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The impact of protein characteristics on the protein network in and properties of fresh and cooked wheat-based noodles

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ABSTRACT

The relation between protein characteristics, protein network formation and wheat noodle properties was studied. Bovine serum albumin (BSA), soy glycinin, ovalbumin, S-ovalbumin and lysozyme were included in the recipe of wheat-based noodles. The characteristics of these non-wheat proteins impacted the type, rate and extent of protein network formation during noodle dough preparation and cooking, and thereby the properties of fresh and cooked noodles. None of the added proteins enhanced the properties of fresh noodles. BSA and soy glycinin enhanced Kieffer-rig extensibility parameters of cooked noodles. Addition of ovalbumin or S-ovalbumin led to excessive protein polymerization in cooked noodles and lowered their quality. Inclusion of lysozyme lowered the rate and extent of polymerization during cooking. Experiments in which urea, olive oil or urea were added in the recipe showed that non-covalent interactions dominate the properties of fresh noodles while covalent cross-links and hydrogen bonds mainly impact the properties of cooked noodles.

1. Introduction

Noodles from wheat (*Triticum aestivum* L.) flour are popular all over the world. In Asia, they are staple foods. To address the demand for healthy protein-rich food, nutritious protein sources are sometimes included in wheat noodle recipes. The proteins they contain can impact noodle quality (Li et al., 2014). The latter is determined by color, symmetry, cooking quality (low water absorption and associated losses into the cooking water) and texture (Oh et al., 1983). The most common protein source included in wheat-based noodle recipes is hen eggs. When present in wheat noodle recipes, eggs have little impact on optimal cooking time and cooking quality but largely increase the Kieffer-rig extensibility of optimally cooked noodles. Addition of hen egg white and egg yolk respectively increases and decreases both optimal cooking time and cooking quality. In contrast, both fractions have little impact on Kieffer-rig extensibility of optimally cooked noodles. These differences in noodle properties have been ascribed to differences in (non-)covalent protein network formation during dough formation and noodle cooking. Different wheat and egg fractions have different impacts on the quality of noodles. In fresh (raw) noodles ionic and hydrophobic interactions largely impact Kieffer-rig extensibility parameters, while disulfide (SS) bonds and hydrogen bonds seem more important in cooked noodles (Lambrecht et al., 2017b). The impact of egg fractions during processing and on product quality has also been studied for pasta (Alamprese et al., 2005, 2009).

Not only eggs, but also several other protein sources can be part of wheat noodle dough. Replacing whole egg by soy isolate decreases cooking quality and noodle hardness (Khouryieh et al., 2006). Substitution of whole egg by 20% minced fish makes cooked noodles softer (Setiady et al., 2007). Inclusion of minced chicken meat in wheat noodle recipes increases the hardness of both fresh and steamed noodles (Khare et al., 2015). When the recipe of wheat noodles contains buckwheat flour, water diffuses faster into the noodles during cooking, thereby lowering the optimal cooking time (Maeda et al., 2009). The chemical composition of egg substitutes apparently impacts noodle quality more than their protein content (Khouryieh et al., 2006). However, the relation between protein characteristics and their impact on noodle properties is poorly understood.

Lambrecht et al. (2017a) demonstrated that specific characteristics of globular proteins are important for their interaction with wheat gluten. For some proteins, the extractability of their mixture

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with gluten decreases more rapidly during heating than would be expected based on the extractability losses of the isolated proteins. For instance, (S-)ovalbumin, bovine serum albumin (BSA), whole egg, egg white, wheat albumins and wheat globulins enhance polymerization in a mixture with gluten (ratio 1:2) during heating in water at 100 °C. In contrast, lysozyme decreases the extent of polymerization in a mixture with gluten (ratio 1:2). Soy glycinin and egg yolk have no significant impact on gluten covalent network formation. The authors concluded that high levels of accessible free sulfhydryl (SH) groups and hydrophobic patches in globular proteins enhance their polymerization with wheat gluten at 100 °C. Support for this theory stems from the fact that ovalbumin enhances the incorporation of gliadin in the protein network even more than does BSA. However, it is not clear whether and how these protein characteristics impact noodle quality.

Against this background, the aim of this paper was to study the relation between protein characteristics, protein network formation and noodle properties. To that end, different well characterized food proteins were included in noodle recipes, and their impact on (non-)covalent protein network formation and noodle properties (optimal cooking time, cooking quality and Kieffer-rig extensibility) was evaluated. BSA (*ca.* 66 kDa, pI 4.7, present in milk and whey) has 17 intramolecular SS bonds and one SH group (Hirayama et al., 1990). It polymerizes very fast at 100 °C in water as a result of SH oxidation and SH-SS exchange reactions and forms aggregates which are unextractable in sodium dodecyl sulfate (SDS) containing medium (Lambrecht et al., 2016). Glycinin [*ca.* 360 kDa (Belitz et al., 2009), pI 4.5–6.0 (Liu et al., 1999)] is one of the most abundant soy proteins. It contains *ca.* 21 SS bonds and no free SH groups (Draper and Catsimpoolas, 1978). Soy glycinin is a hexameric protein of which each monomer consists of a basic and an acidic polypeptide connected through an SS bond (Staswick et al., 1984). During heating in water, less polymers unextractable in SDS containing medium are formed with soy glycinin than with BSA (Lambrecht et al., 2016). Ovalbumin (*ca.* 45 kDa, pI 4.5, the main protein of egg white *ca.* 54%), has one SS bond and four SH groups (Belitz et al., 2009). During heating, ovalbumin rapidly initiates polymerization through SH oxidation reactions and forms polymers extractable in SDS containing medium. No continuous covalent protein network is formed in model systems (Lambrecht et al., 2017a). S-ovalbumin is the thermostable form of ovalbumin which results from isomerization of Ser-164, Ser-236 and Ser-320 into the corresponding D-amino acids (Yamasaki et al., 2003) of which only the isomerization of Ser-164 and Ser-320 contribute to its thermostability (Takahashi et al., 2010). The conversion of ovalbumin into S-ovalbumin during storage impacts the properties of pound cake and its batter (Deleu et al., 2015). Here, we will investigate whether these slight modifications in protein structure of ovalbumin impact its protein network formation in and properties of noodles. Finally, egg white lysozyme (*ca.* 14 kDa, pI 10.7) has four SS bonds and no free SH groups. It is used in some foods because of its antibacterial functionality (Huopalathi et al., 2007). Free SH groups formed by β-elimination reactions after prolonged heating (≥60 min, 100 °C) in water can initiate polymerization of lysozyme (Lambrecht et al., 2017a). In this paper, covalent network formation is evaluated based on the loss of extractability in SDS containing medium. The influence of ionic, hydrophobic or hydrogen interactions between proteins on noodle properties is studied by comparing noodles with and without salt, olive oil or urea addition, respectively.

2. Materials and methods

Kernels from hard wheat cultivar Paragon (RAGT, Ickleton, United Kingdom) were conditioned to 16.0% moisture and milled with a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill to flour with 13.9% protein on dry matter (dm) basis. Glycinin (100.0% protein on dm) was isolated from soy flour (Liu et al., 2007). Lysozyme (from chicken egg white, 100.0% protein on dm) and ovalbumin (albumin chicken egg grade III, 94.1% protein on dm) were from Sigma-Aldrich (Bornem, Belgium). Ovalbumin (6.67 mg/mL) was shaken for 24 h at 55 °C in 0.10 M glycine-sodium hydroxide buffer (pH 9.9) to convert it into S-ovalbumin as described by Takahashi et al. (2005). After dialysis for 24 h against water, S-ovalbumin (91.6% protein on dm) was freeze-dried and ground. BSA (fraction V for biochemistry, 98.2% protein on dm) was from Acros Organics (Geel, Belgium). Moisture and protein contents were determined in triplicate according to AACC-I Approved Method 44–15.02 (AACC, 1999) and 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), respectively. Conversion factors (5.7 for wheat; 6.25 for all other proteins) were used to calculate protein from nitrogen contents. All chemicals were of analytical grade and from Sigma-Aldrich (Bornem, Belgium) unless specified otherwise. Dithiothreitol (DTT), disodium hydrogen phosphate and sodium dihydrogen phosphate were from VWR International (Leuven, Belgium).

Noodles with a constant ratio of flour to model proteins of 2:1, moisture and protein contents of respectively 33.9% and 18.7% on dm were made from recipes containing only wheat flour, model proteins (BSA, ovalbumin, S-ovalbumin, soy glycinin and lysozyme) and deionized water as described by Lambrecht et al. (2017b). Control noodles not containing the model proteins had a protein content of 14.2% on dm. Salt, urea or olive oil (each 3.0% on dm) were added to control noodle recipes and those of noodles containing BSA, ovalbumin or lysozyme. Fresh noodle strands (20.0 g) were cooked in 500 mL deionized water to optimum as well as for 30 s, 1, 3, 6, 12 and 20 min, recovered and immediately cooled in 200 mL deionized water at 23 °C. The optimum cooking time was that needed to gelatinize all starch and determined as the point in time at which an opaque core was no longer visible when squeezing the noodles between two glass plates. Water absorption (*i.e.* weight increase of noodles after cooking), dough pH (*i.e.* pH of a freeze-dried noodle suspension in deionized water), cooking loss (*i.e.* the amount of dry material leached into the cooking water), the level of protein present in the cooking loss (measured with the Dumas method as described above) and proton mobility distributions were determined as in Lambrecht et al. (2017b). Five different proton populations were distinguished using low resolution proton nuclear magnetic resonance (^{1}H-NMR) and assigned to noodle constituents based on insights from studies on starch-water, gluten-water, flour-water (Bosmans et al., 2012) and egg-water model systems (Luyts et al., 2013). For extensibility measurements, ten fresh or cooked noodle strands from each of two different batches were stretched with a Kieffer-rig dough and gluten extensibility rig (Stable Micro Systems, Surrey, UK) using an Instron 3342 (Norwood, MA, USA) as described by Lambrecht et al. (2017b). The maximum force, the extensibility at breakage and the work needed to fracture, *i.e.* total area under curve, were calculated from the force-displacement curves. Significant differences ($\alpha = 0.05$), based on at least three individual measurements, were determined with one-way ANOVA using JMP® Pro 11.2.0 (SAS Institute, Cary, NC, USA). Corresponding Tukey grouping coefficients are given.

The protein extractability in SDS containing medium and its molecular weight (MW) distribution were determined in triplicate as in Lambrecht et al. (2015; 2017b) using size exclusion high performance liquid chromatography (SE-HPLC) and monitoring protein elution at 214 nm. Protein from freeze-dried and ground samples (containing 1.0 mg protein) was extracted with 1.0 mL 0.050 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v)