 5.7 was used to calculate protein from nitrogen
125 content. Moisture contents were determined according to the AACC-I Approved
126 Method 44-19.01 (AACC International, 1999). All analyses were performed in
127 triplicate.

128 *2.3. Noodle production and cooking*

129 Control noodle dough consisted of 100 parts of wheat flour and 33 parts of
130 deionized water. Different levels of (alkaline) salts were added. On flour weight basis,
131 NaCl was added at 0.5, 1.0, 2.0, or 3.0%, while kansui (a 9:1 mixture of sodium to
132 potassium carbonate) was added at 0.2, 0.5, 1.0, and 1.5%. The (alkaline) salts were

133 dissolved in water prior to addition. Ingredients were mixed into crumbly dough using
134 a Kitchen Aid professional mixer (KPM5, St. Joseph, MI, USA). Mixing speed 1 was
135 used for 1 min, followed by mixing speed 2 for 2 min, and then by mixing speed 1 for
136 2 min. During the first resting stage, dough was placed in a plastic bag to rest for 30
137 min at 23 °C. The crumbly dough was then hand kneaded into a stiff mass and passed
138 through a semi-automatic sheeting machine (Model C280 Capitani, Fino Mornasco,
139 Italy) for 5 to 8 times to form and compound a noodle sheet at a 4.0 mm roll gap
140 setting. After the first sheeting stage, the dough sheet was placed in a plastic bag for
141 30 min at 23 °C (second resting stage). It was then successively sheeted through four
142 different roll gaps (2.9, 2.1, 1.5 and 0.9 mm). Immediately after the second sheeting,
143 further referred to as compounding, the sheet was cut into fresh noodle strands (length
144 15.0 cm, width 5.0 mm, thickness 1.0 mm) with a Capitani sheet cutter. Optimal
145 cooking time was defined as the minimum cooking time needed to let the center core
146 disappear when squeezing a noodle between two pieces of clear plastic. It was 390 s
147 irrespective of the noodle composition. Fresh noodles were cooked in deionized water
148 for 390 s and immediately cooled with running tap water. Samples were withdrawn
149 after mixing, second sheeting and cooking, immersed in liquid nitrogen, freeze-dried,
150 ground in a laboratory mill (IKA, Staufen, Germany) and sieved (250 μm).

151 *2.4 Protein extractability in sodium dodecyl sulfate (SDS) containing medium*

152 Wheat flour, freeze-dried noodle dough, and freeze-dried cooked noodles were
153 extracted (60 min) with a 0.05 mol/L sodium phosphate buffer (pH 6.8) containing
154 2.0% (w/v) SDS (Acros Organics, Geel, Belgium). The quantity of buffer used was

155 1.0 mL per 1.0 mg protein in the sample. To determine protein extractability under
156 reducing conditions, samples were extracted under nitrogen atmosphere with the SDS
157 buffer containing 2.0 M urea and 1.0% (w/v) dithiothreitol (DTT; Acros Organics,
158 Geel, Belgium). All resulting samples were centrifuged (10 min, 10,000 g) and
159 filtered over polyethersulfone (0.45 mm, Millex-HP, Millipore, Carrigtwohill, Ireland).
160 The protein extracts were subsequently separated with size exclusion high
161 performance liquid chromatography (SE-HPLC) using a LC-2010 system (Shimadzu,
162 Kyoto, Japan) with automatic injection. The extracts were loaded (60μL) on a
163 Biosep-SEC-S4000 column with a separation range from 15 k to 500 k (300 x 7.8 mm,
164 Phenomenex, Torrance, CA, USA). The elution solvent was acetonitrile/water (1:1,
165 v/v) containing 0.05% (v/v) trifluoroacetic acid. The flow rate was 1.0 mL/min and
166 the column temperature 30 °C. Eluted protein was detected at 214 nm. All analyses
167 were performed in triplicate. Calculating the difference between extractable and total
168 protein content was difficult because extractability was determined in a buffer
169 containing urea, which interferes with the Dumas analysis (N determination).
170 Therefore, protein extractabilities of wheat flour, noodle dough and cooked noodles
171 (under non-reducing or reducing conditions) were always expressed as a percentage of
172 total protein extractability of wheat flour, which was calculated from the peak area of
173 proteins extracted from wheat flour under reducing conditions.

174 *2.5 Levels of lysine, serine, threonine, histidine, lanthionine and lysinoalanine*

175 To freeze-dried samples containing 10.0 mg protein, 1.0 mL 6.0 M HCl
176 containing 0.1 % phenol and 1.5 mM norleucine (as internal standard) was added and

177 samples were flushed with nitrogen. Protein hydrolysis into amino acids, including the
178 cross-linked amino acids lanthionine and lysinoalanine, was by heating for 24 h at
179 110 °C. Reaction mixtures were diluted (200-fold) in deionized water and filtered
180 (Millex-GP, 0.22 µm, polyethersulfone, Millipore). Amino acids were then separated
181 by high performance anion exchange chromatography with integrated pulsed
182 amperometric detection (HPAEC-IPAD), using a Dionex BioLC system (Dionex,
183 Sunnyvale, CA, USA) as in Rombouts et al. (2009). Separation of an injected aliquot
184 (25 µL) was performed at 30 °C with an AminoPac PA10 guard (50 x 2 mm, Dionex)
185 and analytical (250 x 2 mm, Dionex) column at a flow rate of 0.25 mL/min. Four
186 eluents were used for the gradient mobile phases: water of at least 18.2 MΩ resistivity
187 (A), 0.250 M sodium hydroxide (B, Mallinckrodt Baker, Deventer, The Netherlands),
188 1.0 M sodium acetate (C, Dionex), and 0.100 M acetic acid (D). Gradient conditions
189 and detection waveform were as in Rombouts et al. (2009). The standard amino acids,
190 lysinoalanine (Bachem, Weil am Rhein, Germany) and lanthionine (TCI Europe,
191 Zwijndrecht, Belgium) were detected using a gold working electrode and a pH
192 reference electrode. Their levels were calculated using appropriate standards and
193 expressed on dry matter protein (µmol/g protein) as in Rombouts et al. (2009). All
194 analyses were performed in triplicate.

195 *2.6 Levels of cysteine and cystine*

196 Cysteine and cystine residues were oxidized to one and two cysteic acid residues,
197 respectively, which were then released by hydrolysis and chromatographically
198 quantified as described above. The oxidizing medium (3.0 mL, cooled to 0°C)

contained 3.5 % hydrogen peroxide and 90 % formic acid, and was added to freeze dried sample (20.0 mg protein). The reaction mixture was stirred (15 min, 0 °C) and then left overnight (16 h, 0 °C). To reduce the excess of performic acid, 0.5 mL 48 % hydrogen bromide was added, and the mixture was stirred for 30 min. Bromine and formic acid were evaporated at 50 °C, and samples were subjected to amino acid analysis. Because cysteine and cystine were both converted to cysteic acid prior to hydrolysis, only the sum of their levels was determined.

2.7 Levels of free SH groups

Samples containing 0.8 to 1.3 mg protein were first suspended in 1.0 mL sample buffer [0.05 mol/L sodium phosphate buffer (pH 6.5) containing 2.0% (w/v) SDS, 3.0 M urea and 1.0 mmol/L tetrasodium ethylenediamine tetraacetate] and then shaken for 60 min. Next, 100 µL 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) reagent [0.1% (w/v) in sample buffer] was added and the samples were shaken for another 10 min. After filtration over polyethersulfone (0.45 µm, Millex-HP, Millipore), the absorbance at 412 nm (length of light path = 1.0 cm) was read exactly 45 min after adding the DTNB reagent. Absorbance values were converted to concentrations of free SH using a calibration curve made with reduced glutathione. To correct for background absorbance of DTNB and the sample, controls without the DTNB reagent or sample were used.

2.8 Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a Q2000 DSC instrument (TA Instruments, New Castle, DE, USA). Freeze-dried

221 samples [wheat flour mixed with water without additive (control) or containing 0.5,
222 1.0, 2.0, 3.0% NaCl, 0.2, 0.5, 1.0, 1.5% kansui, or 2.0% NaCl and 0.5% kansui] were
223 first suspended in deionized water (ratio of 1:3 w/w dry matter sample/water).
224 Aliquots (10 µL) were then transferred in aluminium pans (Perkin-Elmer, Waltham,
225 MA, USA). The pans were hermetically sealed and equilibrated at 0 °C before heating
226 from 0 to 100 °C at 4 °C/min. An empty pan was used as reference and the system
227 was calibrated with indium. The onset, peak and conclusion temperatures
228 corresponding to the melting of amylopectin crystals (gelatinization) were evaluated
229 from the thermograms using TA Instruments Universal Analysis software.
230 Gelatinization enthalpy was not determined. All samples were analyzed in triplicate.

231 *2.9 Cooking loss*

232 To determine the cooking loss, fresh noodles (25 g) were cooked in 300 mL
233 deionized water for 390 s (in triplicate). The cooking water was collected and its
234 volume adjusted to 500 mL with deionized water. An aliquot (50 mL) of the diluted
235 cooking water was then transferred to a washed, dried and tarred beaker (W_1, in g)
236 and dried in an air oven at 105 °C for 15 h to constant weight (W_2, in g). The cooking
237 loss was expressed as a percentage of the starting material and calculated as 40 x
238 (W_2-W_1).

239 *2.10 Firmness of cooked noodles*

240 Texture properties of cooked noodles were evaluated using an Instron (Norwood,
241 MA, USA) Universal Testing Machine (Model No. 3342) equipped with a 500 N load
242 cell. Data were analyzed using the Instron Bluehill 3 software package (version 3.13).

243 Fresh 15 cm noodle strands were cooked for 390 s and immediately cooled with
244 running tap water. Measurements were then carried out at room temperature exactly 5
245 min after the rinse step. Three cooked noodle strands were placed in parallel on a flat
246 metal platform and compressed by a rectangular aluminum probe (Instron, pasta
247 firmness/stickiness rig, 51×37×10 mm) at a constant speed of 1.0 mm/s to 70% of the
248 original noodle thickness. Firmness (g) was calculated as the force required for 70%
249 compression (Epstein et al., 2002). Analyses were repeated at least six times on three
250 different noodles.

251 *2.11 Statistical analyses*

252 Protein extractabilities in SDS containing media, levels of cysteine, histidine,
253 lanthionine, lysine, lysinoalanine, serine, threonine and free SH groups, cooking
254 losses and texture properties were analyzed by two-way analysis of variance using
255 Statistical Analysis System software 8.1 (SAS Institute, Cary, NC, USA), with
256 comparison of mean values using the Tukey test ($P<0.01$). Relative standard
257 deviations of single analyses of triplicate preparations were not significantly larger
258 than those of triplicate analyses of single preparations, indicating that sample
259 preparation was reproducible.

260

261 **3. Results and discussion**

262 *3.1.Gluten extractability*

263 To evaluate gluten polymerization during production and cooking of noodles, protein
264 extractabilities in SDS containing buffer of noodle dough (after mixing) and fresh

265 noodles (after cutting, *i.e.* prior to cooking) and cooked noodles were determined
266 (**Table 1**). Protein extractability after mixing was not significantly higher than that
267 after cutting, except for noodle dough containing 1.5% kansui. This indicates that
268 processing steps prior to cooking do not induce cross-links between proteins which
269 decrease the protein extractability, except when high kansui levels are added to the
270 recipe. In contrast, cooking strongly decreased the protein extractability of all samples.
271 The extractability loss during cooking can be attributed to heat-induced formation of
272 cross-links between proteins. In addition, noodle dough formulation also impacted
273 gluten network formation during cooking (**Table 1**). When the recipe contained table
274 salt, the extractability loss during cooking was less than that in the control sample.
275 Increasing ionic strength decreases the electrostatic repulsions between charged
276 protein groups by shielding and hence result in more compact protein chain
277 conformations. Specifically for gluten proteins, Wellner et al. (2003) reported that salt
278 increases the level of β-turn secondary structures, which itself increases molecular
279 rigidity and dough strength. We here speculate that the salt-induced changes in
280 secondary structure not only lead to more compact rigid proteins, but as a result also
281 reduce the degree of cross-linking during the cooking stage. When kansui was added
282 to the recipe, the extractability loss during cooking was greater than that in the control
283 sample. Even more, the addition of 1.5% kansui led to a significant extractability loss
284 during fresh noodle production. In agreement with Shiau and Yeh (2001), the
285 increased extractability losses during fresh noodle production and cooking in the
286 presence of alkaline salts are attributed to increased SS cross-linking, *i.e.* increased

287 formation of intermolecular SS bonds. SH oxidation and SH-SS interchange reactions
288 are indeed favored under alkaline conditions as they involve free SH groups under
289 their thiolate anion form (Netto et al., 2007). Whether cooking of noodle dough also
290 induces non-SS cross-links will be discussed in the next section.
291

292 *3.2.Formation of non-reducible cross-links*

293 To evaluate the formation of high molecular weight compounds by formation of
294 non-reducible cross-links between proteins, protein extractability was also determined
295 under reducing conditions, *i.e.* conditions under which all SS cross-links are cleaved.
296 For all samples taken prior to cooking, protein extractability under these conditions
297 was not significantly different from 100%, which indicates that all proteins were
298 extractable from unheated noodle dough. For noodles without additives, with 0.5 to
299 3.0% NaCl, or with 0.2 to 0.5% kansui, cooking had no significant impact on the
300 extractability of proteins under reducing conditions. This observation shows that in
301 these noodles no high molecular weight compounds were formed by non-SS
302 cross-links during cooking which are not extractable in the SDS containing media
303 with reducing agent. In contrast, cooked noodles containing 1.0 and 1.5% kansui had
304 protein extractabilities under reducing conditions of 81% and 50%, respectively. This
305 substantial extractability loss suggested that non-SS cross-linking occurs during
306 cooking of noodles with high kansui levels resulting in the formation of
307 non-extractable high molecular weight proteins. While low kansui levels increased
308 SH-SS interchange reactivity during cooking, that of high kansui levels also led to

309 dehydroalanine-derived cross-linking reactions to an extent which also decreased
310 protein extractability under reducing conditions. In conclusion, the impact of alkaline
311 salts to wheat noodle dough greatly depended on the level added. In instant noodles,
312 kansui is added either at low levels (0.3 – 0.5%) as quality improver, or at high levels
313 (0.5 – 1.0%) to introduce the characteristic alkaline flavor to the final product (Fu,
314 2008). In fresh alkaline noodles, the most popular alkaline noodles, the addition level
315 of kansui is typically 1.0 to 1.5% (Fu, 2008).

316

317 The observed extractability loss under reducing conditions during cooking of noodles
318 with high kansui levels presumably resulted from a β-elimination reaction of *e.g.*
319 cystine followed by formation of non-reducible, dehydroalanine-derived cross-links.
320 The fact that these reactions are favored by high temperature and alkaline pH (Lagrain
321 et al., 2010) is in line with the observation that the extractability loss under reducing
322 conditions only decreased during cooking of noodles with high kansui levels, and not
323 in noodles without additives, with table salt, or with low levels of kansui. In theory,
324 cystine, cysteine, serine and threonine can undergo the initiating β-elimination
325 reaction (Friedman, 1999). Cysteine, lysine and histidine can then react with the
326 resulting (methyl)dehydroalanine. Lanthionine and lysinoalanine are formed by
327 reaction of dehydroalanine with cysteine or lysine, respectively. The levels of
328 potential precursors (cysteine + cystine, serine, threonine, lysine, histidine) and end
329 products (lanthionine, lysinoalanine) of β-elimination and subsequent cross-linking
330 reactions in wheat flour were compared to those in cooked noodles (**Table 2**). No

331 significant differences were noted between precursor levels in noodle dough (after
332 mixing) and those in fresh noodles (after cutting). Neither had the additive level an
333 impact on the level of any precursor in noodle dough or in fresh noodles. In addition,
334 the levels of all potential precursors remained constant during cooking of kansui free
335 noodles. In contrast, the cooking of kansui containing noodles resulted in losses of
336 cysteine and lysine which positively correlated with the level of alkali added (**Table**
337 **2**). Not only did losses of cysteine and lysine occur, also lanthionine and lysinoalanine
338 were formed during cooking. The highest levels (*i.e.* 47 ± 1 µmol lanthionine/g
339 protein and 15 ± 1 µmol lysinoalanine/g protein) of these dehydroalanine derived
340 cross-links were found in the noodles containing 1.5% kansui. It is of note that hard
341 pretzels dipped for 60 s in a 1.0 M NaOH solution at 90 °C prior to baking contained
342 52 µmol lanthionine/g protein and 14 µmol lysinoalanine/ g protein (Rombouts et al.,
343 2012). In kansui containing noodles, lysine not only contributed to lysinoalanine
344 formation but apparently was also involved in other reactions, such as Maillard
345 reactions. No other dehydroalanine-derived cross-links were found. Earlier, Whitaker
346 and Feeney (1983) reported that cystinyl residues are lost much more rapidly in
347 β-elimination reactions than any of the other susceptible amino acid residues. For
348 instance, during heat treatment of wheat gluten in 0.1 M NaOH at 60 °C, cysteine,
349 serine and threonine residues are only consumed in β-elimination reactions at about 3
350 to 7% the rate that cystine is. The kinetics of cross-linking reactions between
351 dehydroalanine and histidine have not been studied. Some guidance here is that
352 lime-processed gelatin contains lower levels of histidinoalanine than of lysinoalanine

353 (Taylor and Wang, 2007).

354

355 To further evaluate the occurrence of β-elimination reactions during production and
356 cooking of kansui containing fresh noodles, the levels of free SH groups in wheat
357 flour, noodle dough after mixing, noodle dough after cutting, and cooked noodles
358 were compared (**Table 3**). Mixing significantly decreased the level of free SH groups
359 in all samples. The decrease in SH content was more pronounced for dough
360 containing 1.0 or 1.5% kansui. As mentioned before, more alkaline conditions
361 increase the ratio of thiolate anions to free SH groups and hence favor oxidation
362 reactions which convert SH groups into SS bonds (Netto et al., 2007). No significant
363 differences between free SH levels of samples taken after mixing and those after
364 cutting were noted (**Table 3**). Also, no significant differences were noted between free
365 SH levels of samples after cutting and those after cooking, except for the samples
366 containing 0.5, 1.0 or 1.5% kansui. For these samples, indications for SH oxidation
367 and β-elimination reactions, which both impact the level of free SH groups, were
368 found. Cooking decreased the level of free SH groups in samples containing 0.5%
369 kansui, but increased it in samples containing 1.0 or 1.5% kansui. That the overall free
370 SH level decreased during the cooking step in the samples containing 0.5% kansui,
371 suggests that β-elimination reactions (which release free SH groups) were
372 quantitatively less important than oxidation reactions (which consume free SH
373 groups). In contrast, the level of free SH groups increased during cooking in samples
374 containing 1.0 or 1.5% added kansui, demonstrating more cystine β-elimination

375 during cooking than oxidation. Thus, the ratio of β-elimination to oxidation reactions
376 during cooking probably increased with increasing kansui level.
377
378 *3.3.Impact of gluten polymerization on product quality*
379 The overall quality of noodles was evaluated based on cooking losses, firmness
380 **(Table 4)** and nutritional profile.
381 Cooking losses increased with increasing salt levels. A contribution to cooking losses
382 is that at least a part of the added salt leached out. Moreover, the presence of (alkaline)
383 salts may also increase cooking losses by increasing the solubility of some α- and
384 γ-gliadins (Ukai et al., 2008). In addition, increased cooking losses can be related to
385 the interplay between the impact of salt on gluten network formation and at the same
386 time on starch gelatinization. On the one hand, with respect to protein network
387 formation, kansui led to increased SS cross-linking of wheat gluten during mixing and
388 cooking due to its impact on pH and, being a salt, probably also affected gluten's
389 secondary structure (**Table 1**). NaCl evidently did not impact pH but, as outlined
390 above, induced changes in the protein secondary structure and thereby led to a more
391 rigid protein network. On the other hand, with respect to starch gelatinization, NaCl
392 and kansui increased its temperature such as estimated by DSC (**Table 5**). This has
393 been attributed to the stabilization of starch polymers by electrostatic interactions
394 between sodium ions and starch hydroxyl groups (Huang and Morrison, 1988; Lai et
395 al., 2002). We here speculate that the gluten network in noodles with (alkaline) salts
396 was already too strong or rigid before starch gelatinization occurred, resulting in

397 increased cooking losses. An analogy is that for pasta a strong protein network is
398 crucial to limit cooking losses, but it must maintain enough resilience to cope with
399 starch swelling during cooking (Bruneel et al., 2010).

400 Both the use of NaCl and kansui increased the firmness of cooked noodles (**Table 4**).
401 Literature relates the firmness of cooked noodles with the starch, protein and lipid
402 constituents (Konik et al., 1992; Ross et al., 1997). In kansui containing noodles,
403 more covalent protein cross-linking occurred than in control samples as reflected in
404 decreased protein extractability (**Table 1).** This may well be the cause of the increased
405 noodle hardness. Remarkably, using a mixture of NaCl and kansui did not yield
406 significantly firmer noodles. Increased insight into the impact of NaCl on starch and
407 lipids would be helpful to better understand its impact on noodle firmness. Noodle
408 appearance and smoothness were improved upon addition of 1.0% NaCl, while
409 optimal quality was observed for noodles with either 0.2 to 1.5% kansui or 2.0% NaCl
410 added.

411 From a nutritional point of view, kansui addition may have some undesired
412 consequences. First, in kansui containing noodles, lysine is involved in
413 dehydroalanine-derived cross-linking and other alkali-induced reactions. Lysine levels
414 decreased from 126 µmol/g protein in fresh control noodles to 107, 103, and 112
415 µmol/g protein in cooked noodles containing 1.0% kansui, 1.5% kansui, and 2.0%
416 NaCl and 0.5% kansui, respectively. With lysine being the limiting essential amino
417 acid of wheat (Kies and Fox, 1970), kansui addition negatively affects the nutritional
418 value of noodles. Secondly, increased protein cross-linking upon alkaline treatment as

419 evidenced by decreased protein extractability may decrease the digestibility of
420 proteins (Savoie et al., 1991). Last but not least, it has been suggested that
421 lysinoalanine may be toxic. However, it is only poorly released by proteolytic
422 enzymes (Savoie et al., 1991).

423

424 ## 4. Conclusions

425 Processing steps prior to cooking do not substantially alter the extractability of gluten
426 of noodles without additives. In contrast, SS bond rearrangements during cooking
427 result in a strong gluten network. The use of table salt reduces the extent of gluten
428 cross-linking during cooking. The hypothesis that changes to the secondary structure
429 upon salt addition lead to more rigid proteins and as a consequence reduce the degree
430 of cross-linking during the cooking stage, remains to be investigated. Use of alkaline
431 salts facilitates gluten network formation during cooking, and at high levels even
432 during fresh noodle production. It increases the level of intermolecular SS bonds and
433 induces β-elimination reactions of cystine followed by formation of lysinoalanine and
434 lanthionine. Even if levels of lanthionine are higher than those of lysinoalanine,
435 literature (Hasegawa et al., 1987) only reported on the presence of the latter in
436 alkaline noodles. In addition, based on decreasing protein extractabilities under
437 reducing conditions in noodles with 1.0 and 1.5% kansui, it is very likely that these
438 dehydroalanine-derived cross-links contribute to the protein network.

439 While the inclusion of table salt or kansui in the recipe increases cooking losses, it
440 positively impacts hardness. Optimal hardness was observed for noodles with either

0.2 to 1.5% kansui or 2.0% NaCl added. Kansui addition reduces the nutritional quality of noodles due to lysine losses and reduces protein digestibility.

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534

535 **7. Tables**

536 **Table 1: Protein extractability in sodium dodecyl sulfate containing buffer of noodle dough, fresh and cooked noodles with various levels**
537 **of NaCl and/or kansui (control: no additive).** Values are reported as percentage of total extractable protein of wheat flour in sodium dodecyl
538 sulfate containing buffer under reducing conditions. Values in the same column with the same letter, and values in the same row with the same
539 numeral, are not significantly different.

540

	Protein extractability (%) of total extractable protein of wheat flour (100%) of					
	Noodle dough after mixing		Fresh noodle (after cutting)		Cooked noodle	
Control	73.9 ± 0.4	1 A	74.4 ± 0.5	1 ABC	21.6 ± 0.4	2 B
0.5% NaCl	75.7 ± 0.9	1 A	75.1 ± 1.1	1 ABC	25.1 ± 0.1	2 A
1.0% NaCl	74.7 ± 1.3	1 A	78.8 ± 1.8	1 A	26.0 ± 0.1	2 A
2.0% NaCl	73.7 ± 1.2	1A	76.4 ± 1.3	1 AB	25.5 ± 0.6	2 A
3.0% NaCl	73.5 ± 0.6	1 AB	75.8 ± 0.4	1 AB	26.4 ± 0.6	2 A
0.2% kansui	72.8 ± 1.0	1 AB	71.1 ± 1.3	1 BC	10.7 ± 0.7	2 CD
0.5% kansui	70.0 ± 0.8	1 AB	69.9 ± 1.5	1 C	9.0 ± 0.2	2 D
1.0% kansui	70.0 ± 0.4	1 AB	69.7 ± 0.2	1 C	12.3 ± 0.1	2 C
1.5% kansui	70.0 ± 0.5	1 B	60.2 ± 0.3	1 D	11.7 ± 0.5	2 C
2.0% NaCl + 0.5% kansui	72.4 ± 0.2	1 AB	73.9 ± 0.3	1 ABC	10.9 ± 0.7	2 CD

541

542 **Table 2: Levels (µmol/g protein) of potential precursors and end products of β-elimination and subsequent dehydroalanine-derived**
543 **cross-linking reactions in noodle dough (after mixing), fresh noodles (after cutting) and cooked noodles.** Amino acid levels with an asterisk
544 are significantly different from the level of this amino acid in control dough after mixing. Amino acid levels of cooked noodles with different
545 additive levels but the same letter are not significantly different.

546

Additive	Sampling after	Cysteine		Histidine		Lysinoalanine		Lanthionine		Lysine		Serine		Threonine	
Control	mixing	187 ± 5		139 ± 3		0 ± 0		0 ± 0		126 ± 3		338 ± 6		202 ± 5	
	cutting	189 ± 15		144 ± 1		0 ± 0		0 ± 0		124 ± 2		337 ± 4		197 ± 1	
	cooking	194 ± 5	A	146 ± 4	A	0 ± 0	D	0 ± 0	D	119 ± 14	AB	334 ± 7	A	196 ± 3	A
3.0% NaCl	mixing	179 ± 7		150 ± 9		0 ± 0		0 ± 0		125 ± 6		355 ± 6		207 ± 3	
	cutting	177 ± 21		157 ± 3		0 ± 0		0 ± 0		128 ± 4		344 ± 12		203 ± 2	
	cooking	210 ± 18	A	163 ± 10	A	0 ± 0	D	0 ± 0	D	127 ± 5	AB	343 ± 11	A	200 ± 1	A
0.2% kansui	cooking	169 ± 7	A	143 ± 16	A	0 ± 0	D	4 ± 1	D	131 ± 7	A	315 ± 18	A	208 ± 9	A
0.5% kansui	cooking	109 ± 11	*B	154 ± 5	A	5 ± 0	*C	15 ± 1	*C	119 ± 2	AB	332 ± 8	A	203 ± 4	A
1.0% kansui	cooking	88 ± 14	*B	152 ± 8	A	7 ± 0	*B	30 ± 1	*B	107 ± 4	*AB	325 ± 6	A	209 ± 3	A

1.5% kansui	mixing	171 ± 3	146 ± 4	0 ± 0	0 ± 0	123 ± 6	348 ± 7	207 ± 3
	cutting	197 ± 27	143 ± 6	0 ± 0	0 ± 0	130 ± 1	348 ± 5	204 ± 1
	cooking	71 ± 11 *B	140 ± 3 A	15 ± 1 *A	47 ± 6 *A	103 ± 3 *B	348 ± 12 A	203 ± 7 A
2.0% NaCl + 0.5% kansui	mixing	178 ± 13	141 ± 6	0 ± 0	0 ± 0	126 ± 13	342 ± 18	195 ± 8
	cutting	182 ± 14	134 ± 4	0 ± 0	0 ± 0	129 ± 3	340 ± 6	191 ± 2
	cooking	109 ± 16 *B	135 ± 7 A	8 ± 1 *B	19 ± 1 *C	112 ± 4 *AB	349 ± 6 A	199 ± 3 A

547

548

549 **Table 3: Levels (µmol/g protein) of free SH groups in wheat flour, noodle dough, fresh and cooked noodles with various levels of NaCl**

550 **and/or kansui (control: no additive).** Values in the same column with the same numeral, and values in the same row with the same letter, are

551 not significantly different. Free SH levels significantly different from that of wheat flour are indicated with an asterisk.

552

Sample	Wheat flour	Noodle dough after mixing	Fresh noodle (after cutting)	Cooked noodle
	5.59 ± 0.14			
Control		2.85 ± 0.51 * A 1	2.96 ± 0.01 * A 1	1.80 ± 0.38 * A 3
3.0% NaCl		2.95 ± 0.34 * A 1	2.43 ± 0.55 * A 1	1.87 ± 0.24 * A 3
0.2% kansui		2.73 ± 0.38 * A 1		1.35 ± 0.10 * A 3
0.5% kansui		3.34 ± 0.26 * A 1		0.83 ± 0.08 * B 3
1.0% kansui		1.24 ± 0.49 * B 2		4.08 ± 0.70 * A 2
1.5% kansui		1.28 ± 0.27 * B 2	1.65 ± 0.34 * A 1	6.46 ± 0.92 A 1
2.0% NaCl + 0.5% kansui		2.73 ± 0.59 * A 1	2.00 ± 0.90 * A 1	1.74 ± 0.02 * A 3

553

554

555 **Table 4: Cooking loss and firmness of cooked noodles with different levels of NaCl and/or kansui (control: no additive).** Values in the

556 same column with the same letter are not significantly different.

557

Sample	Cooking loss (%)				Firmness (g)			
Control	4.22	±	0.04	E	1638	±	96	D
0.5% NaCl	4.30	±	0.07	E	1764	±	74	CD
1.0% NaCl	5.02	±	0.03	D	1910	±	202	BCD
2.0% NaCl	5.59	±	0.04	C	2152	±	173	ABC
3.0% NaCl	5.82	±	0.17	BC	1896	±	129	BCD
0.2% kansui	4.46	±	0.09	E	2304	±	257	AB
0.5% kansui	5.16	±	0.04	D	2443	±	205	A
1.0% kansui	9.00	±	0.16	A	2130	±	250	ABC
1.5% kansui	8.82	±	0.09	A	2151	±	310	ABC
2.0% NaCl + 0.5% kansui	6.11	±	0.09	B	1793	±	187	CD

558

559

560 **Table 5: Onset, peak and conclusion temperatures (°C) corresponding to the melting of amylopectin crystals (gelatinization) of noodle**

561 **dough (after mixing) containing different levels of NaCl and/or kansui (control: no additive).**

562

Sample	Onset T	Peak T	Conclusion T
Control	54.2 ± 0.2	61.7 ± 0.2	70.0 ± 0.3
0.5% NaCl	54.7 ± 0.3	60.8 ± 0.0	69.5 ± 0.2
1.0% NaCl	55.7 ± 0.2	62.1 ± 0.2	70.0 ± 0.3
2.0% NaCl	57.2 ± 0.1	63.0 ± 0.1	71.3 ± 0.3
3.0% NaCl	57.8 ± 0.2	64.3 ± 0.0	72.7 ± 0.3
0.2% kansui	55.2 ± 0.1	65.0 ± 0.1	74.3 ± 0.5
0.5% kansui	55.9 ± 0.3	61.3 ± 0.0	71.1 ± 0.2
1.0% kansui	57.7 ± 0.1	62.9 ± 0.0	72.7 ± 0.2
1.5% kansui	58.8 ± 0.3	64.7 ± 0.1	73.7 ± 0.3
2.0% NaCl + 0.5% kansui	57.6 ± 0.2	65.7 ± 0.0	75.4 ± 0.2

563