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Protein network analysis — A new approach for quantifying wheat dough microstructure

Isabelle Bernklau, Lars Lucas, Mario Jekle *, Thomas Becker

Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology & Process Engineering, 85354 Freising, Germany

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ABSTRACT

Clarification of wheat dough functionalities by visualizing the protein microstructure demands a precise image analysis, which is still challenging. Thus, a novel method for quantifying dough microstructure called protein network analysis (PNA) was established in this study. Hereby, absolute morphological attributes such as junctions' density, branching rate, end-point rate, and lacunarity quantify and characterize the strength of a network. The method was validated in a large range of varying microstructural shapes by increasing the bulk water concentration. In addition, the effect of two different magnifications (objectives with various numerical apparatus) was studied. Resulting values of the branching rate showed a significant linear decrease ($R^2 = 0.97$) by ~40% for both magnifications indicating a decrease in connectivity and cohesion within the network. Rheological measurements, used as reference methods confirmed the loss of a network structure with increasing water addition (e.g. G* decreased by 89%). Additionally, significant correlations between both methods validated the innovative image analysis PNA. With this new approach of image analysis, effects of additives, varying dough ingredients or changing process conditions on gluten network - the most structure-relevant component in wheat dough - can be quantitatively identified, and targeted functionalities can be controlled.

1. Introduction

The characteristics of wheat dough and products strongly depend on their structure and ingredients' formation. Hereby, the formation of the gluten network is fundamental for rheology and gas retention, which highlights the importance of the structure-function relationship. On a macroscopic scale, dough functionality is usually characterized by rheological measurements. In order to get even better insights, confocal laser scanning microscopy (CLSM) returns detailed results by visualization of main structural factors. For analysing microstructure and elucidating interactions of dough components, a precise method of detecting and quantifying various structural shapes is crucial. Although microscopic images of wheat dough can be evaluated qualitatively, a quantification of the main structure-relevant component, the protein network, is challenging.

In earlier studies, CLSM images of protein network of several cereal products have been analysed visually (Baier-Schenk et al., 2005; Beck, Jekle, & Becker, 2012; Dürrenberger, Handschin, Conde-Petit, & Escher, 2001; Parada & Aguilera, 2011). Peighambardoust, van der Goot, van Vliet, Hamer, and Boom (2006) tried to quantify the area fraction of the protein matrix by producing a binary image, but they stated that computing the area fraction was not sufficient to establish a relation between process parameters and dough development. Another approach of quantifying protein areas was pursued by Lee, Ng, Whallon, and Steffe (2001) by using the histogram function of a CLSM software and computing bright pixels over a grey level image. However, the sole description of the protein area does not give enough information to characterize dough functionalities. Jekle and Becker (2011) established a novel analysis method for protein quantification, besides computing the area fraction, taking into account further characteristics like particle count, average size, perimeter, circularity and fractal dimension. By using this methodology, a more detailed knowledge of the protein formation could be reached. For more information about the network structure, Boitte, Hayert, and Michon (2013) used grayscale morphology as texture analysis to obtain the local protein concentration and orientation. First own studies enabled the development of structure function relationships of proteins in wheat dough based on particle related microstructure properties and simple morphological values (Döring, Nuber, Stukenborg, Jekle, & Becker, 2015; Jekle & Becker, 2012). However, a more advanced and adjusted analysis of the protein microstructure focusing on absolute morphological values (such as branching rates) might distinctly increase the predictive significance of the protein network. Furthermore, a more comprehensive understanding of product properties might be provided, and relationships

Abbreviations: AACCi, American Association of Cereal Chemistry international; CLSM, confocal laser scanning microscopy; ICC, International Association for Cereal Science and Technology; PNA, protein network analysis; rel., relative alteration; ROI, region of interest.

* Corresponding author.
E-mail address: mjekle@tum.de (M. Jekle).

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between protein network characteristics and rheological behaviour might be clarified.

In this study, a novel approach of protein network analysis (PNA) was developed to quantify structural alterations. Protein network in wheat dough show a high similarity to other organic structures, like cell systems or blood vessels. Thus, a tool usually used in medical application for characterizing vascular networks was applied on wheat protein networks, and the analysis was adapted to fit the needs of food systems. This method has the advantage of determining not only the protein area, but also the number of junctions and structure regularities for describing the strength of the network. In order to test the capability for detecting protein structures, the applicability of image processing algorithm and their computed attributes were evaluated in detail. For validation of the image analysis, different structure profiles of the gluten network were required. Thus, the bulk water concentration was varied to provoke a large range of microstructural shapes in wheat dough due to a plasticizing effect of water (Jekle & Becker, 2011). As a reference, oscillating rheometry was applied to compare the CLSM results and to combine micro and macrostructural levels. Additionally, different magnifications of CLSM images were compared to study the influence on quantitative results. With this new approach of network analysis, a precise detection of microstructural changes can be provided to elucidate dough functionalities.

2. Materials and methods

2.1. Dough preparation

German commercial wheat flour Type 550 (Rosenmühle, Landshut, Germany) was used for dough preparation. According to methods of the American Association of Cereal Chemistry international (AACCi) and of the International Association for Cereal Science and Technology (ICC), 14.17 ± 0.03 g moisture per 100 g flour (AACCi 44-01), 12.70 ± 0.04 g protein content per 100 g dry flour (AACCi 46-16, N × 5.7), 0.63 ± 0.01 g ash per 100 g dry flour (ICC 104/1), 28.75 ± 0.81 g wet gluten per 100 g flour and a falling number of 407 s (AACCi 56-81) were determined. Dough resistance and water absorption were measured in a Z-kneader (doughLAB; Perten Instruments, Germany) according to AACCi 54-70.01 in order to determine the required kneading time. To reach 500 Farino units, dough consisting of 50.1 g flour and 29.6 g demineralized water were kneaded 180 s at 63 rpm. Bulk water concentration was varied in a range from 59.2 to 89.8 ml per 100 g flour (corrected to 14% moisture) to provoke different microstructural shapes of the protein network. Samples with higher water concentrations should highlight extremes of dough protein structures, like in pre-doughs. To stain the samples for confocal laser scanning microscopy, 5 ml of bulk water was replaced by a Rhodamine B solution (Merck KGaA, Darmstadt, Germany, 0.01 g/100 ml water). All measurements were performed in triplicates.

2.2. Dough rheology measurements

Viscoelastic properties of dough were measured by oscillatory and creep-recovery tests with an AR-G2 rheometer (TA instruments, New Castle, USA) consisting of parallel cross-hatched plates (Ø 4.0 cm) with a constant gap of 2.0 mm and a smart swap Peltier plate temperature system (30 °C constant temperature during measurement). 5 g of dough samples were placed between the plates, the gap was set, the excess of dough was removed and the remaining surface of the dough was treated with paraffin oil to avoid sample drying. After 10 min of resting time for dough structure relaxation, an oscillatory frequency sweep test was performed with a constant deformation of 0.1% (within the linear viscoelastic region) and a frequency ranging from 0.1 to 50 Hz. Fundamental dough rheology properties were characterized by the complex shear modulus G*, the storage modulus G′ and the loss modulus G″ at 1 Hz. Afterwards, a creep-recovery test with a constant shear stress of 250 Pa at 30 °C for 180 s and a relaxation time of 360 s was conducted. The relative elastic part J_{el} was evaluated by the ratio of the creep compliance J_{max} and the creep recovery compliance $J_{r.}$ (c.f. Jekle & Becker, 2011).

2.3. Microstructure analysis by confocal laser scanning microscopy

As described above, dough samples were stained by addition of dye in bulk water with Rhodamine B to visualize proteins. The dough sample (2 g) were transferred to an object carrier with a cylindrical notch (Ø 18 mm, height 7 mm) and sealed with a cover glass. After 10 min of resting time for dough relaxation, samples were analysed by an eclipse Ti-U inverted microscope with an e-C1 plus confocal system (Nikon GmbH, Düsseldorf, Germany) using two different objectives (Plan Apo 20×/0.75 and Plan Apo VC 60×/1.40 Oil). A laser with a wavelength of 543 nm was used for excitation, the emission was detected at 590/50 nm. 10 different images were taken of each dough sample with a resolution of 1024 × 1024 pixel and a size of 215 × 215 μm (for 60× objective) and of 686 × 686 μm (for 20× objective), respectively. Dough samples were produced in triplicates, therefore, 30 images were analysed for one dough type.

2.4. Image processing and analysis

The software-based analysis of CLSM images was performed by AngioTool64 version 0.6a (National cancer Institute, National Institute of Health, Maryland, USA). This tool usually is used for microscopically analysis in medical applications to study changes in vascular networks provoked by angiogenesis (Zudaire, Gambardella, Kurcz, & Vermeren, 2011). AngioTool was applied on CLSM images of gluten network due to high similarities of vascular network.

2.4.1. Implementation details – protein network analysis

Image processing by AngioTool is divided into two main steps; first, the protein network analysis by segmentation and skeleton analysis for identification of protein area, number and density of junctions, protein thread length and number of end-points. Second, the tool analyses the lacunarity for describing irregularities of formation and gaps within the network. Fig. 1 shows a simple network structure before and after segmentation and skeleton analysis by AngioTool. CLSM pictures are converted to grey scale images. Internally, a copy of the image is convoluted with a Gaussian filter to a blurred image. The width of the

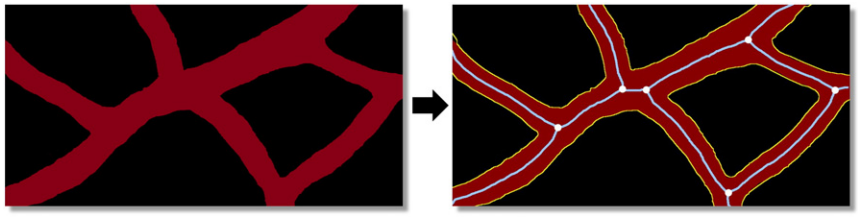

Fig. 1. Simple network structure before and after image processing by AngioTool. Junctions are shown in white, protein skeleton in blue and protein outline in yellow.