The role of wheat and egg constituents in the formation of a covalent and non-covalent protein network in fresh and cooked egg noodles

Marlies A. Lambrecht*, Ine Rombouts, Mieke A. Nivelle, Jan A. Delcour

KU Leuven, Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

*Corresponding author, Tel.: +32 16 376781; fax: +32 16 321997.

E-mail address: marlies.lambrecht@biw.kuleuven.be

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Abstract

Noodles of constant protein content and flour-to-egg protein ratio were made with whole egg, egg white or egg yolk. The optimal cooking time, water absorption and cooking loss of salted whole egg noodles was respectively lower and higher than of egg white and egg yolk noodles. However, cooked whole egg noodles showed the best Kieffer-rig extensibility. Differences in noodle properties were linked to protein network formation. Disulfide bonds in whole egg noodles developed faster and to a larger extent during cooking than in egg yolk noodles but slower and to a lower extent than in egg white noodles. The balance between the rate of protein cross-linking and starch swelling determines cooked noodle properties. Ionic and hydrophobic protein interactions increase the optimum cooking time and total work in Kieffer-rig extensibility testing of fresh noodles. Hydrogen bonds and covalent cross-links are probably the main determinants of the extensibility of cooked noodles.

Key words

Gluten, egg white, egg yolk, polymerization, proton mobility

Practical application

Eggs enhance the flavor, color, texture and nutritional value in pasta and noodles. This paper studies the role of wheat and egg constituents in protein network formation and the potential consequences thereof for noodle quality. Understanding the functionality of wheat flour, whole egg and its fractions in noodles will facilitate the optimization of egg pasta and noodle recipes and the search for egg substitutes.

1. Introduction

Noodles are usually made from common wheat (*Triticum aestivum* L.) flour by dough sheeting while pasta is produced from durum wheat (*Triticum turgidum* L. *var. durum*) semolina by dough extrusion (Fu 2008). Eggs are included to enhance nutritional value, (Li and others 2014) flavor (Alamprese and others 2012) and quality. The latter is typically evaluated in terms of color, symmetry, cooking quality (low water absorption and cooking loss) and texture (Oh and others 1983). Eggs improve the cooking quality of especially soft wheat flour noodles (Dalbon and others 1996). The break load of cooked pasta increases with total egg content (Alamprese and others 2009). Henn eggs consists of two edible fractions, *i.e.* egg white and egg yolk, which are present in a ratio 2 to 1 (Powrie and Nakai 1985). A higher egg white to yolk ratio increases the strength of both fresh (raw) and cooked pasta. However, it also increases the protein and reduces the lipid contents of pasta (Alamprese and others 2009). The underlying mechanisms whereby and to what extent egg constituents impact noodle quality remain to be elucidated.

Noodle and pasta dough are less developed than bread dough due to their lower moisture content (*ca.* 30-35%) and the low energy input during mixing and sheeting or extruding (Icard-Verniere and Feillet 1999; Baik 2010). Protein polymerization upon prolonged heating (such as during pasteurization) strengthens the texture of fresh and cooked egg pasta (Alamprese and others 2005). Pasta with insufficient protein polymerization during cooking lacks a continuous framework resulting in soft and sticky pasta (Resmini and Pagani 1983). Bruneel and others (2010) postulated that an optimum extent of protein network formation during drying and/or cooking is critical for pasta quality. During cooking, glutenin and gliadin, the polymeric and monomeric fractions of wheat gluten, polymerize through SH oxidation and SH-SS exchange reactions. Too much gliadin incorporation tightens the protein network, makes it less flexible to cope with starch swelling during cooking and results in low

pasta quality (Bruneel and others 2016). Both wheat protein content and composition are related to the texture of white salted cooked noodles (Hou and others 2013). While wheat albumin and globulin negatively impact noodle texture (Park and others 2003; Hou and others 2013), the proportions of low-molecular weight glutenin and gliadin have a positive impact (Hou and others 2013). It has also been reported that the composition of high-molecular weight glutenin subunits (HMW-GS) is related to the hardness of white salted noodles (Park and others 2003). Park and Baik (2009) suggested that addition of extra gluten proteins increases protein network formation in fresh noodles and reduces losses during cooking. In spite of the above, the impact of different Osborne fractions on protein network formation and their relation with noodle properties remain to be investigated.

Using a model system approach, Lambrecht and others (2016) concluded that different types of protein can impact each other's network formation. However, the impact of egg and gluten proteins on the rate and extent of protein network formation during noodle cooking is unknown. Egg yolk not only contains protein but also lipids [ca. 33% on dry matter (dm) basis]. However, the role of egg lipids is not necessarily comparable to those of margarine, butter or oil as *ca.* 68% of egg yolk lipids are present as lipoproteins (Anton 2007). Furthermore, Shimoyamada and others (2004) suggested that dry-heated egg white improves texture and the sensorial properties of Chinese noodles through interaction of egg white with gelatinized starch rather than with gluten protein. The interplay between different egg noodle constituents on protein network formation remain to be explored in detail.

This paper studies the functionality of wheat and egg constituents in fresh and optimally cooked noodles. First, the impact of protein quality and quantity of wheat proteins on protein network formation is investigated. Second, the impact of whole egg, egg white and egg yolk on different noodle properties is determined. Third, the impact of egg constituents on protein network formation during noodle cooking is related to noodle properties.

2. Materials and methods

2.1 Material and characterization thereof

Kernels from soft wheat cv Claire (Limagrain, Rilland, The Netherlands) and hard wheat cv Paragon (RAGT, Ickleton, United Kingdom) were conditioned to 16.0% moisture and milled with a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill (Delcour and others 1989) to flour with protein contents of 10.0% and 13.9% on dm respectively. Wheat albumins (36.9% on dm) and globulins (56.3% on dm) were sequentially extracted (60 min, room temperature) from wheat flour (50.0 g) with respectively water or sodium phosphate buffer (0.050 M; pH 7.6) containing 0.4 M sodium chloride (100.0 ml). After three extractions with each solvent and intermediate and final centrifugation (10 000 *g*, 10 min) steps, the combined supernatant of each solvent were dialyzed for 24 h against 0.01% acetic acid. Starch and gluten (Paragon gluten 79.7% protein on dm, Claire gluten 82.6% protein on dm) were isolated with a dough ball method (Pauly and others 2012; Lambrecht and others 2015). Commercial eggs (48.8% protein on dm) were whipped or separated into egg white (89.7% protein on dm) and egg yolk (33.6% protein on dm) with removal of the vitelline membrane. All protein fractions were freeze-dried and gently ground. Lipids were removed from freeze-dried egg yolk (50.0 g) with hexane (250.0 ml). Defatting dried egg yolk with hexane removes mainly triacylglycerol, some phospholipids and cholesterol (Warren and others 1988). After five repetitions of extraction and filtration, the defatted egg yolk (67.2% protein on dm) was air dried. All chemicals were of analytical grade and from Sigma-Aldrich (Steinheim, Germany) unless specified otherwise. Dithiothreitol (DTT), disodium hydrogen phosphate and sodium dihydrogen phosphate were from VWR International (Leuven, Belgium). Moisture contents were determined in triplicate according to AACC-I Approved Method 44-15.02 (AACC international 1999). Protein contents (N x 5.7 for wheat; N x 6.25 for egg protein) were determined in triplicate, using an adaptation of AOAC Official Method 990.03 (AOAC 1995),

with an automated Dumas protein analysis system (EAS Variomax N/CN, Elt, Gouda, The Netherlands). The HMW-GS composition of wheat flour was determined based on Uthayakumaran and others (2006) using an Agilent (Agilent Technologies, Santa Clara, CA, USA) LabChip of a protein 230 kit with an Agilent 2100 Bioanalyzer system. HMW-GS were identified by comparing electrophoresis patterns of flour samples with known subunit compositions.

2.2 Noodle production and cooking

Salted control noodles were prepared from a quantity of wheat flour containing 86.0 g dm and 2.0 g salt. Such noodles made with flour of cv Claire and Paragon, further referred to as Claire and Paragon control noodles, had protein contents of 9.7% and 13.6% of dm and moisture contents of 33.1% and 32.2%, respectively. Whole egg, egg white or (defatted) egg yolk were added to obtain a moisture content equaling that of control noodles, a protein content of 14.6% and 18.0% of dm and a ratio flour to egg protein of 3:2 and 2:1 for Claire and Paragon noodles, respectively. To that end, starch and gluten from the respective wheat cultivars were added. Unsalted noodles prepared with flour of cv Paragon had protein and moisture contents of 18.7% of dm and 33.9%, respectively. In some experiments, olive oil was included in the recipe of unsalted control noodles and such noodles made with defatted egg yolk to obtain a noodle lipid content of 12.4% on dm. Furthermore, gluten, wheat albumin and wheat globulin were included in the recipes of unsalted control noodles to obtain the same protein content as when adding egg fractions. The ingredients were mixed (5 min, 60 rpm) using a Kitchen Aid mixer (KPM5, St. Joseph, MI, USA) with intermediate scraping to include all ingredients adhering to the mixing bowl. After a dough rest of 30 min in a plastic bag at 23 °C, the dough was passed five times through a semi-automatic sheeter (Model C280 Capitani, Lomazzo Como, Italy) with a 2.9 mm roll gap with intermediate refolding. After compounding, the dough was rested a second time (30 min, room temperature) in a plastic bag. It was then

sheeted once through 2.9 mm and each time twice through 2.1, 1.5, and 0.9 mm roll gap sizes successively. After each pass through the rolls, the dough was turned 180°. It was then cut (length 150 mm, width 5.0 mm) with a sheet cutter (Capitani). Fresh noodle strands (20.0 g) were cooked in 500.0 ml deionized water to optimum as well as for 30 s, 1, 3, 6, 12 and 20 min. The optimum cooking time was the minimum time needed to gelatinize all starch and determined as the point in time when an opaque core was no longer visible when squeezing the noodles between two glass plates according to the AACC-I approved method 66-50.01 (AACC international 1999). Cooked noodles were immediately cooled in 200.0 ml deionized water at 23°C.

2.3 Noodle properties

Cooking loss (expressed in %) was determined in triplicate as the dm leached into the cooking water of optimally cooked noodles. Cooking and rinsing water were freeze-dried and the amount of dm was accurately weighed. The protein content (N x 5.7 for gluten noodles, N x 5.9 for other noodles) of cooking loss was determined with the Dumas method (section 2.1). Water absorption was determined in triplicate and calculated by relating the weight increase between dry and optimally cooked noodles to the dm content of dry noodles with correction for the cooking losses as calculated by:

$$WA\ (\frac{g}{g\ dm}) = \frac{CN\ (g) - [FN\ (g) - CL(g, dm)]}{[FN\ (g, dm) - CL\ (g, dm)]}$$

with WA the water absorption, CN the weight of optimally cooked noodles, FN the weight of fresh noodles and CL the cooking loss. The pH was determined in duplicate by suspending freeze-dried fresh noodle samples in deionized water (0.2 g/ml). It was measured after shaking (60 min, room temperature) with a pH meter HI 9025 (Hanna Instruments, Woonsocket, RI, USA). Fresh and cooked noodles were stretched with the Kieffer-rig dough and gluten extensibility rig (Stable Micro Systems, Surrey, UK) using an Instron (Norwood, MA, USA) 3342 with a 50 N load cell. After cooling (10 min, 23°C), adhering water was

gently removed from the surface of cooked noodles with paper. Ten individual noodle strands from each of two cooking batches were clamped between two plates and pulled upwards by a hook at 3.3 mm/s until fracture. The maximum force during extension, the extensibility at breakage and the work needed to fracture, *i.e.* area under curve, were calculated from the force-displacement curves.

2.4 Differential scanning calorimetry

Differential scanning calorimetry measurements were performed in triplicate as in Bosmans and others (2012) with a Q2000 DSC (TA instruments, New Castle, DE, USA).

2.5 Low resolution proton nuclear magnetic resonance

Proton mobility distributions in fresh and cooked noodles were determined as in Bosmans and others (2012) using a Minispec mq 20 low-field pulsed NMR spectrometer (Bruker, Ettlingen, Germany). After cooking, water on the noodle surface was gently removed with paper. The obtained transverse relaxation curves were fitted to a continuous distribution of spin-spin or transverse relaxation times (T_2) using the CONTIN algorithms of Provencher (Provencher 1982). Using the Free Induction Decay (FID) pulse sequence, less mobile proton populations were detected, whereas the Carr-Purcell-Meiboom-Gill (CMPG) pulse sequence was used to measure more mobile protons. The obtained peak areas are proportional to the amounts of protons in the population. Proton populations were assigned to noodle constituents based on starch-water, gluten-water, flour-water (Bosmans and others 2012) and egg-water model systems (Luyts and others 2013).

2.6 Protein extractability in SDS containing medium and molecular weight distribution

The protein extractable in SDS containing medium and its molecular weight distribution were determined in triplicate as in Lambrecht and others (2015, 2016) using size exclusion high performance liquid chromatography (SE-HPLC). Freeze-dried and ground samples (containing 1.0 mg protein) were shaken (60 min, room temperature) in 1.0 ml sodium

phosphate buffer (0.050 M; pH 6.8) containing 2.0% (w/v) SDS (hereafter referred to as SDS containing medium). To evaluate extractability under reducing conditions, 0.10%, 0.25%, 0.50% or 1.00% (w/v) DTT was added to the SDS containing buffer under nitrogen atmosphere. The protein extractable in SDS containing medium (SDS-EP) was calculated from the corresponding peak area and expressed as a percentage of the total area when the corresponding unheated sample was extracted in 1.0 ml SDS containing medium to which 1.00% DTT had been added. Furthermore, a quantity of freeze-dried whole egg noodle containing 10.0 mg protein/ml was extracted sequentially with water and dimethyl sulfoxide (DMSO):propanol:water (ratio 2:1:1, v/v, hereafter referred to as DMSO medium). After dilution (10x) with SDS containing medium, extracts were analyzed as described above. The total area of egg protein extracted in water and the areas of gluten protein extracted in DMSO medium, separated in glutenin, ω-, α- and γ-gliadin according to Lambrecht and others (2016), were integrated. About half of the gluten protein extractable in SDS containing medium was extractable in DMSO medium.

2.7 Kinetics of protein polymerization in cooked noodles

During heating, gluten protein loses extractability in SDS containing medium according to first-order kinetics as demonstrated for fresh pasta (Bruneel and others 2011), bread (Lagrain and others 2008) and gluten-water model systems (Rombouts and others 2012). The extractability decreases towards a minimum but some protein remains extractable. The protein extractability in SDS containing medium (y, calculated from the area in SE-HPLC chromatograms) can be presented as follows:

$$-\frac{dy}{dt} = k[y] \qquad (1)$$

with k the first-order reaction rate constant of polymerization (min^{-1}). Hence, with $[y]_0$ and $[y]_t$ the protein extractability in SDS-containing medium at time zero (unheated fresh noodles) and time t respectively:

205 $\ln[y]_t = -kt + \ln[y]_0$ (2)

206 The protein extractability in noodles decreases towards a minimum during heating time
207 according to the equation

208 $[y]_t = [y]_0 \ e^{-kt} + [y]_{minimal}$ (3)

209 with $[y]_{minimal}$ the extractability of protein which under the experimental conditions resists
210 polymerization.

211 **2.8 Statistical analysis**

212 Significant differences ($\alpha < 0.05$), based on at least three individual measurements, were
213 determined with the one-way ANOVA procedure using JMP® Pro 11.2.0 (SAS Institute,
214 Cary, NC, USA). Corresponding Tukey grouping coefficients are given. Pearson correlation
215 coefficients ($p < 0.05$) were calculated. Trend lines, corresponding equations and goodness of
216 fit (R^2) of protein polymerization kinetics of cooked noodles were estimated on the basis of
217 nonlinear regression analysis of the total extractable protein concentration as a function of
218 heating time. Significant differences in equation parameters were determined with an
219 equivalence test, comparing confidence intervals ($p< 0.05$).

220 **3. Results and discussion**

221 **3.1 Importance of wheat protein network formation for noodle properties**

222 Less protein was extractable in SDS containing medium from salted fresh Paragon noodles
223 (SDS-EP control noodles 70% ± 4%) than from salted fresh Claire noodles (SDS-EP control
224 noodles 84% ± 0%). Paragon gluten contains five HMW-GS including the subunits Dx1, Dx5
225 and Dy10 (Payne and others 1981). This HMW subunit composition is positively related to
226 noodle hardness and negatively to water absorption (Park and others 2003) and indicates a
227 superior gluten quality of Paragon than of Claire which contains the subunits Dx2, Bx7 and
228 Dy12. Also, significantly more force was necessary to stretch out Paragon than Claire control
229 noodle dough (Table 1). After optimal cooking, comparable levels of their gluten protein were

incorporated in the protein networks of salted Paragon (SDS-EP control noodles 27% ± 1%) and Claire (SDS-EP control noodles 30% ± 2%) noodles. However, salted Paragon noodles had higher protein content than Claire noodles. Paragon control noodles seemed to have higher optimal cooking times and absorbed significantly less water after optimal cooking than their Claire counterparts. These observations are in line with those of Park and Baik (2009) who found higher wheat flour protein contents to lead to lower water imbibition during noodle cooking and thereby to increase optimal cooking time. The cooking loss of salted Paragon control noodles was significantly lower than that of the corresponding Claire noodles. In addition, Paragon fresh and cooked noodles showed significantly higher extensibility, maximum force, and total work during Kieffer-rig testing than did Claire noodles. The higher strength of Paragon than of Claire noodles can be ascribed to the better developed protein network in the former.

Addition of wheat gluten to control noodles - Addition of gluten to control noodle recipes appeared to increase the optimal cooking time of the resultant Paragon and Claire extra gluten containing noodles (hereafter referred to as gluten noodles) by 390 s and 510 s respectively. Park and Baik (2009) showed a higher reduction in water imbibition when including gluten in a noodle recipe for soft rather than in one for hard wheat. The impact of gluten addition on the Kieffer-rig parameters was higher in Claire than in Paragon fresh and cooked noodles (Table 1). Figure 1 shows the areas representing protein extracted in SDS containing medium, fitted according to first order kinetics (Equation 3). Protein in gluten noodles polymerized slower (low k value) and more protein was incorporated in the protein network (lower $[y]_{minimal}$ value) than that in control noodles. Also, higher concentrations of DTT in the extraction medium were necessary to extract all protein from gluten noodles than from control noodles heated for 12 min at 100 °C (Figure 2). Thus, more protein was SS bound in gluten noodles than in control noodles. Furthermore, gluten noodles had higher protein content than

control noodles. The higher protein level and degree of polymerization at optimal cooking time in gluten noodles led to more extensible noodles which required more work to fracture than control noodles (Table 1).

In unsalted fresh Paragon noodles, the extensibility at breakage and total work of control and gluten noodles was significantly higher than those in their salted counterparts (Tables 1 and 2). The addition of salt increased the time needed to develop wheat dough (Van Steertegem and others 2013). However, no difference in SDS extractability was noticed between fresh noodles containing a range of salt concentrations (Rombouts and others 2014). The optimum cooking time of unsalted noodles seemed to increase but the Kieffer-rig parameters after cooking were similar to that of salted noodles. Salt can shield ionic interactions. In fresh noodles, non-covalent interactions of the hydrogen bond type, as well as hydrophobic, ionic and Van der Waals interactions can impact Kieffer-rig parameters of noodles. During cooking, starch swells and proteins are covalently incorporated in the protein network. It is suggested that the swollen and gelatinized starch physically hinders non-covalent interactions between proteins and covalent cross-links become more important for the rigidity of the noodle structure.

Inclusion of wheat albumin and globulin in control noodle recipe - The inclusion of wheat albumin in the recipe of control noodles impacted the polymerization kinetics (Figure 1). In the resultant wheat albumin noodles more protein was incorporated and interconnected by SS bonds than in control noodles (Figures 1 and 2). SE-HPLC profiles demonstrated that gliadin was more rapidly incorporated in the protein network in wheat albumin than in control noodles (results not shown). The optimal cooking time increased by 210 s. The swiftly and extensively developed protein network in wheat albumin noodles did not withstand starch swelling. This resulted in low cooking quality and low Kieffer-rig parameters after optimal cooking. When extra wheat globulin was included in the recipe, protein in the resultant wheat

globulin noodles polymerized slower and less protein was incorporated in the network than in control noodles (Figure 1). Also, more protein leached into the cooking water (Table 2). However, to extract all protein from wheat globulin noodles the SDS containing medium needed to contain at least 0.50% DTT while 0.25% DTT sufficed for control noodles (Figure 2). Less work was needed to stretch wheat globulin noodles than control noodles. Probably, protein network formation in wheat globulin noodles was insufficient to ensure noodle strength. The above results confirm that wheat albumin and globulin have a negative impact on noodle properties even if their protein network formation abilities differ.

3.2 Impact of egg constituents on noodle properties

Fresh noodles - Adding egg fractions increased the extensibility at breakage but lowered the maximum force of Claire noodles (Table 1). These effects were more pronounced for egg white. In fresh Paragon noodles, only egg white addition significantly impacted Kieffer-rig parameters. The egg fractions had only little impact on the total work required for rupture of fresh noodles. The positive impact of egg (fraction) addition on the extensibility at breakage apparently outweighed its negative impact on the force measured during extension.

Cooked noodles - The use of whole egg in the salted noodle recipe had little impact neither on the optimal cooking time nor on water absorption and cooking loss. However, salted noodles made from recipes containing egg white or egg yolk had longer or shorter optimal cooking times respectively than did either whole egg or control noodles (Table 1). While egg white use increased and egg yolk use decreased water absorption and cooking loss, the impact of whole egg on cooking quality was intermediate to both.

Cooking increased the total work of whole egg and egg white noodles while it decreased that of egg yolk noodles. The use of whole egg significantly increased the extensibility at breakage, maximum force, and total work of cooked noodles. Egg white use increased the maximum force significantly but did not impact the extensibility of cooked noodles. It

significantly increased total work for cooked Paragon noodles with egg white but not for cooked Claire noodles. In contrast, egg yolk addition had the complete opposite effect, *i.e.* it decreased the extensibility, the maximum force and total work of cooked noodles. It is remarkable that the impact of whole egg on Kieffer-rig parameters, in contrast to that on cooking quality, was not the combined effect of egg white and yolk. Noodles from the same wheat cultivar had equal protein content and flour-to-egg protein ratio, but different protein compositions and lipid contents. The next sections will investigate whether differences in noodle properties can be ascribed to differences in protein network formation.

3.3 Impact of egg constituents on protein network formation

3.3.1 Importance of covalent cross-links

Protein in whole egg noodles polymerized faster and more protein was incorporated in the protein network than in control noodles (Figure 1). Also, more protein was SS bound in whole egg than control noodles. Indeed, higher concentrations of DTT in the extraction medium were necessary to extract all protein (Figure 2). Protein in egg white noodles polymerized more rapidly (Figure 1) and to a larger extent, *i.e.* more protein was incorporated and interconnected by SS bonds (Figure 2), than in whole egg noodles. Less protein was incorporated (Figure 1) and lower concentrations of DTT in SDS containing medium were required to extract protein from egg yolk noodles (Figure 2) than from whole egg and egg white noodles. Furthermore, protein in egg yolk noodles polymerized at a slower rate than protein in whole egg and egg white noodles but still faster than that in control noodles. In complex systems not all protein polymerizes at the same rate nor to the same extent. The extractability of egg protein in water from whole egg noodles and those of glutenin, α-, γ-, and ω-gliadin in DMSO medium were monitored as a function of heating time. Based on the kinetics of polymerization, calculated as in Section 2.8, it was concluded that in whole egg noodles, egg protein polymerized faster than gluten protein (Figure 3). After 6 min cooking,

more egg protein remained extractable in water than gluten protein in DMSO medium. Glutenin polymerized faster than α- and γ-gliadin. Gliadin lacks free SH groups in contrast to glutenin. ω-Gliadin, which lacks both cysteine and cystine residues, remained fully extractable during cooking of whole egg noodles.

The rate and extent of protein polymerization in whole egg noodles corresponded to the values expected based on the data on protein polymerization in egg white and yolk noodles. The fast and extensive protein polymerization in egg white noodles reduced the flexibility of the resultant network and resulted in low cooking quality as it impaired the capacity of the noodles to cope with starch swelling (Table 1). As noted for wheat globulin noodles, less protein in cooked egg yolk noodles was incorporated in the protein network and the extensibility was lower than that of cooked control noodles. The higher extent of cross-linking in cooked egg white and whole egg than in control noodles increased the maximum force for both Claire and Paragon noodles (Table 1). The order of covalent protein network development (egg white > whole egg > egg yolk noodles) was positively related to cooking time, water absorption and cooking loss. These data support the view that high optimal cooking time is related to highly developed protein networks, which slow down water imbibition and therefore starch swelling and gelatinization. No clear differences in the latter were observed (results not shown).

3.3.2 Importance of ionic interactions

Ionic interactions between egg and gluten proteins largely impact dough properties (Van Steertegem and others 2013). They cannot be quantified, but it is possible to estimate attraction and repulsion forces between proteins in a system at a given pH. At pH 6.0, the pH of control and gluten dough (Table 1), gliadin [pH 5.8, calculated pI around 7.8 and in agreement with Wu and Dimler (1963)] has a net positive while glutenin (pH 8.3, calculated pI 5.8) a slightly net negative charge (Figure 4.A). Salt can shield the ionic interactions

between gluten proteins. In fresh unsalted control and gluten noodles, ionic interactions between glutenin and gliadin resulted in more pronounced coherence of the partially developed gluten network and higher Kieffer-rig parameters than in their salted counterparts (Tables 1 and 2). Also, that the gluten network in wheat dough develops more slowly when salt is added (Van Steertegem and others 2013) suggests a less continuous protein network in salted than in unsalted noodles. The latter can explain the shorter optimal cooking times for all salted noodle types (Tables 1 and 4).

Fresh noodles - At the pH of egg yolk noodles (Table 1), the majority of egg yolk protein (Van Steertegem and others 2013) and gliadin have a positive charge while glutenin has a slightly negative charge (Figure 4.B). Egg yolk protein can interact with glutenin the same way gliadin does, *i.e.* it increases dough viscosity and plasticity and reduces its elasticity (Shewry and others 2001). The repulsion between egg yolk and gliadin proteins hinders their interaction. When salt was omitted from the recipe, the maximum force was lower and the extensibility higher (Tables 1 and 3). In egg white noodles (Table 1), glutenin and egg white protein are negatively charged while gliadin is hardly charged (Figure 4.C). Gliadin can still easily interact with glutenin. However, the repulsion between egg white protein and glutenin seem to hinder the development of a coherent protein network. All Kieffer-rig parameters were lower for unsalted fresh egg white than for control noodles (Table 3). Inclusion of salt in the recipe increased the extensibility of fresh egg white noodles but little resistance was measured during stretching (low maximum force and total work) (Table 1). The effect of salt predominated over the combined protein effect. Salt use probably resulted in a less continuous protein network and thereby lowered the total work needed for fracture. The majority of egg protein is negatively charged in whole egg noodle dough (Table 1), shielding the positively charged gliadin (Figure 4.D). The ionic interactions between egg and gluten protein contributed to the coherence of the protein network noodles. Both in salted and unsalted

Paragon noodles, no differences in total work were noted between fresh whole egg and control noodles. Salt addition had a larger impact on Kieffer-rig parameters than whole egg addition (Tables 1 and 3).

Cooked noodles - Salt addition had a different impact on the properties of cooked noodles than on those of fresh noodles. All Kieffer-rig parameters of unsalted cooked egg white and yolk noodles were similar but lower than those of whole egg noodles (Tables 1 and 3). While salt addition did not impact the total work of whole egg and egg yolk noodles, that of egg white noodles was drastically higher when salt had been used. As outlined above, protein networks developed faster in unsalted than in salted noodle dough. This combined with the fast cross-linking of egg white proteins led to a protein network which could not cope with starch swelling during cooking. Finally, it is of note that salt also reduces protein polymerization during noodle cooking (Rombouts and others 2014), thereby improving the flexibility of the protein network and the way it withstands starch swelling.

3.3.3 Importance of hydrophobic interactions

Alamprese and others (2005) suggested that hydrophobic interactions are relevant to egg pasta sheet extensibility. In their view, the hydrophobic patches of proteins in whole egg and egg yolk noodles can interact with egg yolk lipids and thereby reduce the frequency of the occurrence of hydrophobic interactions between proteins.

Defatting egg yolk prior to noodle making - The total work and maximum force of fresh defatted egg yolk noodles were higher and the extensibility lower than those of standard egg yolk noodles. The reduction of egg yolk lipid levels probably increased the level of hydrophobic protein-protein interactions and hence also the stiffness and strength of the protein network. Proteins in defatted egg yolk noodles polymerized faster during heating than that in egg yolk noodles (Figure 1). However, no difference in the level of protein incorporated in the protein network was noticed (Figure 2). Nevertheless, low levels of DTT

405 (0.10%) in SDS containing medium released less protein from the protein network in defatted
406 egg yolk noodles cooked for 12 min than from standard egg yolk noodles cooked for the same
407 time (Figure 2). Thus, defatting egg yolk increased the level of protein-protein cross-links.
408 The rate and extent of protein network formation in defatted egg yolk noodles were lower
409 than those in egg white and whole egg noodles (Figures 1 and 2). We verified that the hexane
410 treatment itself when tested on noodles containing (hexane-treated) bovine serum albumin did
411 not impact protein network formation of isolated proteins during noodle making (results not
412 shown). Defatting egg yolk prior to using it in the noodle recipe increased the optimal
413 cooking time and significantly decreased the water absorption (Table 3). Also, less protein
414 leached from noodles to the cooking water from defatted egg yolk noodles than from standard
415 egg yolk noodles. Defatting significantly impacted neither the maximum force nor total work
416 of optimally cooked egg yolk noodles.

417 **Addition of olive oil to noodle recipes -** To further investigate the impact of lipids on protein
418 polymerization and noodle properties, olive oil was included in the recipes of control and
419 defatted egg yolk noodles. The noodles with olive oil contained the same lipid level as
420 standard egg yolk noodles. Olive oil in fresh control noodles caused a greater extensibility
421 and reduced the maximum force and total work (Table 3). It seems to plasticize the noodle
422 structure by obstructing protein-protein interactions. During cooking, the inclusion of oil in
423 the recipe decreased the rate of protein polymerization significantly while no difference in the
424 level of protein incorporated in the protein network was observed (Figure 1). However, more
425 protein was extractable with 0.10% DTT in SDS containing medium from oil containing than
426 from control noodles (Figure 2). Thus, the use of oil slowed down protein network formation
427 and decreased cross-linking between gluten proteins in noodles. As for control noodles, all
428 protein of olive containing control noodles was extractable with 0.25% DTT in SDS
429 containing medium. The optimal cooking time slightly decreased when the control noodles

contained olive oil. No differences in cooking quality were noticed, except that more protein leached into the cooking water. While the use of olive oil did not impact the gelatinization temperature of starch (results not shown), the inferior protein network in the cooked noodles led to a lower maximum force and total work than for control noodles even if the extensibility of cooked noodles was not influenced by the use of olive oil (Table 3).

The inclusion of olive oil in the recipe of fresh defatted egg yolk noodles increased the extensibility and decreased the maximum force and total work (Table 3). No significant differences were noted between extensibility parameters of standard egg yolk noodles and defatted egg yolk noodles containing olive oil. The rate of protein polymerization during cooking of defatted egg yolk noodles with oil was significantly lower than those for defatted egg yolk noodles and even standard egg yolk noodles (Figure 1). The extent of protein incorporation in the protein network in defatted egg yolk noodles containing olive oil was equal to that in defatted egg yolk noodles but lower than that in standard egg yolk noodles. More protein was extractable with 0.10% DTT in SDS containing medium from defatted egg yolk noodles with olive oil than from defatted egg yolk noodles (Figure 2). The level of protein extracted in 0.10% DTT in SDS containing medium of the former was similar as in standard egg yolk noodles. However, the protein network of defatted egg yolk noodles containing olive oil still was superior to that in egg yolk noodles (Figures 1 and 2).

Olive oil use decreased the optimal cooking time and water absorption more in defatted egg yolk noodles than in control noodles. In addition, it significantly impacted noodle maximum force and total work when used in control noodles, but not in defatted egg yolk noodles (Table 3). The fatty acid composition of olive oil differs from that in egg yolk lipids (Belitz and others 2009). Because olive oil impacts protein network formation and properties of defatted egg yolk noodles differently than those of control noodles, it is hypothesized that

454 rather than only exerting physical hindrance, lipids interact with egg yolk lipoprotein and
455 hinder their subsequent polymerization.

456 ### 3.3.4 Importance of hydrogen bonds

457 The role of hydrogen bonds in gluten networks is undisputed (Belton 1999). Dough
458 constituents interact with water. This reduces its mobility. Depending on the degree of
459 interaction, different proton populations can be distinguished (Bosmans and others 2012).
460 Based on the model systems described by Bosmans and others (2012) and Luyts and others
461 (2013) the proton populations in fresh and optimally cooked noodles (Figure 5) could be
462 assigned. Proton population A, with lowest mobility (lowest T_2), is not in contact with water
463 and contains rigid non-exchanging CH protons of starch, gluten and egg protein. This
464 population decreases during cooking as a result of starch gelatinization (Bosmans and others
465 2012; Luyts and others 2013). The remainder are rigid CH protons in the protein network and,
466 in cooked and cooled noodles, crystalline amylose. Proton populations B and C are similar
467 and consist of CH protons in amorphous starch regions and protein aggregates in little contact
468 with water. Population D contains exchangeable water protons within and directly
469 surrounding starch granules and protein aggregates. The relative fraction of proton
470 populations B, C and D decreased during cooking as noodle constituents increasingly came
471 into contact with water (Figure 5, Table 4). Finally, protons in population E had the highest
472 mobility and contained lipid protons in fresh noodles. The absorbed cooking water was
473 responsible for the increase in proton population E during cooking. While bulk water has a T_2
474 of about 2.5-3.0 s (Luyts and others 2013), the absorbed cooking water was bound in the
475 noodle structure with T_2 around 60 ms.

476 **Fresh noodles -** The main differences in NMR spectra of fresh control and gluten noodles
477 were noted for populations C and D. In fresh gluten noodles, more protons were in little
478 contact with water (population C) and less protons were exchanging with those of water

(population D) than in control noodles (Table 4). However, the average mobility of both populations was higher in fresh gluten than in control noodles. Thus, a higher degree of hydrogen bond exchange between noodle constituents occurs as a result of the use of extra gluten. Whether this contributes to the improved dough properties upon wheat gluten addition remains unclear at present. Also in fresh whole egg noodles population C had higher mobility than in control noodles (Figure 5, Table 4). No distinction between populations C and D could be made in fresh egg white noodles. Water protons were less mobile, thus more strongly bound, in fresh egg white noodles than in all other noodle types. Also, defatting decreased the proton mobility and the fraction of protons occurring in population D in egg yolk noodles. Proton population E, which contained lipid protons, was highest and most mobile for fresh egg yolk noodles, and lowest for control, gluten, egg white and defatted egg yolk noodles.

Cooked noodles – The moisture gradient during cooking of noodles homogenizes after cooking (Kojima and others 2001). During cooking, noodles absorb water, starch swells and gelatinizes, and proteins extend their covalent network, resulting in decreases in proton populations A, B, C and D, and increases in proton population E for all noodles (Figure 5, Table 4). Differences in cooking time led to differences in water absorption and levels of protons in population E. However, it should be noted that the rate of water diffusion in wheat noodles decreases over cooking time (Maeda and others 2009). Also, the mobility of population E protons generally seemed to increase with cooking time except for whole egg noodles (Tables 3 and 4). Such noodles, which had the same optimal cooking time as control noodles (Table 3), had lower T_2 values for population E after cooking (Table 4, Figure 5). In noodles cooked for 20 min, and thus beyond the optimum cooking time, the T_2 values of this population were similar for all noodle types except for whole egg noodles, in which water protons were more homogeneously bound and thus had a lower average T_2 value (results not shown). As demonstrated above, as starch swells and the noodle volume increases, ionic and

hydrophobic protein-protein interactions had little impact on total work in Kieffer-rig extensibility testing (Table 3). The NMR data indicate that hydrogen bonds between noodle flour and egg constituents and water together with the formed covalent network can determine the coherence and flexibility of the noodle structure.

4. Conclusion

This work demonstrated that ionic, hydrophobic and hydrogen interactions between proteins, as well as protein cross-linking reactions, impact extensibility parameters and cooking characteristics of noodles. During cooking, starch swells limiting the importance of ionic and hydrophobic interactions on noodle extensibility. Cooking quality and properties of cooked noodles are largely determined by the balance between covalent protein network formation and starch gelatinization. The covalent protein network of whole egg noodles was inferior to egg white but superior to egg yolk noodles. However, the rapidly formed protein network in egg white noodles could not cope with starch swelling. This resulted in high optimal cooking times, low cooking quality and low total work during Kieffer-rig extensibility tests. In contrast, the covalent protein network in egg yolk noodles was only moderately developed resulting in low optimal cooking times and noodle strengths. It is suggested that besides the timing and extent of covalent network formation also hydrogen interactions are the main determinants of extensibility of cooked noodles.

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Authors contributions

M.A. Lambrecht, I. Rombouts and M.A. Nivelle conducted the experiments. M.A. Lambrecht designed the experiments, interpreted the results and wrote the manuscript. I. Rombouts and J.A. Delcour reviewed the manuscript.

References

AACC international. 1999. Approved methods of analysis. Methods 44-15.02, 66-50.01 and 76-31.01. St. Paul, MN, USA, 11th edn AACC International.

Alamprese C, Casiraghi E and Rossi M. 2009. Modeling of fresh egg pasta characteristics for egg content and albumen to yolk ratio. Journal of Food Engineering 93(3): 302-307.

Alamprese C, Casiraghi E and Rossi M. 2012. Mechanical and rheological properties of fresh egg pasta as affected by shell egg production factors. International Journal of Food Science and Technology 47(12): 2503-2509.

Alamprese C, Iametti S, Rossi M and Bergonzi D. 2005. Role of pasteurisation heat treatments on rheological and protein structural characteristics of fresh egg pasta. European Food Research and Technology 221(6): 759-767.

Anton M. 2007. Composition and structure of hen egg yolk. Bioactive egg components. R. Huopalathi, R. López-Fandiño, M. Anton en R. Schade. Berlin, Springer-Verlag**:** 1-5.

AOAC. 1995. Official methods of analysis. Washington, DC, USA, AOAC.

Baik BK. 2010. Effects of flour protein and starch on noodle quality. Asian noodles: Science, technology, and processing. G. G. Hou. Hoboken, NJ, John Wiley and Sons.

Belitz H-D, Grosch W and Schieberle P. 2009. Food chemistry 4th edition. Berlin, Germany, Springer.

Belton PS. 1999. On the elasticity of wheat gluten. Journal of Cereal Science 29(2): 103-107.

552 Bosmans GM, Lagrain B, Deleu LJ, Fierens E, Hills BP and Delcour JA. 2012. Assignments
553 of proton populations in dough and bread using nmr relaxometry of starch, gluten, and
554 flour model systems. Journal of Agricultural and Food Chemistry 60(21): 5461-5470.
555 Bruneel C, Buggenhout J, Lagrain B, Brijs K and Delcour JA. 2016. Redox agents and n-
556 ethylmaleimide affect protein polymerization during laboratory scale dry pasta
557 production and cooking. Food Chemistry 196: 646-653.
558 Bruneel C, Lagrain B, Brijs K and Delcour JA. 2011. Redox agents and n-ethylmaleimide
559 affect the extractability of gluten proteins during fresh pasta processing. Food
560 Chemistry 127(3): 905-911.
561 Bruneel C, Pareyt B, Brijs K and Delcour JA. 2010. The impact of the protein network on the
562 pasting and cooking properties of dry pasta products. Food Chemistry 120(2): 371-
563 378.
564 Dalbon G, Grivon D and Pagani MA. 1996. Continuous manufacturing process. Pasta and
565 noodle technology. J. E. Kruger, R. B. Matsuo en J. W. Dick. St. Paul, MN, USA,
566 American Association of Cereal Chemists**:** 13-58.
567 Delcour JA, Vanhamel S and Degeest C. 1989. Physicochemical and functional-properties of
568 rye nonstarch polysaccharides. I. Colorimetric analysis of pentosans and their relative
569 monosaccharide compositions in fractionated (milled) rye products. Cereal Chemistry
570 66(2): 107-111.
571 Fu BX. 2008. Asian noodles: History, classification, raw materials, and processing. Food
572 Research International 41(9): 888-902.
573 Hou GG, Saini R and Ng PKW. 2013. Relationship between physicochemical properties of
574 wheat flour, wheat protein composition, and textural properties of cooked chinese
575 white salted noodles. Cereal Chemistry 90(5): 419-429.

576 Icard-Verniere C and Feillet P. 1999. Effects of mixing conditions on pasta dough
577 development and biochemical changes. Cereal Chemistry 76(4): 558-565.
578 Kojima TI, Horigane AK, Yoshida M, Nagata T and Nagasawa A. 2001. Change in the status
579 of water in japanese noodles during and after boiling observed by nmr micro imaging.
580 Journal of Food Science 66(9): 1361-1365.
581 Lagrain B, Brijs K and Delcour JA. 2008. Reaction kinetics of gliadin-glutenin cross-linking
582 in model systems and in bread making. Journal of Agricultural and Food Chemistry
583 56(22): 10660-10666.
584 Lambrecht MA, Rombouts I and Delcour JA. 2016. Denaturation and covalent network
585 formation of wheat gluten, globular proteins and mixtures thereof in aqueous ethanol
586 and water. Food Hydrocolloids 57: 122-131.
587 Lambrecht MA, Rombouts I, Van Kelst L and Delcour JA. 2015. Impact of extraction and
588 elution media on non-size effects in size exclusion chromatography of proteins.
589 Journal of Chromatography A 1415: 100-107.
590 Li M, Zhu KX, Guo XN, Brijs K and Zhou HM. 2014. Natural additives in wheat-based pasta
591 and noodle products: Opportunities for enhanced nutritional and functional properties.
592 Comprehensive Reviews in Food Science and Food Safety 13(4): 347-357.
593 Luyts A, Wilderjans E, Waterschoot J, Van Haesendonck I, Brijs K, Courtin CM, Hills B and
594 Delcour JA. 2013. Low resolution h-1 nmr assignment of proton populations in pound
595 cake and its polymeric ingredients. Food Chemistry 139(1-4): 120-128.
596 Maeda I, Horigane AK, Yoshida M and Aikawa Y. 2009. Water diffusion in buckwheat
597 noodles and wheat noodles during boiling and holding as determined from mri and
598 rectangular cylinder diffusion model. Food Science and Technology Research 15(2):
599 107-116.

600 Oh NH, Seib PA, Deyoe CW and Ward AB. 1983. Noodles .1. Measuring the textural
601 characteristics of cooked noodles. Cereal Chemistry 60(6): 433-438.
602 Park CS, Hong BH and Baik BK. 2003. Protein quality of wheat desirable for making fresh
603 white salted noodles and its influences on processing and texture of noodles. Cereal
604 Chemistry 80(3): 297-303.
605 Park SJ and Baik BK. 2009. Quantitative and qualitative role of added gluten on white salted
606 noodles. Cereal Chemistry 86(6): 646-652.
607 Pauly A, Pareyt B, De Brier N, Fierens E and Delcour JA. 2012. Starch isolation method
608 impacts soft wheat (triticum aestivum l. Cv. Claire) starch puroindoline and lipid
609 levels as well as its functional properties. Journal of Cereal Science 56(2): 464-469.
610 Payne PI, Holt LM and Law CN. 1981. Structural and genetical studies on the high-
611 molecular-weight subunits of wheat glutenin. 1. Allelic variation in subunits amongst
612 varieties of wheat (triticum aestivum). Theoretical and Applied Genetics 60(4): 229-
613 236.
614 Powrie WD and Nakai S, Eds. (1985). Characteristics of edible fluids of animal origin: Eggs.
615 Food chemistry (2nd ed.). New York, NY, USA, Marcel Dekker.
616 Provencher SW. 1982. Contin - a general-purpose constrained regularization program for
617 inverting noisy linear algebraic and integral-equations. Computer Physics
618 Communications 27(3): 229-242.
619 Resmini P and Pagani MA. 1983. Ultrastructure studies of pasta - a review. Food
620 Microstructure 2(1): 1-12.
621 Rombouts I, Jansens KJA, Lagrain B, Delcour JA and Zhu KX. 2014. The impact of salt and
622 alkali on gluten polymerization and quality of fresh wheat noodles. Journal of Cereal
623 Science 60(3): 507-513.

624 Rombouts I, Lagrain B and Delcour JA. 2012. Heat-induced cross-linking and degradation of
625 wheat gluten, serum albumin, and mixtures thereof. Journal of Agricultural and Food
626 Chemistry 60: 10133-10140.
627 Shewry PR, Popineau Y, Lafiandra D and Belton P. 2001. Wheat glutenin subunits and dough
628 elasticity: Findings of the eurowheat project. Trends in Food Science & Technology
629 11(12): 433-441.
630 Shimoyamada M, Ogawa N, Tachi K, Watanabe K, Yamauchi R and Kato K. 2004. Effect of
631 dry-heated egg white on wheat starch gel and gluten dough. Food Science and
632 Technology Research 10(4): 369-373.
633 Uthayakumaran S, Listiohadi Y, Baratta M, Batey IL and Wrigley CW. 2006. Rapid
634 identification and quantitation of high-molecular-weight glutenin subunits. Journal of
635 Cereal Science 44(1): 34-39.
636 Van Steertegem B, Pareyt B, Brijs K and Delcour JA. 2013. The effects of fresh eggs, egg
637 white, and egg yolk, separately and in combination with salt, on mixogram properties.
638 Cereal Chemistry 90(3): 269-272.
639 Warren MW, Brown HG and Davis DR. 1988. Solvent-extraction of lipid components from
640 egg-yolk solids. Journal of the American Oil Chemists Society 65(7): 1136-1139.
641 Wu YV and Dimler RJ. 1963. Hydrogen ion equilibria of wheat glutenin and gliadin.
642 Archives of Biochemistry and Biophysics 103(3): 310-318.

Table 1. Dough pH, optimal cooking time, water absorption, cooking loss, extensibility until fracture, maximum force and total work needed for fracture determined on fresh and optimal cooked salted noodles. Noodles with different egg proteins and gluten were made with flour of soft wheat cultivar Claire and hard wheat cultivar Paragon.

Cultivar	Noodle type	Dough pH	Optimal cooking time	Water absorption (g/g dm)	Cooking loss (% on dm)	Extensibility (mm)		Maximum force (N)		Work (J)	
						Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
Claire	Control	6.0 c	5 min 30 s	2.76 c	10.9 b	10.5 d	17.9 c	0.47 b	0.56 c	2.8 c	5.4 b
	Gluten	6.1 c	14 min	3.02 bc	9.9 b	25.0 a	30.6 a	1.05 a	0.74 b	15.1 a	13.1 a
	Whole egg	7.0 b	5 min 30 s	3.25 b	9.3 b	16.0 c	24.7 b	0.33 cd	0.99 a	2.8 c	13.3 a
	Egg white[a]	7.7 a	9 min	4.15 a	14.0 a	24.7 a	20.3 c	0.25 d	0.72 b	3.4 bc	7.9 b
	Egg yolk	6.3 c	2 min 30 s	1.60 d	6.0 c	21.4 b	11.5 d	0.34 c	0.43 d	4.3 b	2.3 c
Paragon	Control	6.2 c	6 min 30 s	1.96 bc	6.5 b	20.2 c	26.7 c	1.15 b	0.92 b	14.3 b	13.6 c
	Gluten	6.22 c	13 min	2.34 b	6.8 b	31.7 b	37.5 a	1.75 a	1.15 a	33.7 a	24.6 a
	Whole egg	6.9 b	7 min	2.02 bc	6.3 b	19.3 c	32.3 b	1.09 b	1.24 a	11.9 b	22.5 ab
	Egg white[a]	7.8 a	13 min	3.78 a	11.3 a	38.7 a	27.0 c	0.28 c	1.22 a	7.1 c	19.1 b
	Egg yolk	6.2 c	3 min	1.16 c	4.4 b	21.3 c	18.2 d	1.17 b	0.79 b	13.2 b	7.7 d

In each column, values for one wheat cultivar with the same letter are not significantly different from one another (α=0.05). When relevant, significant differences between Paragon and Claire control noodles are mentioned in the text

[a] Noodles with a higher moisture content of 37.1%.

Table 2. Optimal cooking time, water absorption, cooking loss and the portion of protein present in the cooking loss, extensibility until fracture, maximum force and total work for optimal cooked unsalted noodles made with different protein enriched Osborne-fractions of wheat flour cv Paragon.

Noodle type	Dough pH	Optimal cooking time	Water absorption (g/g dm)	Cooking loss (% on dm)	Protein in cooking loss (% on dm)	Extensibility (mm)		Maximum force (N)		Work (J)	
						Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
Control	5.9 c	9 min	2.11 b	5.4 b	9.0 b	29.1 c	23.9 b	1.19 b	0.87 b	23.0 b	11.5 b
Wheat albumin	6.7 a	12 min 30 s	2.86 a	11.6 a	6.4 c	39.8 b	18.5 c	0.90 c	0.61 c	25.1 b	5.7 d
Wheat globulin	5.6 d	9 min	2.42 ab	6.4 ab	15.7 a	23.5 d	19.6 c	1.29 b	0.86 b	19.0 b	8.5 c
Gluten	6.0 b	15 min 30 s	2.65 a	5.6 b	10.5 b	50.3 a	35.1 a	1.78 a	1.14 a	60.1 a	23.1 a

Table 3. Optimal cooking time, water absorption, cooking loss, protein content of cooking loss and protein extractabilities in sodium dodecyl sulfate containing medium (SDS-EP) of optimal cooked unsalted Paragon noodles made with different edible egg fractions and/or addition of olive oil. Extensibility until fracture, maximum force and total work of fresh and optimal cooked noodles.

Noodle type	Optimal cooking time (min)	Water absorption (g/g dm)	Cooking loss (% on dm)	Proteins in cooking loss (% on dm)	Extensibility (mm)		Maximum force (N)		Work (J)		SDS-EP Cooked (%)
					Fresh	Cooked	Fresh	Cooked	Fresh	Cooked	
Control	9 min	2.11 b	5.4 b	9.0 d	29.4 b*	23.9 b	1.19 b	0.87 b	23.0 a*	11.5 b	23.3 c
Whole egg	9 min	1.89 b	4.9 b	11.7 ab	30.0 b*	31.8 a	1.27 b*	1.56 a*	22.3 a*	27.8 a	16.9 e
Egg white	15 min	3.37 a	9.7 a	12.9 a	25.5 c*	20.8 cd*	1.04 c*	0.84 b*	16.7 bc*	9.3 bc*	14.0 f
Egg yolk	4 min 30 s	1.25 c	3.6 b	12.6 a	26.9 bc*	19.4 de	0.82 d*	0.86 b	12.6 d	8.5 bc	30.7 a
Defatted egg yolk	11 min	1.93 b	5.8 b	10.6 bc	20.0 d	15.1 f	1.46 a	0.95 b	16.8 b	7.4 c	22.8 d
Defatted egg yolk + oil	7 min	1.09 c	4.5 b	11.7 abc	27.3 bc	16.6 ef	0.74 d	0.86 b	11.2 d	7.4 c	22.9 c
Control + oil	8 min	2.10 b	5.0 b	10.4 c	40.8 a	22.5 bc	0.54 e	0.60 c	13.8 cd	7.3 c	24.7 b

Column values with the same letter are not significantly different (α=0.05).

*Values are significantly different with the corresponding parameter of salted Paragon noodles within the same type (Table 2)

Table 4. Spin-spin relaxation times (T_2) and corresponding peak areas for different proton populations present in fresh and optimally cooked unsalted Paragon noodles made with different edible wheat and egg fractions. AU, arbitrary units. Standard deviations are between brackets.

Noodle type		Population A		Population C		Population D		Population E	
		T_2 (μs)	Area (AU)	T_2 (ms)	Area (AU)	T_2 (ms)	Area (AU)	T_2 (ms)	Area (AU)
Control	Fresh	13 (0)	19623 (204)	0.45 (0.02)	909 (5)	4.32 (0.06)	6703 (19)	60.00 (0.00)	61 (4)
	Cooked	14 (0)	3701 (392)	0.50 (0.10)	813 (91)	4.87 (0.51)	1173 (94)	56.53 (4.37)	14403 (209)
Gluten	Fresh	13 (0)	18620 (244)	0.61 (0.05)	1377 (181)	5.00 (0.36)	6478 (100)	63.33 (11.55)	79 (10)
	Cooked	13 (1)	3586 (150)	0.57 (0.06)	694 (73)	6.30 (1.04)	991 (45)	71.37 (5.69)	14883 (298)
Whole egg	Fresh	13 (0)	18476 (447)	0.60 (0.05)	1147 (160)	3.52 (1.04)	6932 (605)	76.67 (4.62)	452 (14)
	Cooked	13 (1)	4023 (143)	0.53 (0.15)	696 (37)	4.73 (0.75)	1154 (115)	43.20 (1.57)	14111 (174)
Egg white	Fresh	13 (0)	20103 (124)			1.64 (0.09)	7453 (104)	53.33 (5.77)	67 (5)
	Cooked	13 (1)	3514 (199)	0.40 (0.00)	355 (28)	6.33 (1.53)	875 (93)	66.83 (3.72)	15844 (99)
Egg yolk	Fresh	13 (0)	16995 (276)	0.49 (0.01)	866 (33)	4.32 (0.05)	6912 (104)	87.33 (0.58)	1077 (29)
	Cooked	13 (1)	4331 (308)	0.49 (0.09)	978 (40)	4.50 (0.26)	1495 (114)	43.10 (0.98)	13205 (169)
Defatted egg yolk	Fresh	13 (0)	18914 (49)	0.44 (0.02)	1013 (63)	4.11 (0.08)	6682 (39)	56.67 (15.28)	54 (13)
	Cooked	13 (1)	3547 (80)	0.49 (0.02)	910 (60)	4.40 (0.17)	1447 (63)	50.13 (2.73)	11995 (973)

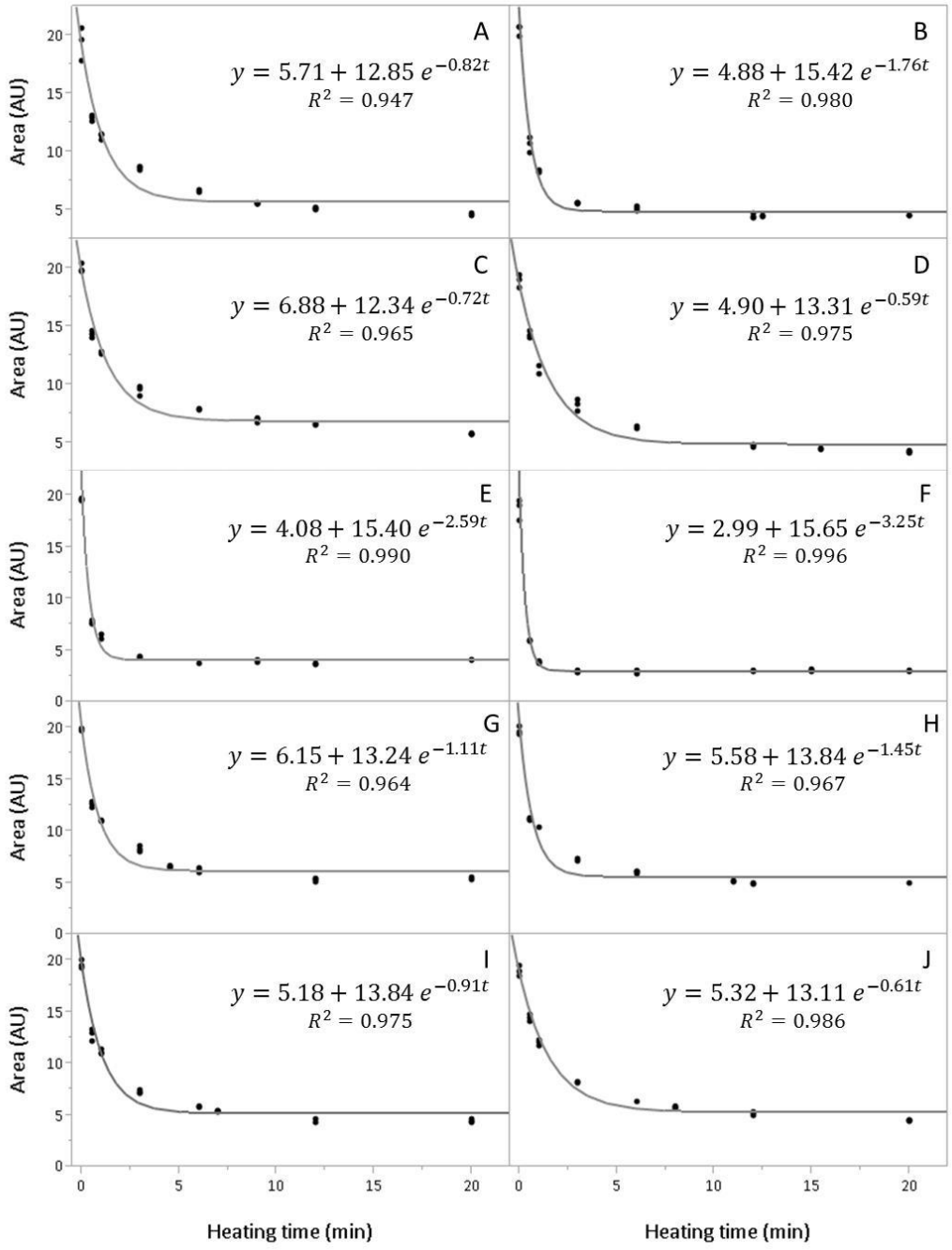

Figure 1. Extractabilities (areas in SE-HPLC chromatograms) of protein in sodium dodecyl sulfate containing medium from unsalted noodles made with flour of cultivar Paragon only (control noodles, A) and flour containing additional wheat albumin (B), wheat globulin (C), wheat gluten (D) whole egg (E), egg white (F), egg yolk (G), defatted egg yolk (H), defatted egg yolk with oil (I) and oil (J) after cooking at 100 °C. Trend lines and their corresponding formula, expressed as equation 3, and goodness of fit (R^2) were achieved applying first-order kinetics. AU, arbitrary units.

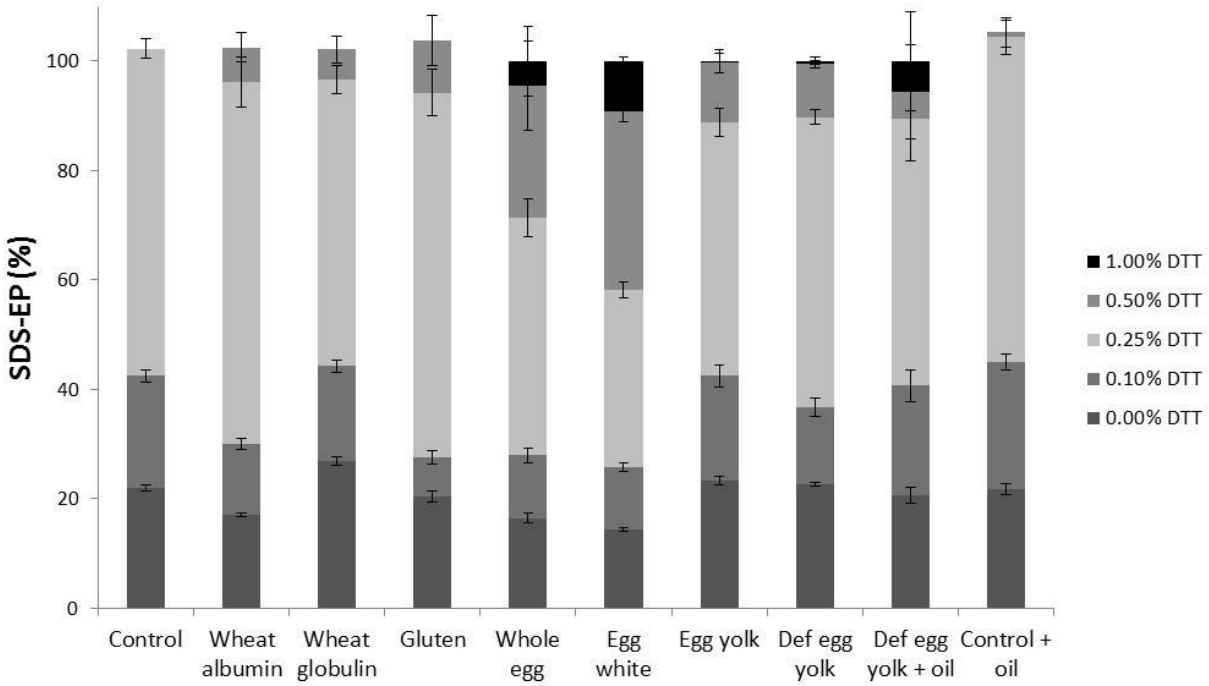

Figure 2. The increase in extractability of proteins in sodium dodecyl sulfate containing medium (SDS-EP) with increasing dithiothreitol (DTT) concentrations on unsalted noodles made with flour of cultivar Paragon only (control noodles) and flour with protein enriched Osborne-fractions of wheat, *i.e.* wheat albumin, wheat globulin and wheat gluten cooked and flour with whole egg, egg white, egg yolk, defatted (def) egg yolk, def egg yolk with oil and oil for 12 min at 100 °C. The SDS-EP is expressed as a percentage on the protein extractabilities of the corresponding samples in SDS medium with 1.00% DTT. Standard deviations are given with error bars.

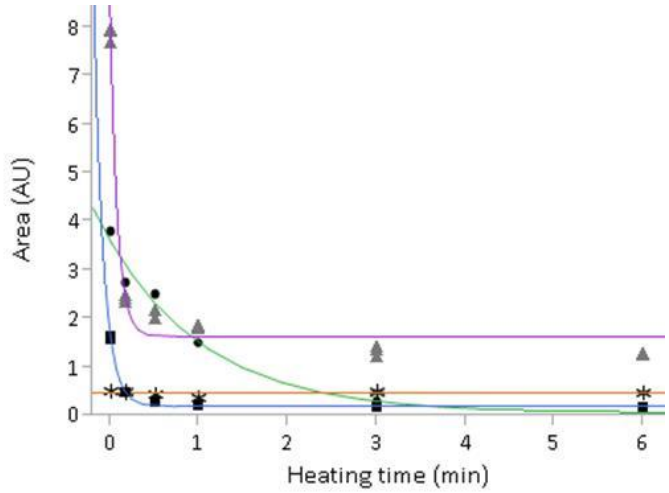

Figure 3. Extractabilities (areas in SE-HPLC chromatograms) of egg proteins in water (Δ) and glutenin (□), ω-gliadin (∗), α- and γ-gliadin (○) in dimethyl sulfoxide:propanol:water (ratio 2:1:1, DMSO containing medium) of unsalted whole egg noodles made with flour of cultivar Paragon in water after heating for various times at 100 °C. Trend lines were fitted according to first-order kinetics. AU, arbitrary units.

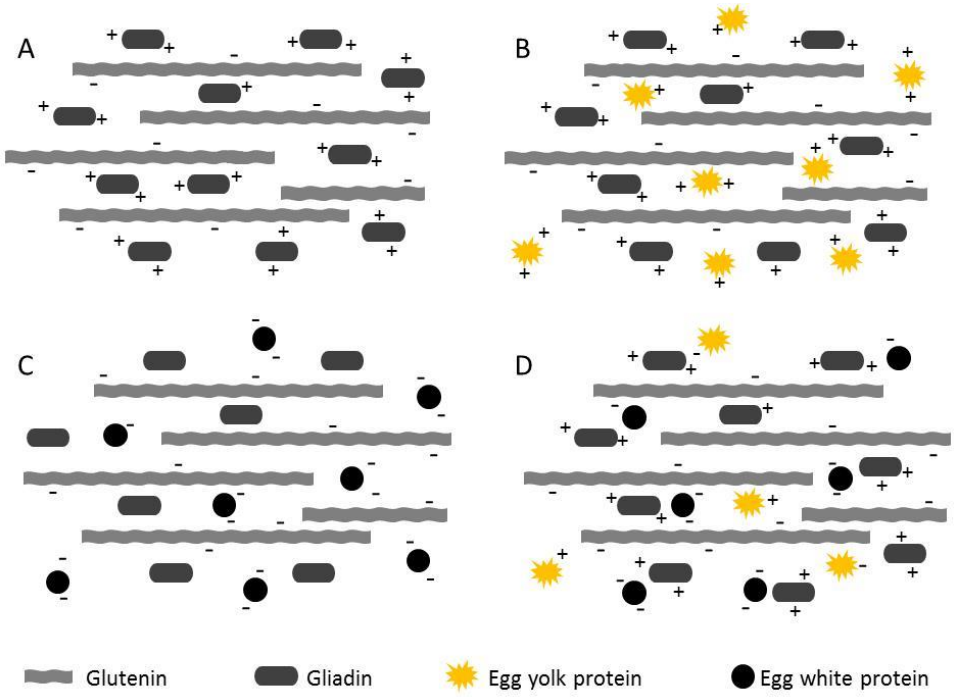

Figure 4. Visualization of ionic interactions in control (A), egg yolk (B), egg white (C) and whole egg noodles (D). Negative (-) and positive (+) surface charges are based on the average isoelectric point of protein mixtures and the actual dough pH.

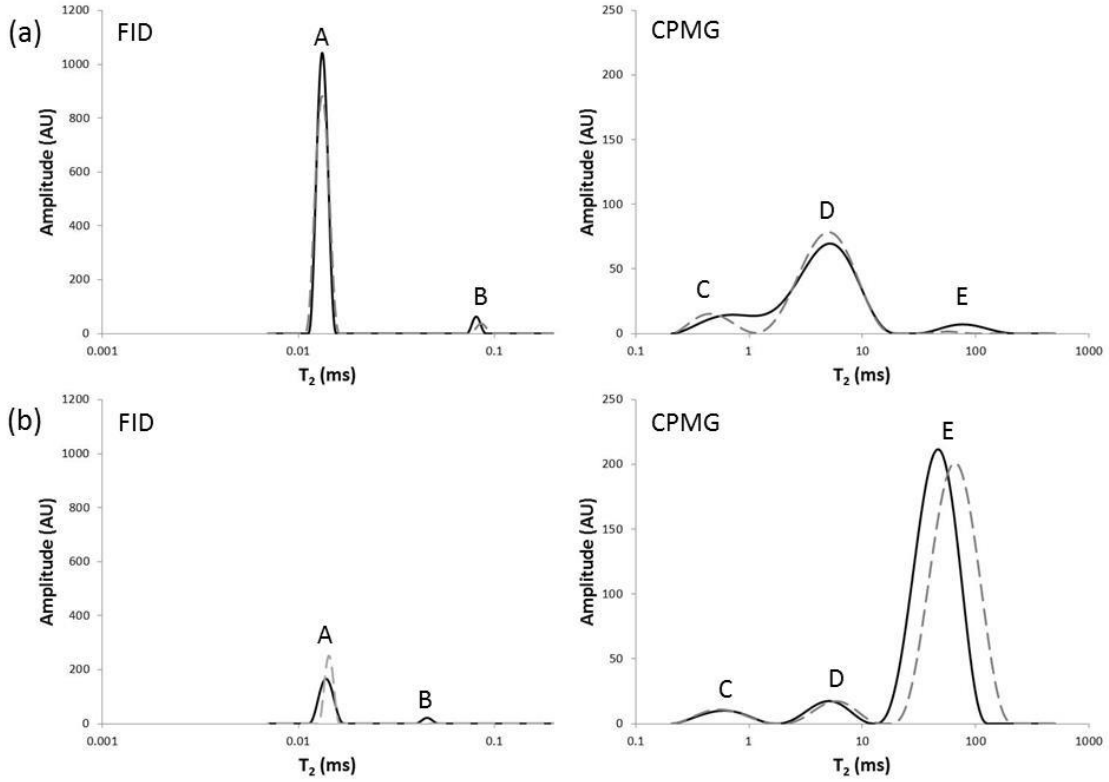

Figure 5. Free induction decay (FID) and Carr-Purcell-Meiboom-Gill (CPMG) spectra of fresh (a) and optimally cooked (b) control (---) and whole egg (──) noodles. The different proton populations are indicated with capital letters in order of increasing mobility. AU, arbitrary units.