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Cross-linking of wheat gluten proteins during production of hard pretzels

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Running title

Protein cross-links in pretzels

Abstract

The impact of the hot alkaline dip, prior to pretzel-baking, on the types and levels of cross-links between wheat proteins was studied. Protein extractability of pretzel dough in sodium dodecyl sulfate containing buffer decreased during alkaline dipping [45 s, 1.0 % (w/v) NaOH, 90°C], and even more so during baking (3 min at 250 °C) and drying (10 min at 135 °C). As reducing agent increased the extractability partly, both reducible and non-reducible protein cross-links had been formed. The decrease in cystine and Lys levels suggested β-elimination of cystine releasing Cys and dehydroalanine (DHA). Subsequent reaction of DHA with Lys and Cys, forming the potentially cross-linking unusual amino acids lysinoalanine (LAL) and lanthionine (LAN), respectively, was observed both in alkaline dipped dough (7 µmol LAN/g protein) and in end product (9 µmol LAL and 50 µmol LAN/g protein). The baking/drying step increased sample redness and decreased Lys levels more than expected based on LAL formation, suggesting that Maillard-derived cross-links also contribute to the observed extractability loss. Higher dipping temperatures, longer dipping times, and higher NaOH concentrations increased protein extractability losses and redness, as well as LAL and LAN levels in the end product. However, no indications for Maillard-derived cross-links or LAL in pretzel dough immediately after dipping were found, even when severe dipping conditions were used.

Keywords

Gluten

Beta-elimination

Dehydroalanine

Lysinoalanine

Lanthionine

1. Introduction

Hard pretzels are popular savory wheat-based snacks, often with the shape of a knot or a stick. The dough, which typically consists of wheat flour, water, shortening, salt and a leavening agent, is shaped by relatively low pressure extrusion, treated with hot alkaline solution, and baked. Baking is divided into a rapid initial bake at high temperature, followed by a slow drying process at lower temperature (Delcour and Hoseney 2010; Walsh 1993; Seetharaman et al. 2004). The unique taste and hard shiny surface of pretzels result from the alkaline dipping prior to baking, typically for 30 to 45 s in a 1.0 % (w/v) NaOH at 80 to 90 °C. This treatment gelatinizes starch granules at the dough surface, dissociates amylose-lipid complexes, decreases levels of reducing sugars, and induces Maillard and caramelization reactions (Yao et al. 2006; Walsh 1993). Although the impact of such alkaline dip on the starch fraction is relatively well understood, the impact on the protein fraction is not clear. While conditions during pretzel production induce protein hydrolysis (Yao et al. 2006), it remains to be elucidated whether and/or to what extent they affect amino acid degradation and protein cross-linking. This study focuses on protein cross-linking which already has been shown to greatly determine the texture and structure of various wheat-based foods (Lindsay and Skerritt 1999; Kuktaite et al. 2004) including bread (Payne et al. 1987) and pasta (Cubadda et al. 2007).

Gluten is the complex heterogeneous mixture of wheat storage proteins. It consists of monomeric gliadins and polymeric glutenins. Heat-induced gluten cross-linking is mainly ascribed to formation of cysteine disulfide (SS) cross-links (Schofield et al. 1983), which are favored by high pressure and temperature (Kieffer et al. 2007), and alkaline pH (Visschers and de Jongh 2005). Indeed, SS cross-linking results from oxidation of reactive sulfhydryl (SH) groups and interchange reactions between SH groups and SS bonds (Lagrain et al. 2008). The reactivity of SH groups, which have a pK_a of 8.35 when fully exposed to water (Belitz et al. 2009a), is pronounced at alkaline pH, hence our interest in evaluating the impact of dipping

66 conditions on formation of SS cross-links in pretzels. Previous research using gluten model
1
2 67 systems has suggested that the severe heat/alkali conditions during pretzel dipping may well
3
4 68 induce β-elimination of cystine (Rombouts et al. 2010; Lagrain et al. 2010). In such reaction,
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6
7 69 the hydrogen atom of the chiral carbon of an intra- or intermolecular SS bond is first abstracted,
8
9 70 and a persulfide in β-position of the chiral carbon is eliminated, yielding the amino acid
10
11
12 71 dehydroalanine (DHA). Then, elimination of sulfur from the newly formed persulfide leads to
13
14 72 Cys containing a free SH group, which can initiate SH oxidation or SH-SS interchange
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16
17 73 reactions. This way, SS cross-links can be formed, but DHA residues can also react with *e.g.*
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19 74 Cys or Lys to form the cross-links lanthionine (LAN) or lysinoalanine (LAL), respectively. An
20
21
22 75 important distinction between SS and DHA derived cross-links is that only the first are
23
24 76 reducible. Although cross-linking as a result of β-elimination reactions is important for wool
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26
27 77 (Horn et al. 1941) and other (Linetsky et al. 2004; Zimmermann et al. 1993) proteins, literature
28
29 78 about such cross-linking in food products is scarce and mainly limited to LAL formation (Maga
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31
32 79 1984; Friedman 1999; Sternberg et al. 1975). Furthermore, pretzel production conditions induce
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34 80 reactions between amino (NH_2) groups of proteins and reducing sugars, which initiates a
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36
37 81 number of consecutive reactions classed under the term 'Maillard reaction'. High temperature,
38
39 82 low moisture content (mc), and alkaline conditions all promote this reaction, which *inter alia*
40
41 83 involves the ε-NH_2 group of Lys and yields cross-links and colored products (Vaclavik and
42
43
44 84 Christian 2008).
45
46 85 The main objective of our work was to investigate the impact of the hot alkaline dip on the
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48
49 86 formation of cross-links between proteins in hard pretzels. To that end, protein extractability,
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51 87 amino acid levels and cross-links, and pretzel color were monitored during the pretzel-making
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54 88 process. In addition, the effect of possible deviations from a standard pretzel production process
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56 89 on protein cross-linking was studied.
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2. Materials and Methods

Materials

All solvents, chemicals and reagents were at least of analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany) or VWR International (Leuven, Belgium), unless specified otherwise.

Commercial wheat flour [9.3 % protein (N x 5.7) on dry matter basis (db), mc 11.2 %] was from Meneba (Hasselt, Belgium), and commercial wheat gluten [74.7 % protein (N x 5.7, db), mc 7.4 %] was from Syral (Aalst, Belgium). Sugar was from Tiense Suikerraffinaderij (Tienen, Belgium), shortening (all vegetable, Crisco) was from J. M. Smucker (Orville, OH, USA), and compressed yeast was from Bruggeman (Ghent, Belgium).

Determination of Protein and Moisture Contents

Protein contents were determined in triplicate, using an adaptation of the AOAC Official Method 990.03 (AOAC 1995) to an automated Dumas protein analysis system (EAS Variomax N/CN, Elt, Gouda, The Netherlands). A conversion factor of 5.7 was used to calculate protein from nitrogen content. The determination of mc was as described by AACC-I Approved Method 44-19 (AACC 2000).

Pretzel Production

Flour (100.0 g), sugar (2.5 g), shortening (2.5 g), and yeast (0.5 g) were mixed for 3 min using a pin mixer (National Manufacturing, Lincoln, NE, USA). After addition of 50 ml water, the dough was mixed for 1 min, fermented for 20 min (30 °C, 90 % relative humidity), and sheeted (gap width 2.5 mm, National Manufacturing sheeter). Pretzel sticks [length 56.4 (± 0.8) mm, height 2.6 (± 0.1) mm, width 3.9 (± 0.0) mm], were cut out of the dough. After 10 min fermentation, pretzel sticks were dipped for 15, 30, 45, or 60 s in 0.0, 0.5, 1.0, or 1.5 % (w/v)

NaOH at 50, 65, 80, or 90 °C. The dipped pretzel sticks were baked for 3 min at 250 °C, and dried for 10 min at 135 °C.

In addition, gluten-water dough was prepared by manually mixing 100.0 g commercial gluten with 60 ml water. After a 20 min rest (30 °C, 90 % relative humidity), the gluten dough was rolled into balls (500 mg), which were dipped for 45 s in 1.0 % (w/v) NaOH at 90 °C.

All samples were freeze dried and ground in a laboratory mill (250 µm, IKA, Staufen, Germany) prior to further analysis.

Determination of Protein Extractability

To determine the protein extractability in SDS containing media and the protein molecular weight distribution, size-exclusion high-performance liquid chromatography (SE-HPLC) was performed as described by Lagrain and co-workers (2005), using an LC-2010 system (Shimadzu, Kyoto, Japan) with automatic injection. Freeze dried samples [1.0 mg protein (db)/ml] were extracted (60 min, 20 °C) with a 50 mM sodium phosphate buffer (pH 6.8) containing 2.0 % (w/v) sodium dodecyl sulfate (SDS) (further referred to as SDS buffer). The extractability of proteins in SDS containing media under reducing conditions was determined under nitrogen atmosphere. In this case, 2.0 M urea and 1.0 % (w/v) dithiothreitol were added to the SDS buffer. All analyses were performed in duplicate. After centrifugation (10 min, 11,000*g*) and filtration over polyethersulfone (Millex-HP, 0.45 µm, Millipore, Carrigtwohill, Ireland), supernatants were loaded (60 µl) on a Biosep-SEC-S4000 column with separation range from 15,000 to 500,000 (Phenomenex, Torrance, CA, USA). The elution solvent was acetonitrile/water (1/1, v/v) containing 0.05 % (v/v) trifluoroacetic acid. The flow rate was 1.0 ml/min and the column temperature 30 °C. Protein elution was monitored at 214 nm. Extractability in SDS containing media (under non-reducing and reducing conditions) of any given sample was calculated from its peak area and expressed as percentage of total

extractability. The latter was taken as the peak area of the unheated pretzel dough, extracted in SDS buffer under reducing conditions. All analyses were performed in duplicate.

Amino Acid Analysis and Determination of LAN and LAL

Amino acids were first liberated by heating (24h, 110 °C) freeze dried samples (15.0 mg protein) in 1.0 ml 6.0 M HCl containing 0.1 % phenol and 1.5 mM norleucine (as internal standard) after flushing the samples with nitrogen. Reaction mixtures were diluted (200-fold) in deionized water and filtered (Millex-GP, 0.22 µm, polyethersulfone, Millipore). Amino acids, including LAN and LAL, were then separated by high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD), using a Dionex BioLC system (Dionex, Sunnyvale, CA, USA) as described by Rombouts and co-workers (2009). Separation of 25 µl samples was performed at 30 °C with an AminoPac PA10 guard (50 x 2 mm, Dionex) and analytical (250 x 2 mm, Dionex) column at a flow rate of 0.25 ml/min. Four eluents were used for the gradient mobile phases: water of at least 18.2 MΩ resistivity (A), and solutions of NaOH (B; 0.250 M), sodium acetate (C; 1.0 M), and acetic acid (D; 0.100 M). Gradient conditions were as in Rombouts and co-workers (2009) and the detection waveform as in Ding and co-workers (2002). LAN and LAL were detected using a gold working electrode and a pH reference electrode. Their levels were calculated using appropriate standards, and expressed on dry matter protein (µmol/g protein). All analyses were performed in triplicate. Relative standard deviations of amino acid levels did not exceed 10 %.

Determination of Cys

Cys residues were first oxidized to Cys sulfonic acid and subsequently hydrolyzed and chromatographically 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

165 sample (20.0 mg protein). The reaction mixture was stirred (15 min, 0 °C) and then left
166 overnight (16 h, 0 °C). To reduce the excess of performic acid, 0.5 ml 48 % hydrogen bromide
167 was added, and the mixture was stirred for 30 min. The remaining bromine and formic acid
168 were evaporated at 50 °C, and samples were subjected to amino acid analysis.
169

170 **Determination of DHA**

171 Gluten samples (100 mg) were heated in sealed reaction tubes in 1.5 N HCl (0.50 ml) at 110
172 °C for 120 min to liberate DHA as pyruvic acid, which was then determined colorimetrically
173 after a clarification step (Rombouts et al. Accepted, 01/04/2011).
174

175 **Color determination**

176 Average color parameters [L* (luminosity), a* (redness), b* (yellowness)] of ground samples
177 were determined after five-fold measurement with a colorimeter (Colourquest 45/0 LAV,
178 CQ/UNI-1600, HunterLab, Reston, VA,USA). Standard deviations of the average readings of
179 three individual samples did not exceed 0.2.
180

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65

3. Results and Discussion

Protein extractability loss during conventional pretzel-making

Proteins of unheated pretzel dough, alkaline dipped (45 s, 90 °C, 1.0 % NaOH) pretzel dough, and baked pretzel were extracted in SDS containing buffer, separated based on molecular weight using SE-HPLC, and quantified (Figure 1a). The main proteins in pretzel dough are gluten proteins. Under the used experimental conditions, polymeric glutenin and monomeric gliadin eluted before and after 7.2 min, respectively (Lagrain et al. 2005). The SE-HPLC profiles showed decreasing extractabilities of both fractions during alkaline dipping and baking. The glutenin proteins in baked pretzel were no longer extractable. The observed extractability loss is a result of the formation of either SS or non-SS protein cross links. To distinguish between SS and non-SS cross-links and to evaluate their separate impact on extractability loss, proteins were also extracted under reducing conditions, *i.e.* after cleavage of SS bonds (Figure 1b). In what follows, extractabilities are reported as percentages of the total extractability of proteins in unheated pretzel dough under reducing conditions. In unheated pretzel dough, 79.4 % of the proteins were extractable under non-reducing conditions. The alkaline dip reduced their extractabilities under non-reducing and reducing conditions to 46.6 and 92.5 %, respectively, and baking reduced them further to 9.1 and 24.0 %, respectively. The substantial increase of protein extractability upon reducing SS bonds illustrates the importance of SS cross-links for gluten network formation. High temperatures and alkaline pH, such as during pretzel production, favor SH-SS interchange reactions, and hence the formation of SS cross-links (Lagrain et al. 2011). That reduction did not completely restore protein extractability of the dipped pretzel and the end product, illustrated that also non-SS cross-links were formed during dipping and baking.

206 **Amino acid degradation and cross-linking during conventional pretzel-making**

207 Although various amino acids are susceptible to degradation reactions under alkaline 208 conditions (Whitaker and Feeney 1983), we only observed Lys and Cys losses during pretzel-209 making. Dipping (45 s, 90 °C, 1.0 % NaOH) did not affect Lys levels, but the additional baking 210 step decreased them from 176 µmol/g protein in unheated dough to 119 µmol/g protein in the 211 final product. Dipping decreased the Cys level from 144 µmol/g protein in the unheated dough 212 to 134 µmol/g protein and subsequent baking reduced it to 51 µmol/g protein. The cross-links 213 LAL and LAN (see Figure 2) were formed during pretzel-making. No LAL, but 7 µmol LAN/g 214 protein was found in alkaline dipped pretzel dough. The final product contained 9 µmol/g 215 protein LAL and 50 µmol LAN/g protein. Sternberg and co-workers (1975) detected 2 µmol 216 LAL/g protein in pretzels. Unfortunately, they did not report on the applied pretzel-making 217 conditions.

218 Pretzel-making conditions thus clearly induce β-elimination and subsequent cross-linking 219 reactions. In gluten model systems, each mole of cystine yields one mole of DHA (Lagrain et 220 al. 2010), which can react with Lys or Cys to form LAL or LAN, respectively. Thus, β-221 elimination and the subsequent cross-linking reactions do not alter the sum of the levels of 222 cystine, DHA, LAN and LAL (Lagrain et al. 2010). DHA levels were difficult to determine 223 accurately in pretzel dough, probably due to interference of non-protein components during the 224 colorimetric assay. Dipping gluten-water dough for 45 s at 90 °C in 1.0 % NaOH produced 4.0 225 µmol DHA/g protein, so the cystine loss (10 ± 1 µmol/g protein) during dipping corresponded 226 to the formation of DHA (4.0 ± 0.3) and LAN (7 ± 1 µmol/g protein). In contrast, β-elimination 227 and the subsequent cross-linking reactions could not explain the great cystine loss during 228 baking. Similarly, unchanged Lys levels after dipping corresponded to the absence of LAL in 229 dipped dough, but the formation of 7 µmol LAL/g protein during baking only partly explained 230 the loss of 57 µmol Lys/g protein. During baking, cystine and Lys are probably also involved in

other heat-induced reactions, including the Maillard reaction. The potential consumption of Lys by the Maillard reaction is discussed in the next section.

Color changes during conventional pretzel-making

The Maillard reaction, which could explain the disproportionate Lys loss during baking, mainly yields orange and red colored products, some of which are cross-links (Biemel et al. 2001; Dai et al. 2007), and is thus correlated with increasing a* values on the Cielab color scale (Belitz et al. 2009b; Lamberts et al. 2008; Mestdagh et al. 2008). Dipping did not increase the a* value, probably because high humidity disfavors the Maillard reaction (Anese et al. 1999). In this context, it is of note that dipping did not affect the Lys levels either. Yet, redness increased from -1.0 to 5.8 as a result of baking, so Maillard-derived cross-links may well have been formed in this process step. Figure 2 gives an overview of reactions contributing to protein cross-linking during pretzel-making.

The impact of pretzel-making conditions on protein cross-linking

Reaction temperature, time, and pH impact cross-linking reactions in gluten (Lagrain et al. 2010; Lagrain et al. 2011). We here investigated the impact of deviations from the standard conditions on protein cross-linking during alkaline dipping. Pretzel dough was dipped for 45 s in 1.0 % NaOH at 50, 65, 80, or 90 °C. Firstly, no extractability loss was observed after dipping at 50 °C (Figure 3). Secondly, dipping at 65 °C decreased the extractability under non-reducing conditions (Figure 3a), but this could be restored by reduction (Figure 3b), suggesting that mainly new SS cross-links were formed during dipping at lower temperature. Thirdly, dipping at 80 or 90 °C decreased the extractability under non-reducing as well as reducing conditions, so higher temperatures induced SS as well as non-SS cross-link formation. The impact of dipping time on protein extractability was investigated by dipping pretzel dough for

256 15, 30, 45, or 60 s in 1.0 % NaOH at 90 °C. Protein extractability under non-reducing
257 conditions decreased as a function of dipping time (Figure 4a). Under reducing conditions
258 (Figure 4b) no decrease was noted during the first 15 s of the alkaline dip, which suggested that
259 initially mainly new SS cross-links caused the extractability loss. Longer dipping decreased the
260 extractability as a function of dipping time, so after prolonged dipping also non-SS cross-links
261 became detectable. Finally, dipping pretzel dough for 45 s in 0.5, 1.0, or 1.5 % NaOH at 90 °C
262 decreased protein extractability under non-reducing conditions from 79.4 to 45.4 %, and
263 subsequent baking further reduced it to 9.1 % (Figure 5a), irrespective of the applied NaOH
264 concentration.

265 Under the experimental conditions, extractability loss under non-reducing conditions was
266 apparently not impacted by NaOH concentration, possibly because the dipping solutions with
267 0.5, 1.0, and 1.5 % (w/v) NaOH only had a slightly different pH, *i.e.* 13.1, 13.4, and 13.6,
268 respectively. However, higher NaOH concentrations increased extractability losses during
269 dipping under reducing conditions (Figure 5b), indicating that they led to more non-SS cross-
270 links. The non-SS cross-links formed during dipping may well be LAN, of which the level in
271 the dipped pretzel dough increased with dipping temperature, time, and NaOH concentration
272 (Figure 6). No indications for non-SS cross-links other than LAN were found in any sample
273 immediately after dipping. Indeed, even dipping for 60 s (90 °C, 1.0 % NaOH), or in 1.5 %
274 NaOH (45 s, 90 °C), did not induce noticeable LAL formation (Figure 7) or increased a* values
275 (results not shown). In conclusion, the impact of dipping conditions on protein cross-linking
276 during dipping is limited.

277

278 Baking reduced the protein extractability under non-reducing conditions further to a minimum
279 of 9.1 %, irrespective of the applied dipping temperature, duration, or concentration (Figures
280 3a, 4a, 5a). In contrast, the protein extractability loss under reducing conditions was larger

281 when higher temperatures, longer dipping times, or higher NaOH concentrations were applied,
282 probably due to more non-SS cross-link formation (Figures 3b, 4b, 5b). More severe dipping
283 conditions yielded pretzels with higher redness and higher LAN (Figure 6) and LAL (Figure 7)
284 levels. Indeed, the formation of Maillard reaction products increases redness, and LAN and
285 LAL are potential cross-links. The linear correlations between the extractability loss under
286 reducing conditions and the sum of LAN and LAL levels (r = 0.988) supported the hypothesis
287 that the formed LAN and LAL links are at least partly intermolecular, and hence contribute to
288 the protein network. Maximum LAL levels (14 µmol/g protein) were found in the pretzels
289 dipped for 45 s at 90 °C in 1.5 % NaOH, while LAN formation was maximal (52 µmol/g
290 protein) after dipping for 60 s at 90 °C in 1.0 % NaOH. Another interesting observation was
291 that even dipping at 50 °C (45 s, 1.0 % NaOH), or dipping in 0.5 % NaOH (45 s, 90 °C), or
292 dipping for 15 s (90 °C, 1.0 % NaOH) led to end products with non-reducible cross-links.
293
294 The above results demonstrated the key role of non-SS cross-links into the protein network in
295 pretzels which are dipped in a hot alkaline solution prior to baking. To further investigate the
296 impact of pH, we evaluated protein cross-linking during dipping for 45 s in water at 90 °C and
297 subsequent baking and drying. The dip at neutral pH decreased protein extractability under non-
298 reducing conditions from 79.4 to 72.9 %, and subsequent baking further decreased it to 35.1 %
299 (Figure 5a). This extractability loss was caused by formation of SS cross-links, as protein
300 extractability under reducing conditions remained constant during the dip in water and
301 subsequent baking (Figure 5b). No LAN or LAL were found in the products obtained by
302 dipping in water and subsequent baking (Figures 6 and 7). Thus, in comparison to alkaline
303 dipping, dipping in water limited SS cross-linking and even prevented non-SS cross-linking.
304 These results suggest that the protein network in bagels, which are boiled in water prior to

baking, is probably less strong than that in pretzels. The alkaline conditions during dipping make pretzel production unique.

Abbreviations used

dehydroalanine (DHA)

high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD)

lysinoalanine (LAL)

lanthionine (LAN)

moisture content (mc)

sodium dodecyl sulfate (SDS)

size-exclusion high-performance liquid chromatography (SE-HPLC)

sulfhydryl (SH)

disulfide (SS)

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Figure Captions

Figure 1 SE-HPLC profiles of proteins in unheated pretzel dough, pretzel dough after alkaline dip [90 °C, 45 s, 1.0 % NaOH (w/v)], and baked pretzels. Proteins were extracted in SDS containing buffer under non-reducing **(a)** and reducing **(b)** conditions (see text). A.U.: arbitrary units.

Figure 2 Overview of reactions during pretzel-making resulting in protein cross-links.

Figure 3 Protein extractabilities in SDS containing buffer under non-reducing **(a)** and reducing **(b)** conditions of unheated pretzel dough, pretzel dough after alkaline dip, and baked pretzels. Pretzel dough was dipped for 45 s in 1.0 % (w/v) NaOH at 50, 65, 80 or 90 °C. Extractabilities of samples indicated with the same letter are not significantly different ($P > 0.05$, $\alpha = 0.05$).

Figure 4 Protein extractabilities in SDS containing buffer under non-reducing **(a)** and reducing **(b)** conditions of unheated pretzel dough, pretzel dough after alkaline dip, and baked pretzels. Pretzel dough was dipped for 15, 30, 45, or 60 s in 1.0 % (w/v) NaOH at 90 °C. Extractabilities of samples indicated with the same letter are not significantly different ($P > 0.05$, $\alpha = 0.05$).

Figure 5 Protein extractabilities in SDS containing buffer under non-reducing **(a)** and reducing **(b)** conditions of unheated pretzel dough, pretzel dough after (alkaline) dip, and baked pretzels. Pretzel dough was dipped for 45 s in 0.0, 0.5, 1.0, or 1.5 % (w/v) NaOH at 90 °C. Extractabilities of samples indicated with the same letter are not significantly different ($P > 0.05$, $\alpha = 0.05$).

Figure 6 Impact of temperature and concentration of NaOH concentration, and duration of dipping, on lanthionine (LAN) levels (µmol/g protein) in pretzel dough after (alkaline) dip, and after baking. Pretzel dough was dipped for 45 s in 1.0 % (w/v) NaOH at 90 °C, unless specified otherwise.

Figure 7 Impact of temperature and concentration of NaOH concentration, and duration of dipping, on lysinoalanine (LAL) levels (μmol/g protein) in pretzel dough after (alkaline) dip, and after baking. Pretzel dough was dipped for 45 s in 1.0 % (w/v) NaOH at 90 °C, unless specified otherwise.

449 **Figure 1**

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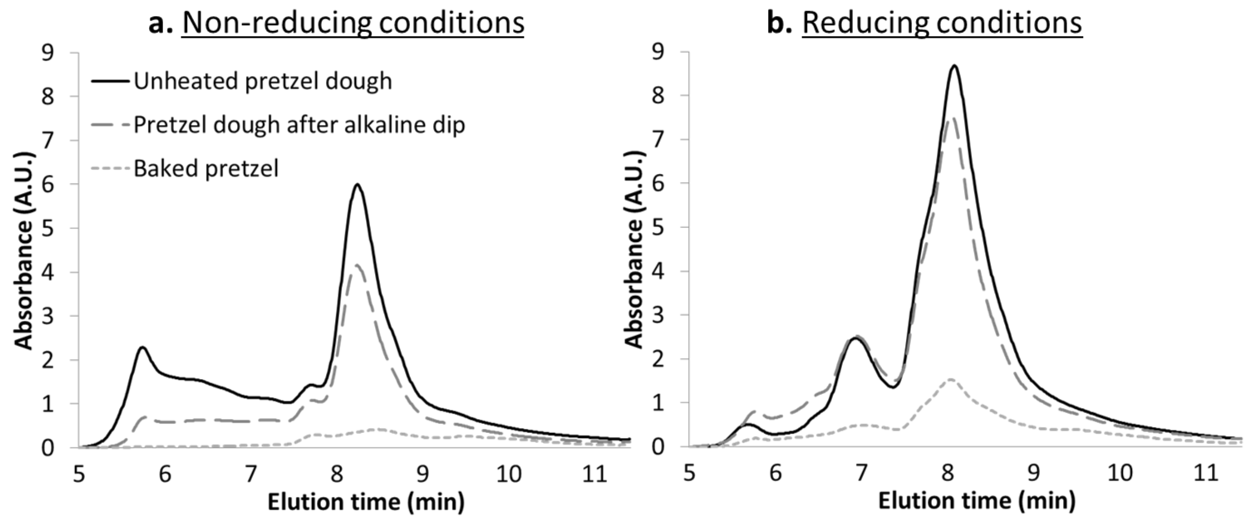

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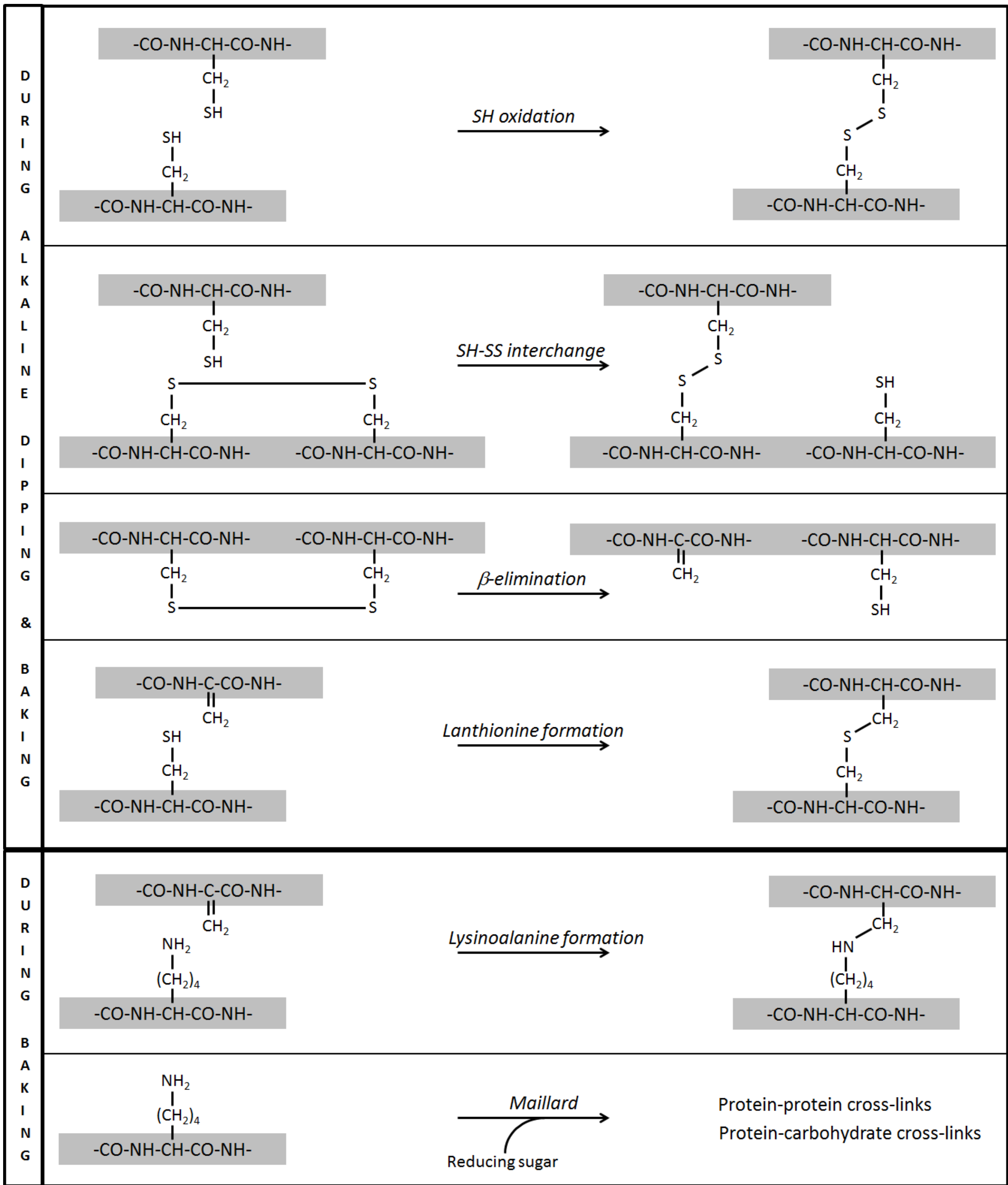
452 **Figure 2**



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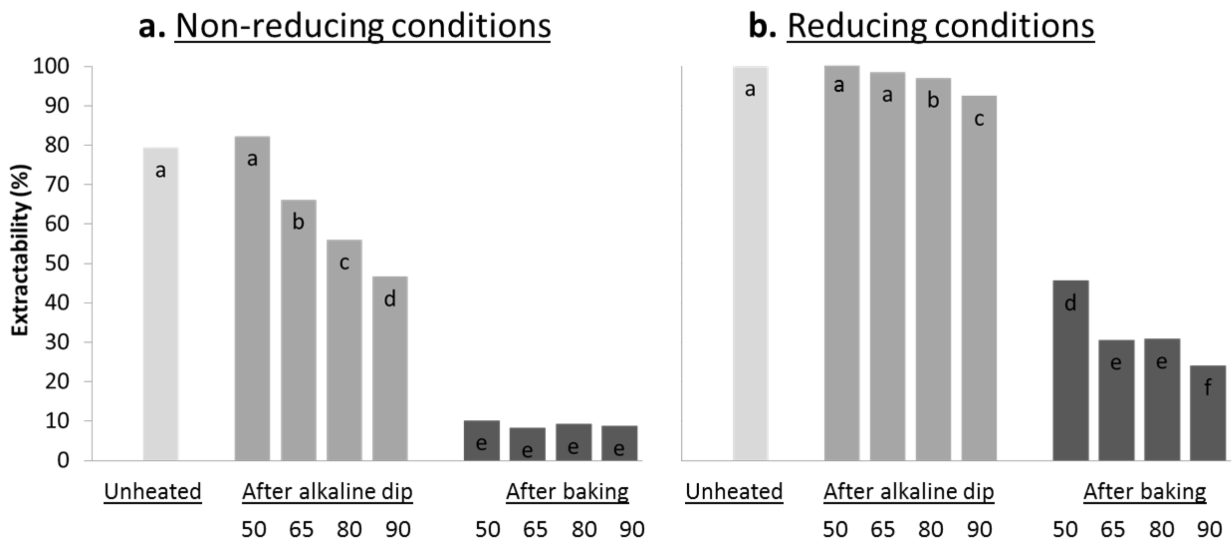
455 **Figure 3**



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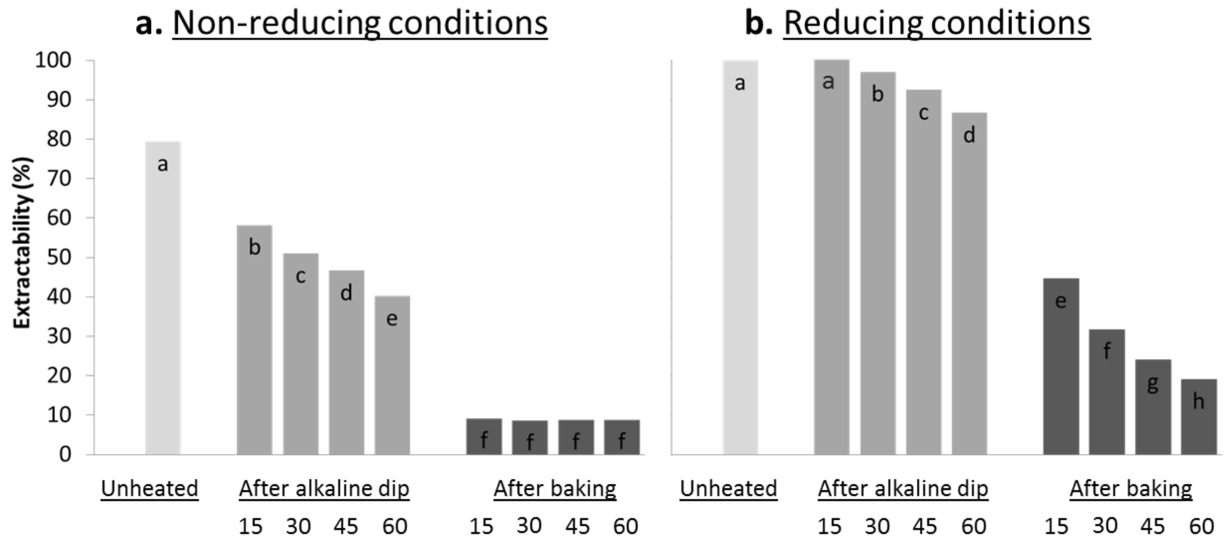
458 **Figure 4**



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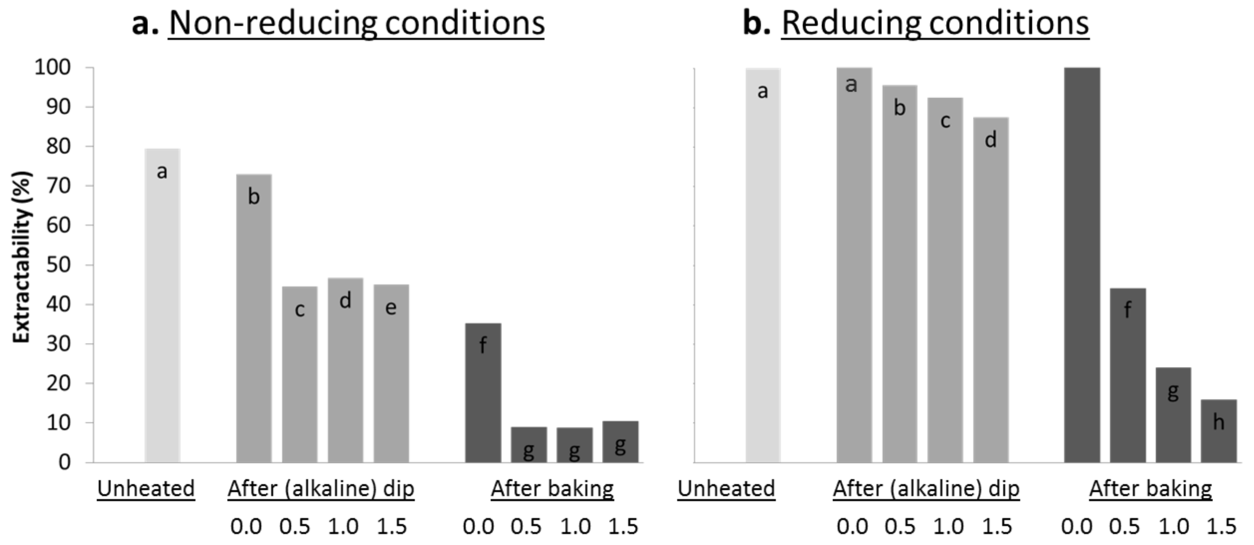
461 **Figure 5**



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464 **Figure 6**

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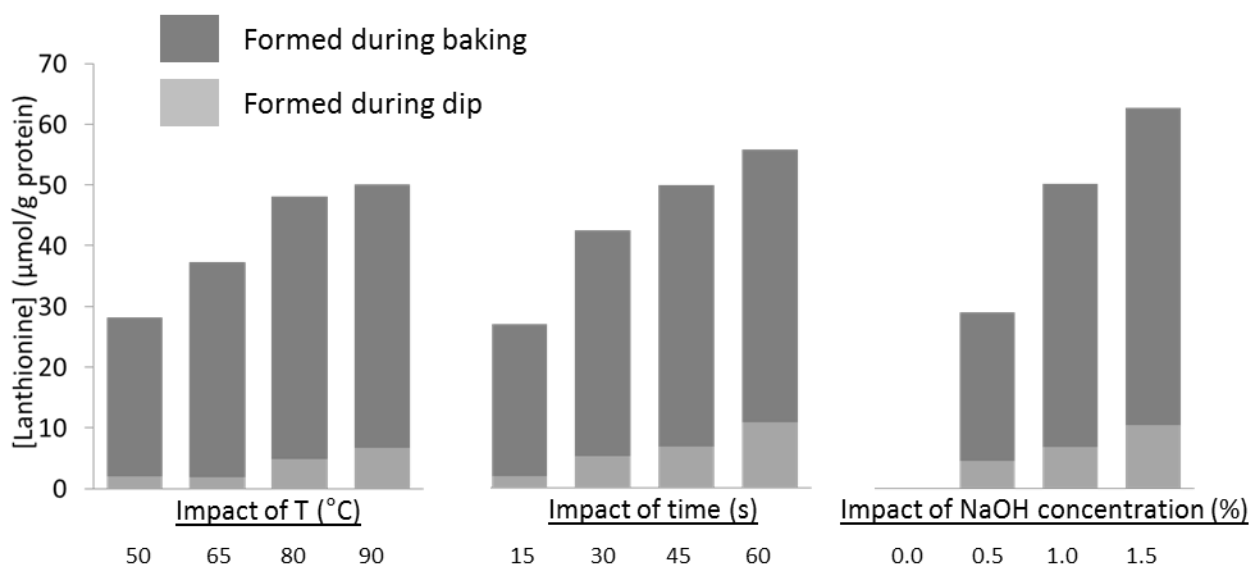

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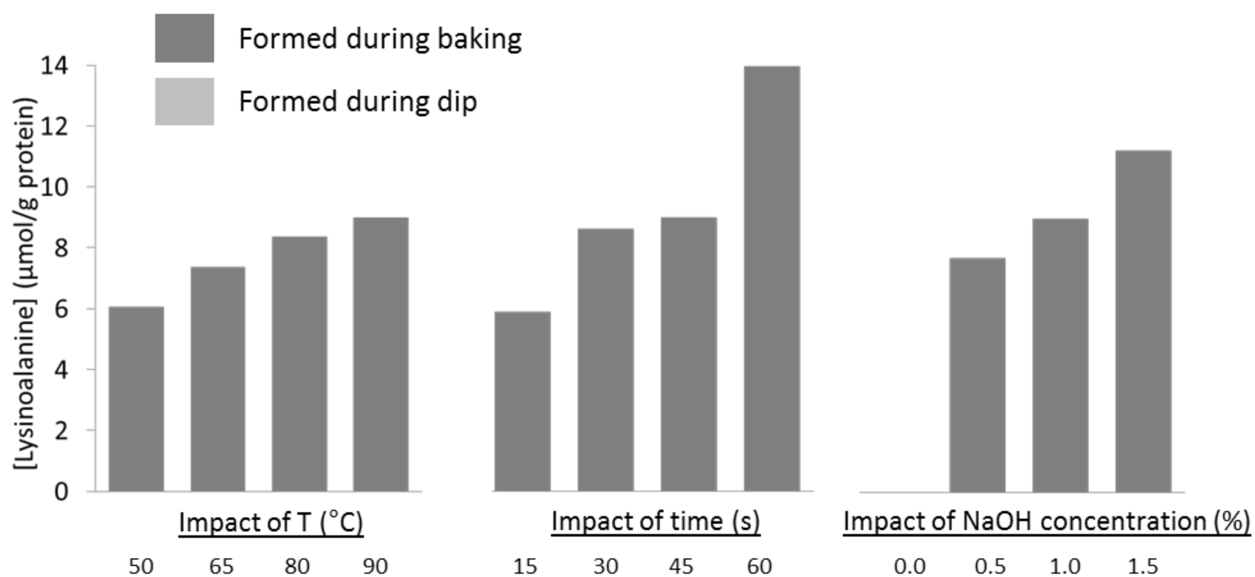
467 **Figure 7**



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