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Engineering the gluten matrix: Advanced insights into formation and shear stress dynamics

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Preface and Peer-Reviewed Publications

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This cumulative thesis is based on the following peer-reviewed publications:

I. Vidal, L. M., Braun, A., Jekle, M., & Becker, T. (2022). Micro-Scale Shear Kneading: Gluten Network Development under Multiple Stress-Relaxation Steps and Evaluation via Multiwave Rheology. Polymers 2022, Vol. 14, Page 846, 14(4), 846. doi: 10.3390/POLYM14040846

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Contents

Abbreviations

Abbreviation	Full meaning
A	Area
AACC	American Association of Cereal Chemists
A_f	Network strength
DDT	Dough development time
F	Force
G	Elastic Modulus
G*	Complex Modulus
G’	Storage Modulus
G’’	Loss Modulus
GS	Glutenin Subunit
h	Hight
HMW-GS	High Molecular Weight – Glutenin Subunit
K	Strength coefficient
LMW-GS	Low Molecular Weight – Glutenin Subunit
LVR	Linear Viscoelastic Region
n	Strain-hardening exponent
r	Radius
RH	Relative humidity
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
rpm	Revolutions per minute
S	Gel strength
SAOS	Small Amplitude Oscillatory Shear
SHI	Strain-hardening index
SME	Specific mechanical energy
t	Time
TNoJ	Total number of junctions
x	Displacement
z	Network connectivity

Equation Characters

Characters	Full Meaning
γ	Strain
$\dot{\gamma}$	Strain rate
δ	Shift Angle
ε	Biaxial Strain
η	Viscosity
σ	Stress
ϕ	Phase Angle

Summary

Precise and rapid determination of wheat flour and dough properties is crucial for the bakery industry to produce high quality baked goods and ensure consistent consumer acceptance. Analyzing these properties helps detect fluctuations in flour quality due to wheat growing conditions, milling, and storage. Variations in these parameters lead to changes in the gluten network development during dough preparation, which is based on the molecular cross-linking of the proteins contained in interaction with the starch fraction of the flour. However, the established analysis methods have considerable disadvantages: 1) they require high sample volumes with more than 500 g 2) they require several independently operating and expensive analysis devices and 3) they require extensive user knowledge for operation of these devices and analysis of measurement values. Therefore, the objective of this study was to develop a novel method for inline kneading and analysis to overcome these drawbacks. The core of the work is an adapted rheometer that enables network development under simple shear, resulting in further advantages. On the one hand, the method allows further process steps connected to the kneading process, such as proofing and baking the samples, to be carried out inline. On the other hand, with a minimal sample size of less than 0.5 grams, it is possible to perform an analysis that covers the entire process chain from flour to the finished baked product on micro-scale. This is achieved with a single device, a commercially available rheometer, which operates on this micro-scale and makes additional sample handling unnecessary. The rheometer is equipped with standard plane geometry on top and an adapted cone geometry at the bottom.

As a first step, an inline shear kneading and analysis method was developed in a conventional rheometer. Through simple shear stress steps, with implemented pauses for matrix relaxation, fully developed doughs could be produced. By implementing multi-wave frequency sweeps and analyzing the network connectivity z [-] - a measure for the interacting rheological units in the matrix - the direct comparability of shear-kneaded to classically kneaded dough was demonstrated. For both doughs, sheared and classically kneaded, z was in the range of 5.0 and 5.5 at the optimum stage of development which was in good agreement with literature. In addition to the analysis of the rheological parameters describing the dough development, the experimental setup was further validated in the second part of this work by microscopic inline protein network analysis. For this purpose, a confocal laser scanning microscope

(CLSM) was combined directly with the rheometer. This setup allowed kneading, rheological classification, and optical network analysis inline for the first time without sample transfer or interruption of the kneading process. The total number of junctions (TNoJ) investigated by image analysis was between 1300 and 2000 for all flour samples, picturing a fully connected network depending on the protein content. The encouraging results of these experiments were the motivation for further improvements to the experimental set-up. These results led to the development of the shear kneading technique, which opened new opportunities for research into fermentation and baking processes on micro-scale. Therefore, the third part of this study focused on microanalytical baking tests. Less than 0.5 g of flour samples were required to analyze the influence of the composition on the achievable sample volume after kneading, proofing and baking. It was demonstrated that volume increases of 80 - 95 % of the original sample size could be achieved for this microanalytical baking line, which is comparable to classic baking tests with yeast as leavening agent. In baking tests with chemical leavening agents, consisting of baking soda and powdered acid, similar results were obtained. For further validation of the developed microanalytical baking line in a complex system, the contained acid was replaced by an alternative in conventional baking powder. The exchange allowed the chemically induced leavening behavior of a new substance in the dough to be microanalytically investigated. Further tests also provided additional insights into the gas retention capacity of the dough network. This was then investigated as a fourth step in this work. For the first time, it was possible to draw conclusions about the structure-strengthening properties of the flour and dough samples in a rheometer using a method involving pure shear stress on the sample. The shear method in the rheometer was validated with lubricated squeeze flow analyses as an established procedure. Finally, all the results of the previous stages of this work were compared using a correlation analysis of the determined flour, dough, and processing properties. The correlation between the protein composition of the flours and the achievable network strength of the dough, as well as the achievable volume yields of the baked samples, was demonstrated for the flour samples examined. The results of this work provide the wheat breeding, milling and baking industries with important mechanistic insights into the development of the dough network and a powerful analytical tool for the prediction of process and baking properties of flour samples using sample volumes in the micro range.

Zusammenfassung

Eine präzise und schnelle Evaluation der Weizenmehl- und Teigeigenschaften ist für die Backwarenindustrie von entscheidender Bedeutung, um Backwaren mit hoher gleichbleibender Qualität und Verbraucherakzeptanz produzieren zu können. Damit lassen sich Mehlqualitätsschwankungen in Abhängigkeit der Anbaubedingungen, dem Mahlprozess sowie den Lagerungsbedingungen erkennen, die zu einer veränderten Netzwerkentwicklung bei der Teigherstellung führen können. Verantwortlich für die Knet- und Teigeigenschaften der Mehle sind die enthaltenen, netzwerkbildenden, Proteine im Zusammenspiel mit der Stärkefraktion des Mehls.

Die etablierten Analysenmethoden zur Bestimmung der Mehl- und Teigeigenschaften weisen jedoch erhebliche Nachteile auf: 1) sie benötigen hohe Probenvolumina von mehr als 500 g 2) sie erfordern mehrere unabhängig voneinander arbeitende und teure Analysegeräte, sowie 3) sie setzen ein umfangreiches Anwenderwissen für den Betrieb und die Auswertung der Messergebnisse der verschiedenen Messgeräte voraus. Das Hauptziel dieser Arbeit bestand darin, eine innovative Methode für die Herstellung und Inline-Analyse von Teig zu entwickeln, die die bisherigen Nachteile vermeidet. Im Zentrum dieser Methode steht ein handelsübliches Rheometer, das durch einfache Scherung die Entwicklung des Netzwerks ermöglicht. Die Vorteile der Methodik sind unter anderem die Verwendung einer minimalen Probenmenge von unter 0.5 g Mehl. Zusätzlich ist eine umfassende Analyse der gesamten Prozesskette, von der Mehlaufbereitung bis zum gebackenen Zustand, unter Einsatz eines standardisierten Rheometers möglich. Dies geschieht alles im Mikro-Maßstab und erfordert kein zusätzliches Handling der Probe wie Überführung des Teiges in andere Geräte, was eine effiziente und präzise Untersuchung ermöglicht. Durch dieses Verfahren können weitere dem Knetprozess angeschlossene Prozessschritte wie die Gare und das Backen der Proben ohne Prozessunterbrechung im selben Gerät durchgeführt werden.

Im ersten Schritt der Arbeit wurde eine Inline – Scherknet- und Analysemethode in einem konventionellen Rheometer entwickelt. Durch einfache Scherbelastungsschritte, mit implementierten Pausen zur Relaxation der Matrix, konnten vollständig entwickelte Teige produziert werden. Durch in diese Relaxationsschritte integrierte Multiwellen-Frequenzsweeps und die Analyse der

Netzwerkkonnektivität z [-], ein Maß für die miteinander interagierenden rheologischen Einheiten in der Matrix, konnte eine direkte Vergleichbarkeit von schergeknetetem zu klassisch geknetetem Teig nachgewiesen werden. Für beide Teige lag z im optimalen Entwicklungsstadium im Bereich von 5.0 und 5.5 und somit im optimalen Bereich. Neben der Analyse der rheologischen Parameter, welche die Teigentwicklung beschreiben, wurde im zweiten Teil dieser Arbeit der Versuchsaufbau durch eine mikroskopische Inline-Protein-Netzwerkanalyse weiter validiert. Zu diesem Zweck wurde ein konfokales Laser-Scanning-Mikroskop mit dem Rheometer gekoppelt. Dieser Aufbau erlaubte, das Kneten, die rheologische Klassifizierung und die optische Netzwerkanalyse erstmals inline und ohne Probentransfer oder Unterbrechung des Knetprozesses durchzuführen. Die analysierte Gesamtzahl der bildanalytisch ausgewerteten Knotenpunkte (TNoJ) lag dabei für alle Mehlproben zwischen 1300 und 2000, abhängig vom Proteingehalt, was auf ein vollständig entwickeltes Netzwerk im Teig schließen lässt. Die Ergebnisse dieser Versuche führten zu weiteren Anpassungen des Versuchsaufbaus. Die Scherknettechnik eröffnet neue Möglichkeiten zur Analyse von Fermentations- und Backprozessen im Mikrobereich. Daher zielte der dritte Teil dieser Studie auf mikroanalytische Backtests ab, welche ohne Änderungen des Aufbaus durchgeführt werden konnten. Mit der entwickelten Analysemethode konnte der Einfluss der Mehlzusammensetzung auf das erreichbare Probenvolumen nach dem Kneten, der Gare und des Backprozesses analysiert werden. Es zeigte sich, dass für diese mikroanalytische Backstraße, kongruent mit der klassischen Backversuchen, vergleichbare Volumenzuwächse von 80 - 95 % der ursprünglichen Probengröße erreicht werden konnten. Um die entwickelte mikroanalytische Backstraße in einem komplexen System weitergehend zu validieren, wurde der Säuregeber in konventionellem Backpulver ausgetauscht. Durch den Austausch konnte das chemisch induzierte Triebverhalten einer neuen Substanz im Teig mikroanalytisch untersucht werden. Die weiterführenden Versuche gaben zudem weitere Einblicke in das Gasrückhaltevermögen des Teignetzwerks. Ein wesentlicher Faktor für die Fähigkeit des Weizenteigs, Gas während der Gare und des Backprozesses zurückzuhalten, ist die Strukturverfestigung der, die Gasblasen umgebenden, Teiglamellen während ihrer Expansion. Diese Strukturverfestigung wurde dann in einem vierten Schritt in dieser Arbeit untersucht. Durch eine Methode, die ausschließlich auf der Scherbelastung der Probe basiert, konnten

Schlussfolgerungen über die strukturverfestigenden Eigenschaften von Mehl- und Teigproben in einem Rheometer gezogen werden. Die Schermethode im Rheometer wurde dafür mit etablierten Verfahren, unter anderem der Lubricated-Squeeze-Flow-Analyse, validiert. Zuletzt wurden alle Ergebnisse der vorangegangenen Teilschritte dieser Arbeit, mittels einer Korrelationsanalyse der ermittelten Mehl-, Teig- und Verarbeitungseigenschaften, gegenübergestellt. Dabei konnte der Zusammenhang zwischen der Proteinzusammensetzung der Mehle und der erreichbaren Netzwerkstärke des Teiges, sowie der erzielbaren Volumenausbeuten der gebackenen Proben, für die untersuchten Mehlproben aufgezeigt werden.

Die Ergebnisse dieser Arbeit liefern der Weizenzucht-, Mühlen- und Backindustrie wichtige mechanistische Einblicke in die Entwicklung des Teignetzwerks sowie ein starkes analytisches Werkzeug für die Vorhersage von Prozess- und Backeigenschaften von Mehlproben unter Einsatz von Probenvolumina im Mikrobereich.

1 Introduction

Hybridization between *Triticum turgidum* and *Aegilops tauschii* led to the hexaploid *Triticum aestivum*, which formed the basis for the worldwide cultivation of one of the most important crops. Since the first planned cultivation of hexaploid wheat some 10,000 years ago, methods to improve yield, protein content, and the plant's resistance to weather and pests have evolved dramatically. The journey of this crop from wild to domesticated genotypes has resulted in a resilient and versatile plant that plays a crucial role in addressing global food security challenges. For more than a century, the improvement of wheat, in yield and composition, as one of the most important crop species went from empirically, random try and error breeding, to scientific driven, target focused, methods [1], [2]. In times of growing consumer awareness, increasing price competition, and advancing globalization, very high demands regarding flour and baked goods quality are made. Fundamentally, the appeal of hexaploid wheat is rooted in its extraordinary potential for high yield. With three sets of chromosomes, this genetic characteristic translates into heightened grain production per plant, positioning it as an invaluable asset for efficient food production [2], [3]. This advantage, coupled with the wheat's adaptability to diverse environmental conditions, ranging from different soil types to various climates, empowers professionals to cultivate wheat across a wide geographical spectrum, thereby enhancing global food security [4]. However, the importance of bread wheat reaches far beyond agricultural production. Its versatility in end-use, serving as a foundational ingredient in various culinary and industrial applications, exemplifies its adaptability to meet the dynamic demands of global consumers. Researchers can harness its genetic complexity to develop improved varieties, incorporating traits like disease resistance, drought tolerance, and enhanced yield [5]. Especially protein content is one of the key factors of research and breeding. It is the price and usage demanding parameter for present grain and flour traders and producers of baked goods. The protein content, often referred to as gluten content, plays a crucial role in determining the flour's ability to be processed into dough, adhesives, films, or other products [6]. On the one hand, for flour traders the total protein content marks as the only crucial parameter for price and quality determination. On the other hand, for the processors it’s the gluten´s quality, not only the amount, which determines the resulting dough or gluten film properties by its versatile protein

composition. Therefore, the gluten content and quality act as key parameters for producers of baked goods and related products. This protein quality significantly influences dough development and the manageability of the kneaded dough. As it has direct impact on the network evolution during kneading and therefore the behavior of the dough during and after the kneading process. Understanding and achieving specific dough characteristics necessitates a focus on the evolution of the flour's network structure and kneading parameters. Research often centers on this aspect ([7], [8], [9]). Thereby, one of the challenges is to reduce the actual required sample size of more than 10 g flour for kneading and further analysis ([10], [11]). With the reduction of the sample size, the evaluation of new breeds or small batches of flour would help to determine the gluten quality in an early-stage material and cost-effective. Various mechanical mixers are available for processing wheat flour doughs for analytical and industrial purposes. These range, as a small excerpt, from pin mixers in research to classic Z-mixers in bakeries and high-speed mixers in industry. Each kneader type introduces energy into the dough in a unique way aiding in the development of the gluten network and resulting in the desired dough consistency [12]. The complexity of energy transfer and the need to first knead a dough and then transfer it, for scientific analysis, to other measuring devices, leads to the task of simplifying energy transfer during kneading and the possibility of device combinations. By developing a better understanding of network evolution, we can enhance our measurement capabilities to address the previously mentioned challenges effectively. This thesis aims to demonstrate a new and combined dough kneading system integrated into a rheometer to combine network development measurements and the kneading process in one device for an inline, automated and independent measuring with the additional benefit of significantly lower sample size as required for classical farinograph analysis. This inline kneading and measuring system enables the continuous evaluation of the network development during the energy transfer from the kneader to the dough system. With the reduction to micro-scale and the possibility to combine the rheometer with other analysis instruments, a deeper investigation of the kneading and network development is possible. The combination of the kneading and measuring system in a rheometer with a confocal laser scanning microscope (CLSM) makes inline optical evaluation of the network development possible for the first time. Micro-scale baking trials show the applicability of the kneading system for a fully comprehensible flour analysis regarding foaming and baking properties without sample

transfer and with precise temperature profile control during the whole process. Further stress-related material responses of doughs are discussed to enlighten the influence of deformation during kneading and processing on the network attributes of gluten starch matrices.

Therefore, the key parameters to develop the protein network in wheat flour doughs are described in the next chapter. Thereafter, the crosslinking processes of kneaded gluten-starch blends and wheat flour with water under load are discussed. Finally, the underlying hypotheses of this thesis, as well as the outline, are presented.

1.1 Components in *Triticum aestivum*

The wheat kernel contains various ingredients, each with a distinctive functionality within the development from kernel to plant. The whole grain contains, summed up by Van Hung et al. [13], around 65.4 – 78.0 % carbohydrates, 7.0 – 19.3 % protein, 7.8 – 14.8 % Water, 1.2 – 3.0 % Minerals and 0.9 – 3.3 % lipids. These main ingredients are the key factors for the quality of the breeds and the resulting processability of the milled flour and the gluten or starch products obtained from it.

1.1.1 Carbohydrates

In the endosperm, the contained carbohydrates are free sugars, gluctofructans (1 – 2 % w/w), and starch. The starch in wheat kernels mainly consists of the two major components amylose and amylopectin. Both are α-1,4-glucose polymers and occur in a range of 25 – 28 % amylose and 72 – 78 % amylopectin, varying among starch from different plant sources as well as from different wheat breeds [14], [15]. In the starch granules, amylose contributes to gel firmness and retrogradation, while amylopectin influences gel viscosity and stability ([16], [17]). The size and shape of the starch granules, which vary among wheat varieties, also impacts the water absorption capacity and swelling power of the flour, thereby affecting dough consistency and stability ([16], [17], [18], [19]). With a bimodal size distribution, in contrast to most plant starches, wheat starch contains small spherical (B) granules with a diameter up to 10 µm, while the large (A) granules are lenticular with a diameter of up to 40 µm [20], [21], [22]. The starch granules can be damaged during milling and processing of the flour. This damaged starch highly influences the water absorption and swelling of the granules which has an impact on the achievable dough properties and quality of baked goods [23]. During baking, gelatinization of starch is a process that involves the

breakdown of the intermolecular bonds within the starch molecules in the presence of water and heat, leading to the absorption of water and swelling of the starch granules. This process results in a change in the physical properties of the starch, including increased viscosity, solubility, and translucency [24].

1.1.2 Proteins

Proteins in the wheat grain fulfill several important functions related to the structure, nutrition, and functionality of the grain. These functions contribute to the overall growth, development, and survival of the wheat plant [25]. The proteins provide structural integrity, serve as storage reserves, regulate biochemical processes, protect against pests, influence technological properties and contribute to the overall growth and development of the wheat plant [26], [27], [28]. As described by Osborne in 1907 the wheat proteins are traditionally divided into four Osborne fractions according to their solubility [29]: albumins, globulins, gliadins and glutenins. These major protein fractions in wheat play different roles in the gluten network development (see 1.1.4). The albumin/globulin fraction contains mainly regulatory, metabolic and protective proteins. Gliadins classified as prolamins, undergo further subdivision into α-, γ-, $\omega_{1,2}$-, and ω_5-gliadins based on their molecular weights falling within the range of 25 – 75 kDa [30], [31]. They can also be divided into monomeric proteins and oligomeric proteins [32]. In their native form, prolamins mainly exist as monomers, while glutenins take on a polymer structure characterized by subunits of both low (30 – 50 kDa) and high molecular weight (70 – 140 kDa) [31].

1.1.3 Protein functionality in the grain

Some proteins, including enzyme inhibitors in the albumin/globulin fraction, act as part of defense mechanisms against pests by hindering their digestive processes [33]. During grain growth and plant development, gliadins and glutenins play pivotal roles by participating in essential biochemical and physiological processes. Gliadins and glutenins are rich in nitrogen and sulfur, and during grain filling, they serve as a reservoir for these essential nutrients. The disulfide bonds in the glutenins contribute to the proper folding and stabilization of proteins in the seed. This is crucial for maintaining the viability of the seed during storage and ensuring successful germination. During germination, the gliadins and glutenins, along with other seed storage proteins, act as an energy source. The breakdown of these proteins releases

energy that is utilized for metabolic activities, sustaining the seedling until it can engage in photosynthesis independently.

1.1.4 Protein functionality in the dough

The wheat proteins glutenin and gliadin form the continuous gluten phase when mixed with water and are responsible for dough elasticity and firmness during bread making [34], [35], [36]. The continuous gluten phase contributes to the formation of a stable bread structure. Gliadins and glutenins influence the technological properties of wheat flour regarding achievable network properties. They directly influence dough rheology and therefore the quality of baked goods [37], [38], [39]. They play a role in determining the texture, volume, and overall quality of baked goods. They can be divided into the High Molecular Weight Glutenins (HMW-GS) and Low Molecular Weight Glutenins (LMW-GS). HMW-GS are characterized by their large molecular size and their complex, glutamine- and proline-rich structure and act as key components of the gluten network. These proteins play a pivotal role in the strength and elasticity of gluten by forming the backbone of glutenin macropolymers. This molecular scaffolding is crucial for maintaining the structural integrity of the network and, ultimately, influences the dough's handling properties. LMW-GS are smaller in size compared to their high molecular weight counterparts. Their structure, marked by repetitive domains and cysteine residues, contributes to the diversity of gluten composition. Functionally, LMW-GS enhance the overall structure of gluten, providing additional layers of complexity. As part of the gluten network, these proteins, based on their cysteine-rich regions, participate in the formation of disulfide bonds, reinforcing the dough´s structural framework. In contrast to glutenins, gliadins are smaller, monomeric proteins with a simpler structure. Grouped into α-, γ-, $\omega_{1,2}$-, and ω_5-gliadins based on their solubility and molecular weight (see 1.1.2), these proteins impart a different set of functionalities to the grain. Gliadins contribute to the extensibility and viscosity of gluten, affecting the hydration and handling properties of the dough. Their influence on the rheological characteristics of the grain extends to the final texture of baked products.

At the molecular level, the gluten development process during dough kneading involves intricate chemical processes that shape the composition and structure of the gluten network. Hydrogen bonds form between the amino acid residues of gliadin and glutenin. These bonds arise from the attraction between the positively charged hydrogen atoms and the electronegative atoms in amino acids. Although hydrogen

bonds are relatively weak, they are crucial in the nascent stages of protein interaction, serving as the foundation for the subsequent structural organization of gluten [40], [41], [42]. Hydrophobic interactions come into play as certain regions of gliadin and glutenin exhibit a repulsion toward water. Hydrophobic parts of these proteins tend to associate with each other, leading to the creation of a hydrophobic core. This association enhances the stability of the protein structure, effectively shielding hydrophobic segments from the surrounding aqueous environment. The resulting hydrophobic collaboration significantly contributes to the overall cohesiveness of the gluten network [30], [43], [44]. Disulfide bonds, representing covalent connections, are formed between the sulfur-containing amino acids, specifically cysteine residues. These bonds are pivotal for conferring strength and stability to the gluten network. During the kneading process, disulfide bonds from because of the oxidation of sulfhydryl groups on the gluten proteins, which leads to the formation of disulfide bonds between adjacent protein strands. Acting as molecular "cross-links," they provide a robust interconnected framework between different protein chains, reinforcing the gluten structure against the mechanical stresses encountered during dough manipulation [28], [30], [45], [46]. In essence, the chemical process of gluten development entails the establishment of hydrogen bonds as the initial scaffolding, followed by the stabilization of the protein structure through hydrophobic interactions. The covalent integrity of the gluten network is then fortified by the formation of disulfide bonds, creating a molecular architecture that determines the strength, elasticity, and overall quality of the dough.

1.2 Dough processing

To achieve consumer satisfaction with baked goods and to maintain constant product quality, dough processing can be subdivided into three main steps: mixing and kneading of the ingredients, foaming of the mixed matter, and baking of the shaped end products [47].

1.2.1 Mixing and kneading

The dough kneading process can be broken down into the chemical and physical aspects of the network's evolutionary development. Figure 1 shows the ongoing kneading process from mixing the ingredients with water to a fully developed network and beyond [48]. After reaching the optimum network development state, defined as the momentum at which the matrix best withstands external deformation, the

weakening of the network starts due to breaking of bindings and released water from the matrix. This weakening is caused by the additional incorporated energy from the kneader [49].

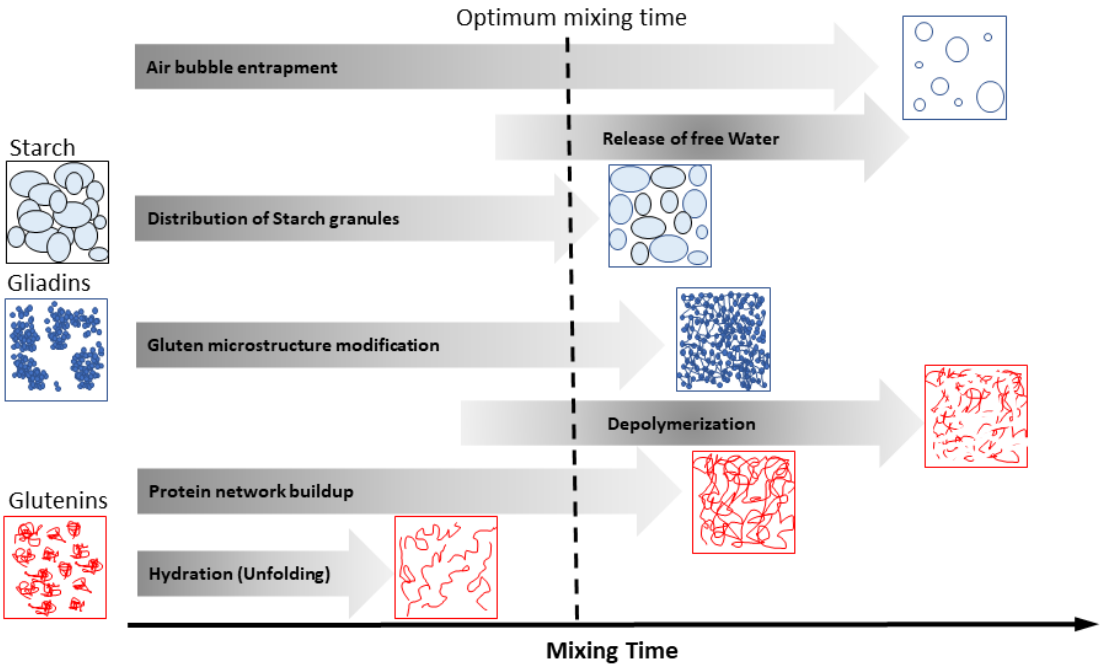

Figure 1: Gluten network development from flour to dough over time. Processes during the evolution and degradation of the network caused by energy transfer to the matrix (according to Schiedt et al. [48]**).**

Firstly, the kneading process starts by mixing the dry ingredients and the water. When the hydration of the flour particles starts, the hydrophobic and hydrophilic amino acid residues of both glutenin and gliadin start to interact with the added water as polar solvent. Thereby, the oxygen atom in water has a partial negative charge, while the hydrogen atoms have partial positive charges. This polarity enables water to interact with the charged and polar groups in proteins [50]. Glutenin and gliadin molecules swell after contact with water. After penetrating the proteins' structure, the water disrupts hydrophobic interactions within the proteins and exposes the hydrophilic regions, resulting in the mentioned swelling. Along the hydration and further mixing of the matter, water molecules and polar amino acid residues (e.g. serine, threonine, and tyrosine) form hydrogen bonds. Additional hydrogen bonds are formed with the amide groups of the protein backbone and stabilize the water protein interaction. With ongoing mixing and hydration, the water surrounds the hydrophobic regions, preventing the proteins from aggregating and promoting their solubilization. The

induced conformational changes in glutenin and gliadin lead to a hydrated and extended protein structure. With these changes, a supportive environment for subsequent protein-protein interactions during gluten formation is created. The hydrated glutenin and gliadin molecules, which are now in a more flexible and extended state, are prepared for interaction. This is the basis for gluten formation during the kneading process, in which the mechanical energy aligns and organizes the proteins into a continuous network. Through the energy input caused by the movement of the kneader geometry, the matter is exposed to shear, tensile and compression forces. Also, rigid body motions without energy transfer to the matter can be observed [51]. The rotating blades of highspeed mixers, mostly in industrial dough production, transfer energy predominantly by shear [52]. In contrast, to the highspeed mixers, hook kneaders incorporate the crucial energy to form a dough mostly by tension and compression [53], [54]. Especially the shear forces lead to the alignment of the proteins in a specific direction depending on the movement of the kneader geometry. These forces act on hydrated proteins and induce further changes in their arrangement and conformation. With the alignment of the proteins, hydrogen bonds form between amino acid residues within and between glutenin and gliadin molecules. The prevalence of these relatively weak bonds lead to an overall increase of strength in the gluten network. The stress-related structure strengthening of the dough matrix is also called strain-hardening. When the matrix is subjected to shear and tensile stresses during kneading, strain-hardening is defined as the phenomenon where the stress increases more than proportionally to the strain under an applied constant strain rate and increasing strain [55]. Strain-hardening is also caused by the mentioned alignment and orientation of the proteins [56]. Simultaneously, disulfide bonds start to form between cysteine residues in glutenin and gliadin molecules [57]. These covalent bonds are stronger than hydrogen bonds and contribute significantly to the strength and stability of the gluten network. Disulfide bonds act as cross-links between different protein chains, providing structural integrity to the evolving protein network. The cohesiveness of the network is augmented through the linkage of adjacent protein chains. As cross-linking and orientation of the protein strands continue, the network becomes more organized and structured [37], [58]. Within this evolving matrix, air bubbles get entrapped and distributed. These air bubbles serve as crucial nuclei for the aeration of the dough during foaming (see 1.2.2) [59], [60]. The contained starch granules are distributed within the protein network and serve as filling particles [61]. Caused by the

energy input to the matter these starch granules get in close contact with each other and the continuous gluten phase and are responsible for an increased friction within the matrix [62].

1.2.2 Foaming

In leavened baked goods, additional gas, mostly carbon dioxide, is produced either by chemical leavening agents or by fermentation with baker's yeast (*saccharomyces cerevisiae*) and diffuses into the entrapped gas nuclei in the dough matrix [63]. Sugars extracted from the grain are converted into carbon dioxide and other metabolic products during the yeast fermentation. On the chemical side the most common leavening agent is sodium bicarbonate in combination with an acid in the wet or dry state. Thereby, CO_2 is produced through an acid-base reaction as well as thermal degradation during the heating process in the beginning of the baking process [64]. Due to bubble mechanics, the incorporation of the mentioned gas nuclei during the kneading process is crucial for the growth of these gas bubbles, as the pressure to form new bubbles within the matrix would require infinite pressure, which is impossible [47]. Thus, the inflation of the dough matrix during fermentation and chemical leavening occurs through the growth of individual gas bubbles in the continuous gluten network [65]. The produced CO_2 diffuses through the liquid phase of the dough into the bubbles and causes a volume increase of the sample and expansion of the matrix. This diffusion already occurs before reaching the saturated state of solved CO_2 in the liquid phase [66]. The gas bubbles are surrounded by the continuous gluten phase, and the thin films on the bubble surface are stretched and thinned during the expansion. The capability of the gluten film to withstand this deformation strongly depends on the protein content and quality of the used flour. With ongoing gas production and volume increase of the bubbles, some gas bubble walls cannot withstand the tensile and shear forces and rupture. Before rupturing, the bubble walls, consisting of the continuous gluten phase, undergo also strain-hardening phenomena (see 1.2.1). During this stretching, proteins align in the direction of the stretched film, and the forces to further expand the bubbles increase. Also, driven by concentration gradients, the CO_2 diffuses from smaller to bigger bubbles, causing them to grow even more. This phenomenon is called Ostwald ripening and can destabilize the system and cause an inhomogeneity in bubble size distribution [55]. The foaming is mainly a fermentation-driven process by baker's yeast at moderate temperatures around 30°C. For most chemical leavening

agents, higher temperatures are required, and the gas production only starts at the beginning of the baking process.

1.2.3 Baking

The baking process begins with the transfer of the sample into an oven or heating device and is characterized by a hot or heated environment and a continuous increase in sample temperature. During the baking process, several processes take place within the dough matrix. As mentioned above (see 1.2.2) the production of CO_2 causes the dough to rise and gain volume. The heating causes water to evaporate and diffuse into bubbles and the already trapped gas expands further. As a result, the volume of the sample increases even further. When the temperature within the matrix rises, the chemical conformation of the contained proteins begins to change, and they start to denature. The denaturation occurs at temperatures ranging from 65 to 80 °C [67]. Parallel to the denaturation, the starch gelatinization takes place during heating around 60 – 95 °C [68]. It involves the irreversible loss of the molecular order of starch granules, also known as crystallinity. This process is linked to a glass transition, shifting from an ordered state to a disordered, almost melting-like state, requiring both water and heat [24]. As the temperature of the matter rises in the oven, the starch granules begin to absorb water. This absorption is facilitated by thermal energy, which disrupts the hydrogen bonds holding the starch molecules in a highly ordered structure. The study of Jekle et al. [61] suggest that during the initial phase of gelatinization, gluten forms a coating around starch granules. This layer acts as a barrier, impeding the absorption of water by the starch granules. Consequently, this restriction of water diffusion into the starch granules results in a delayed onset of the gelatinization process [61]. Initially, the granules swell as they take up water, causing a noticeable increase in viscosity. This process involves two main starch components: amylose and amylopectin. Amylose leaches out as temperature rises, forming a gel network crucial for structure and moisture retention. Amylopectin swells more, enhancing gel viscosity and texture and stays within the granules to support their structure [69]. Gelatinization is completed around 60-80 °C, with full crumb strength developing upon cooling and starch recrystallization. Concurrently, the Maillard reaction occurs on the baked product's surface, typically above 140 °C [70]. The Maillard reaction between amino acids and reducing sugars leads to browning and flavor development, influenced by temperature, pH, and the reactants involved.

1.3 Rheological Assessment of dough development

To enhance our understanding of mechanical dough development, various rheological testing methods are utilized, categorized into empirical and fundamental approaches. The empirical rheological methods involve assessing dough mixing characteristics using a recording mixer such as the Farinograph or similar devices, as well as analyzing starch gelatinization features using a heated stirrer like the Amylograph. Additional tests, employing instruments like a texture analyzer or an Extensograph, measure dough properties including rupture, sponginess, or stretchability. These empirical approaches are in close alignment with standard dough and flour evaluation practices in bakeries, as per the methods outlined by the American Association of Cereal Chemists (AACC) and play a crucial role in forecasting baking performance. However, their reliance on arbitrary units hampers compatibility with other methodologies and measuring devices. In contrast, fundamental rheological methods operate with standardized SI units, enhancing the comparability of results. In fundamental rheological methods, the stress applied to material results from deformations caused by tension, compression, or shearing. Shear flow is characterized by multiple layers of fluid moving over one another, where each successive upper layer moves more quickly than the one beneath it (see Figure 2). In this scenario, the bottommost layer is typically stationary, while the uppermost layer exhibits the greatest velocity. This phenomenon occurs because of shear force being applied to the fluid.

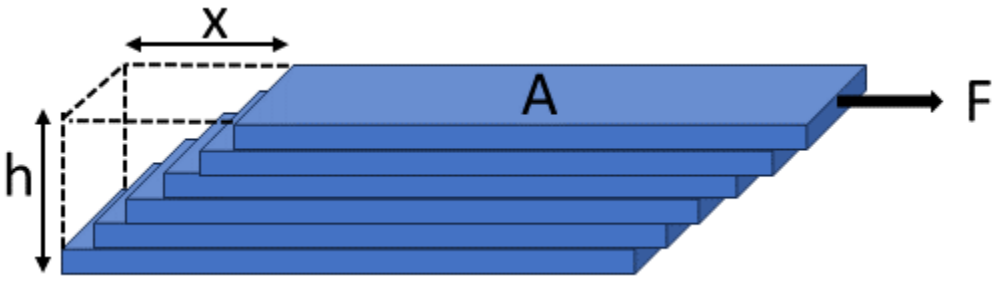

Figure 2: Quantification of deformation for layers of fluid sliding over one another over the unit area A with applied force F.

The shear stress σ calculated from the area involved corresponds to the material face (**A**) parallel to the applied force vector (**F**), i.e., with surface normal vector perpendicular to the force (see Figure 2: Quantification of deformation for layers of fluid sliding over one another over the unit area A with applied force F..

$$\sigma = \frac{F}{A}\ [Pa] \tag{1}$$

The displacement gradient formed within the sample, referred to as shear strain (γ), arises because the top layer exhibits the greatest response to the applied force, while the bottom layer remains unresponsive. This gradient of height h and displacement x, can be denoted as follows:

$$\gamma = \frac{x}{h}\ [-] \tag{2}$$

The shear strain (γ) represents the differential movement across the layers of the material. In classical solids, which behave as a unified mass, the strain becomes infinite under stress, rendering flow infeasible. Conversely, in fluids, where components can slip past one another, shear strain progressively increases for the duration (t) of the applied strain. This ongoing increase leads to a velocity gradient known as the strain rate ($\dot{\gamma}$), expressed as the rate of change of strain over time:

$$\dot{\gamma} = \frac{d\gamma}{dt}\ [s^{-1}] \tag{3}$$

When shear stress is applied to a fluid, it initiates momentum transfer; the shear stress equates to the rate at which this momentum flux is imparted to the fluid's top layer. This momentum then cascades downward through the fluid's layers. However, kinetic energy, and thus the velocity of each layer, decreases between layers. This reduction is due to the energy lost in collisions between the fluid's particles. The relationship between strain rate and shear stress is quantified by the shear viscosity, also known as dynamic viscosity (η):

$$\eta = \frac{\sigma}{\dot{\gamma}}\ [Pa \cdot s] \tag{4}$$

This parameter reflects the internal friction within the fluid's layers. A higher shear viscosity indicates increased damping, which translates to greater losses of kinetic energy within the system. The mechanical response of wheat dough to strain is characterized by both elastic and viscous flow properties acting as viscoelastic matter. Under shear conditions, the elastic, spring-like (see Figure 3) flow behavior adheres to Hooke's Law, represented by the equation:

$$\sigma = G * \gamma\ [Pa] \tag{5}$$

In this formula, σ represents stress, G is the elastic modulus, and γ is the strain. When the strain is removed, the dough completely recovers its original form, demonstrating the reversible nature of elastic flow. The viscous, damped (see Figure 3) flow can be described by Newton's law:

$$\sigma = \eta * \gamma \, [\dot{P}a] \tag{6}$$

The formation of stress in a viscous flow is a time-dependent process, reliant not only on the viscosity, denoted as η, but also on the rate of strain, symbolized as $\dot{\gamma}$. Unlike elastic flow, where the material can revert to its original state post-deformation, viscous flow is characterized by its irreversible nature. In viscous flow, as the strain rate increases, the internal resistance of the material, determined by its viscosity, plays a crucial role in how stress develops over time. This irreversibility is due to the permanent realignment of the material's internal structure, which doesn't return to its pre-stressed condition once the strain is removed. Substitute models consisting of springs and dampers (see Figure 3) are used to describe complex materials in flow analysis because they provide a simplified and effective way to model the mechanical behavior of materials with viscoelastic properties. These models capture the interplay between elastic (solid-like) and viscous (fluid-like) responses.

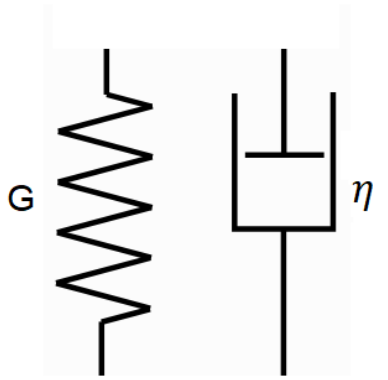

Figure 3: Spring and damper as the representation of the Hookean and Newtonian behavior of materials.

Wheat dough exhibits both elastic and viscous properties, necessitating the use of models that combine a spring and a damper to accurately describe its response to deformation (see Figure 4). The Kelvin-Voigt model, which aligns these elements in parallel, captures the dough's immediate elastic response and subsequent slower viscous behavior, with recoverable deformations influenced by viscosity. Conversely,

the Maxwell model places the elements in series, depicting an initial viscous response followed by elastic recovery. This model includes a time-dependent relaxation process, where recovery might be incomplete if stress is removed rapidly, indicating some irreversible flow.

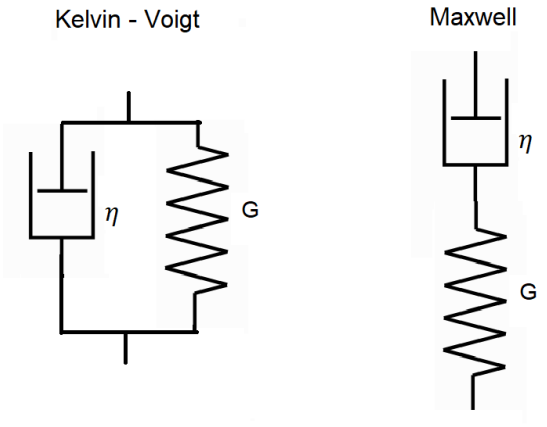

Figure 4: Viscoelastic materials, both solids and fluids, can be characterized using two fundamental models: the Kelvin-Voigt model and the Maxwell model.

Both the Kelvin-Voigt and Maxwell models are crucial for understanding viscoelastic materials, as they elucidate the interplay between elastic and viscous properties under varying stress and strain conditions. These models are applicable to a diverse array of materials, including polymers, biological tissues, and specific dough types. Dough's consistency largely dictates its perception as either solid or liquid [71]. The Kelvin-Voigt model, with a spring and damper in parallel, represents dough's solid-like aspects, combining immediate elastic recovery with a time-dependent viscous response. In contrast, the Maxwell model, aligning the spring and damper in series, is better suited for liquid-like dough, depicting its partially reversible deformation due to the irreversible nature of the viscous component. A rheometer primarily consists of a drive motor connected to a spindle or geometry that holds or interacts with the sample, such as a plate, cone, or cylinder. This setup applies controlled stress or strain to the sample. Additionally, it includes a highly sensitive torque sensor to measure the resistance of the material to the applied force. These mechanical components are crucial for determining the rheological properties of various substances by measuring how they deform or flow under stress. In a controlled strain measurement, the rheometer is set to rotate at a predetermined speed. As it rotates, the device measures the torque required to maintain this constant speed despite the resistance offered by the sample. This method is particularly useful for understanding how the sample behaves under a

specific rate of deformation. Alternatively, in a controlled stress measurement, the rheometer applies a defined torque to the sample. The focus here is on observing the resulting rotational speed of the plates. This setup is beneficial when the interest lies in understanding how the sample responds to a specific amount of applied stress. In dynamic tests, unlike static tests, the strain applied to the material is not steady or constant. Instead, it oscillates in a sinusoidal pattern, making the strain a time-dependent variable. This approach allows for the examination of the material's response under varying strains over time.

$$\gamma = \gamma_0 * \sin(\omega t) \tag{7}$$

In equation 7 γ_0 is the strain at time t = 0 and ω representing the angular frequency. In case of a viscoelastic sample subjected to sinusoidal deformation in an oscillatory test, the resultant oscillating stress will display a phase shift characterized by the angle δ and calculated with the stress at time t = 0 and the angular frequency ω. This shift occurs due to the viscoelastic nature of the material, reflecting its combined elastic and viscous responses:

$$\sigma = \sigma_0 * \sin(\omega t + \delta) \tag{8}$$

The total stress experienced by a viscoelastic sample under sinusoidal deformation can be separated into two distinct components. The first component aligns perfectly in phase with the strain, while the second component is out of phase. This can be mathematically represented as:

$$\sigma = \sigma'_0 * \sin(\omega t) + \sigma''_0 * \sin(\omega t) \tag{9}$$

Here, σ'_0 is the in-phase stress component, and σ''_0 is the out-of-phase stress component, with ω representing the angular frequency and t being time. From this decomposition, the loss factor can be calculated as:

$$\tan\delta = \frac{\sigma''_0}{\sigma'_0} = \frac{G''\gamma_0}{G'\gamma_0} \tag{10}$$

Thereby the loss factor reflects the ratio of the out-of-phase to the in-phase stress component. By substituting σ with the equation representing Hooke's Law for elastic materials (equation 5), the loss factor tanδ can be reformulated. This reformulation allows for a more precise understanding of the material's viscoelastic properties, particularly how its viscous and elastic behaviors interact under oscillatory shear

conditions. With these measures over time, the development of the gluten matrix can be observed, and the formation of the network can be tracked. G' represents the storage modulus and G'' the loss modulus, respectively, in viscoelastic material analysis. G', the storage modulus, corresponds to the elastic aspect of the material and is in-phase with the oscillating strain, indicating the energy stored in the material during deformation. Conversely, G'', the loss modulus, represents the viscous component that is out-of-phase with the strain. This out-of-phase component signifies the energy dissipated as heat during the oscillation, reflecting the material's internal friction. Both G'' and G'' are crucial indicators of the network behavior under load and can be directly linked to dough characteristics by the complex modulus G*. With the storage and loss modulus as an additional parameter the complex modulus G* can be calculated:

$$G^* = G' + i * G'' = \sqrt{(G')^2 + (G'')^2} \tag{11}$$

The complex modulus G* provides a singular value that encapsulates the dough's resistance to deformation under both elastic and viscous conditions. A higher G* value indicates a dough with stronger overall viscoelastic properties, suggesting it can withstand greater deformation forces without permanent structural changes. This is particularly important in baking and food processing, where the balance between elasticity and viscosity in dough dictates the texture, shape retention, and overall quality of the final baked product. The most common technique for assessing these viscoelastic properties with a rotational rheometer is the Small Amplitude Oscillatory Shear (SAOS) test. This method involves continuously oscillating a sample around its neutral, or equilibrium, position in a repeated cycle, allowing for detailed analysis of its viscoelastic behavior. For this test, it is essential that the strain amplitude γ_0 falls within the material's Linear Viscoelastic Region (LVR). The LVR is identified through an amplitude sweep test conducted prior to the frequency sweep test. Within the LVR, both the storage modulus (G') and the loss modulus (G'') remain constant and unaffected by changes in the applied strain. This constancy ensures accurate assessment of the material's viscoelastic properties. In view of the functional properties of the flour components and the complexity of the rheological behavior of viscoelastic samples, a highly sensitive rheometer seems to be the perfect tool for dough development and inline testing. With the ability to detect the smallest deviations in the rheological properties of the dough in the highly controlled environment of the rheometer, the possibilities for flour and dough analysis are exceptional.

2 Motivation and Thesis Outline

In the previous sections, the mechanisms of dough and gluten network development and the influence of applied deformation have been described in detail. Based on this, the initial situation and motivation of this thesis can be summarized as follows:

- In principle, dough development and the network formation during kneading are known. However, the investigation of network formation is based, on the one hand, on a separate investigation in which dough production and measuring instrument are two or more different devices. On the other hand, the most widely used method to determine dough development is an empirical method, which only gives highly specific information about the dough and network conditions. It is therefore more of an estimate or forecast than a detailed analysis.

- The visual evaluation of dough development on a microscopic is already an established method. In classical analysis, during the sample transfer from the kneader to the microscope, the dough matrix might be influenced and differ from the natural state at the specific point of interest. A transfer-free and inline investigation of optical and rheological dough development is not state of the art and, therefore, there is a lack of *in situ* knowledge regarding network development of wheat flour dough under load.

- Natural and chemical leavening agents deviate from one another in producing gas to leaven the product. Also, the influence of the raw material on dough behavior during leavening is remarkable. Accessing the foaming ability of the leavening agents, thereby mostly depends on manual tests and empirical measurements. The influence of the baker performing the test is largely unpredictable and the quantity of sample material is very high due to the repeatability and size limitations of the AACC.

- The ability to accurately predict the final volume of baked products based on the gas retention capacity of the gluten matrix in wheat flour doughs is essential for optimizing baking quality. However, effectively evaluating strain-hardening, a key factor in understanding this capacity, remains challenging in wheat flour dough systems. An involuntary release of the gases in the dough matrix will

likely happen, resulting in qualitative and quantitative differences in foaming properties and product quality.

Based on this initial situation, the main objective of this thesis is formulated as follows:

Development of an inline shear kneading and measuring device for integration into a conventional rheometer to enable detailed analysis of network development and properties across the entire dough processing and baking.

To accomplish this objective, we need to devise solution approaches for the following research questions:

1. How can wheat flour and water be mixed and kneaded in a conventional rheometer with small sample size to form a fully developed gluten matrix with a straightforward analysis of the network evolution?
2. How can this network formation be inline validated on a rheological and microscopically basis?
3. Does the reduction of the sample size on a microscale influence the foaming and baking properties of the kneaded samples?
4. How does the flour composition influence the dough properties, particularly the strain-hardening and gas-holding capacity?

A detailed plan was formulated based on these research questions to accomplish this objective. To obtain a comprehensive overview of the topics, the following steps were taken:

1. Different shear stresses were applied to wheat flour-water samples in a rheometer, and a shear-kneading setup was developed to produce a fully developed dough at the micro-scale. An in-line multi-wave frequency test was used to gain rheological insights into the structural network development processes during the shear-kneading process.
2. The used rheometer was coupled with a confocal-laser-scanning-microscope (CLSM) to elucidate the network formation during the shear-kneading of the samples and to validate the shear-kneading setup in comparison to classical offline kneader systems.

3. The influence of different leavening agents on foaming and baking properties was investigated with regard of total volume growth and growth speed for different flours in microscale baking trials. Additionally, a new leavening acid L-galactono-1,4-lactone (GGL) was tested as an alternative to common substances to test the sensitivity and applicability in product development of the method.

4. The strain-hardening phenomena occurring during kneading and foaming were investigated with focus on the establishment of a shear-only measurement to predict the achievable gas retention capacity of different flour with varying protein content. Also, a correlation analysis was carried out to reveal the key factors of flour composition on the network development and network attributes based on the findings of the previous work.

3 Methods

The used commercial flours were analyzed for their micro nutritional content according to AACC-methods (Used in the publications I, II, III, IV).: AACCi 54–70.01 to determine the required dough development time (DDT) and water addition in an DoughLAB® torque recording mixer. The moisture (AACCi 44-01) as well as the ash (ICC 104/1) and the wet gluten content (AACC 38-12A) were analyzed. The protein content according to the Kjeldahl method from AACC 46-16 was evaluated as well. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) analysis was performed to determine the protein composition of the flours as described by Schuster *et al.* [38] (III).

All rheological measurements were carried out in a MCR502 rheometer (Anton Paar, Ostfildern, Germany) with parallel plate geometries (I, II, III, IV). To maintain constant humidity and temperature, a CTD 180 Humidity Ready (Anton Paar, Ostfildern, Germany) chamber was set to 30 °C and 80 % relative humidity for all trials (I, II, III, IV).

The classical and shear-kneaded samples were analyzed by an eclipse Ti-U inverted microscope (II) with an e-C1 plus confocal system (Nikon GmbH, Düsseldorf, Germany) using a laser with a wavelength of 543 nm for excitation, the emission was detected at 590 nm with a 50 nm bypass filter. To stain the samples for CLSM, 5 mL of bulk water was replaced by a rhodamine B solution (Merck KGaA, Darmstadt, Germany) with 0.01 g/100 mL water.

The software-based analysis of CLSM images was performed by AngioTool64 version 0.6a (National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA).

The dough volume was calculated according to a body volume by the silicon oil displacement method (III). After baking, the volume of the loaf was analyzed with a TexVol Instruments BVM-L370. The baked crumb structure was analyzed using a Basler acA2500-60ue camera and a MATLAB code to process the pictures and calculate the pore size distribution.

For the lubricated-squeezing-flow measurements a TA.XT.Plus with a 50 kg load cell (Stable Microsystems, Godalming, UK) was used (IV) and the results were calculated according to Chattraei *et al.* [72].

Mathematical and statistical evaluation (I, II, III, IV) was performed by using Matlab (R2018a, MathWorks Inc., Natick, MA, USA) and Origin (2018b, OriginLab Corporation, Northampton, MA, USA). Results were evaluated statistically with Origin(Pro), Version Version 2022. (OriginLab Corporation, Northampton, MA, USA), with Pearson Correlation coefficient calculation. All values (I, II, III, IV) are represented with the standard error of the mean (SEM).

Unless otherwise stated, all analyses were performed in triplicates.

4 Results

4.1 Summary of the main results

Part 1: Micro-Scale Shear Kneading—Gluten Network Development under Multiple Stress–Relaxation Steps and Evaluation via Multiwave Rheology

Pages 26 – 38

The aim of the study was to develop an innovative approach to analyzing wheat flour dough development [49]. The objective of the research was to introduce a new shear-kneading technique, which was implemented in a rheometer to evaluate the development of the dough matrix in-line. The evaluated method, which is performed in a conventional shear rheometer and includes successive stress relaxation steps and multi-wave frequency sweeps, allows for a more detailed and efficient analysis compared to conventional kneading methods. The successful implementation of the shear kneading process marks a significant advancement over traditional kneading methods. The technique allows for an in-depth, real-time analysis of dough matrix evolution, a critical factor in gluten network development. Key findings from the research demonstrate that this innovative method enables the development of a dough matrix that closely mirrors the characteristics of dough classically kneaded for an optimal duration. The use of linearization techniques and power-law fitting in the analysis of the relaxation modulus provides a more accurate representation of the gluten network's development. With the successful production of an optimally developed dough matrix close to the reference kneading time with comparable network attributes, this insight is crucial for understanding the crosslinking processes occurring during kneading. The study's approach to dough analysis significantly enhances the understanding of the energy-dependent development of the dough matrix. It opens new possibilities in the investigation of dough development and further processing steps on a microscale.

Authors´ contribution:

Leonhard Maria Vidal designed the working hypothesis, research project and experimental approach after critical discussion with the co-authors. He developed the applied methodology and conducted the main experiments. Furthermore, he evaluated the resulting data. As the principal author of this publication, he wrote the manuscript and submitted it. All authors reviewed and approved the final manuscript.

Part 2: Microscopic analysis of gluten network development under shear load - combining confocal laser scanning microscopy with rheometry

Pages 39 – 48

The second study [73] focuses on the microscopic analysis of gluten network development in wheat flour dough under shear stress. The goal was to enhance the understanding of dough development by combining confocal laser scanning microscopy (CLSM) with rheometry for *in situ* analysis.

The research revealed that the shear kneading technique in a rheometer of paper one [49] can efficiently produce a fully developed dough matrix efficiently. This approach was extended with an *in-situ* optical analysis, allowing real-time examination of the evolving gluten network. For this reason, a coupling device for the CLSM and the rheometer was developed and produced together with Anton Paar, Ostfildern, Germany. Previous HPLC analysis of the flours used provided a deeper insight into the role of individual Osborne fractions of the protein contained in the network formation and the achievable dough matrix properties. The study demonstrated that the developed shear-kneading method enables the evaluation of the rheological and optical protein network evolution without interrupting or transferring the sample, a significant improvement over previous methods.

The study successfully established a thorough and non-invasive approach to analyse dough development. This method provides deeper insights into the gluten network's evolution, which is crucial for improving the quality and consistency of wheat flour-based products. The findings suggest the potential for expanded use of the shear-kneading technique in flour and dough evaluation, owing to its ability to provide a more accurate and detailed understanding of dough characteristics.

Authors´ contribution:

Leonhard Maria Vidal designed the working hypothesis, research project and experimental approach after critical discussion with the co-authors. He developed the applied methodology for the main experiments. Furthermore, he evaluated the resulting data. As the principal author of this publication, he wrote the manuscript and submitted it. All authors reviewed and approved the final manuscript.

Part 3: A dynamic micro-scale dough foaming and baking analysis – Comparison of dough inflation based on different leavening agents

Pages 49 – 56

The third publication [47] describes a micro-scale approach to analyze the dough foaming and baking process based on the shear-kneading technique from publication one [49]. The study aims to compare the effect of different leavening agents on dough inflation and quality, using minimal sample sizes in a conventional rheometer.

The results demonstrate the comparability of the micro-scale method with traditional baking tests, especially when using yeast as a leavening agent. The study explores the efficacy of different leavening agents, including yeast and baking powder components, in influencing dough characteristics. One significant finding is the potential of GGL, an alternative acidifying agent in baking powder, which promises to be applied in certain baking scenarios due to its slower carbon dioxide release.

This innovative approach offers a more efficient and less resource-intensive method for analyzing baking performance and the impact of various leavening agents, potentially beneficial for the baking industry through improved quality control and product development.

Authors´ contribution:

Leonhard Maria Vidal designed the working hypothesis, research project and experimental approach after critical discussion with the co-authors. He developed the applied methodology for the main experiments. Furthermore, he evaluated the resulting data. As the principal author of this publication, he wrote the manuscript and submitted it. All authors reviewed and approved the final manuscript.

Part 4: Structure Strengthening Phenomena of Gluten Matrices under Different Stress Types

Pages 57 – 66

The fourth publication [56] investigates the relationship between wheat flour dough's strain hardening and its gas retention capacity, which is crucial for baking performance. The study employs a novel approach, combining simple shear methods and biaxial extension tests, to evaluate the impact of high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits on dough's response to different deformation speeds. The results demonstrate a significant correlation between these subunits and the dough's structural properties, highlighting their role in dough strengthening and stability.

This research provides new insights into dough behavior influenced by flour composition, presenting potential applications in improving baking quality and consistency.

Authors´ contribution:

Leonhard Maria Vidal designed the working hypothesis, research project and experimental approach after critical discussion with the co-authors. He developed the applied methodology and conducted the main experiments. Furthermore, he evaluated the resulting data. As the principal author of this publication, he wrote the manuscript and submitted it. All authors reviewed and approved the final manuscript.

4.2 Thesis Publications

4.2.1 Micro-Scale Shear Kneading—Gluten Network Development under Multiple Stress–Relaxation Steps and Evaluation via Multiwave Rheology

polymers

MDPI

Article

Micro-Scale Shear Kneading—Gluten Network Development under Multiple Stress–Relaxation Steps and Evaluation via Multiwave Rheology

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Abstract: To evaluate the kneading process of wheat flour dough, the state of the art is a subsequent and static measuring step on kneaded dough samples. In this study, an in-line measurement setup was set up in a rheometer based on previously validated shear kneading processes. With this approach, the challenge of sample transfer between the kneader and a measurement device was overcome. With the developed approach, an analysis of the dynamic development of the dough is possible. Through consecutive stress–relaxation steps with increasing deformation, a kneading setup in a conventional rheometer is implemented. Fitting of the shear stress curve with a linearization approach, as well as fitting of the relaxation modulus after each kneading step, is a new way to evaluate the matrix development. Subsequently, multiwave rheology is used to validate the kneading process in-line. The shear kneading setup was capable of producing an optimally developed dough matrix close to the reference kneading time of 150 ± 7.9 s (n = 3). The linearization approach as well as the power-law fit of the relaxation modulus revealed gluten network development comparable to the reference dough. With this approach, a deeper insight into gluten network development and crosslinking processes during wheat flour dough kneading is given.

Keywords: dough development; shear kneading; rheology; wheat dough; protein network

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1. Introduction

The most widespread usage of wheat flour is as kneaded dough, as it is consumed as processed food in the form of baked goods. Through mechanical energy input and hydration, wheat flour–water mixtures produce viscoelastic dough matter. The wheat dough owes its unique viscoelastic properties to its crosslinked protein network [1] and enables a variety of food applications. In the beginning of the dough kneading procedure, the mechanical energy input together with the hydration of the flour particles causes a crosslinking of glutenins and gliadins to a continuous protein network. This network is known as the continuous gluten phase [2–4]. The basis for all baked goods is the production of an optimally developed gluten phase in the dough matrix. Therefore, the optimum consistency of a dough is achieved when the dough best withstands the deformation of the kneading geometry. This development stage of the dough matrix is often indicated by the mentioned energy maximum in a torque-recording kneading machine and is therefore the peak of dough consistency [5]. The gluten phase also embeds starch granules, which promote internal friction during kneading. In addition, small gas nuclei are introduced during kneading and are evenly distributed in the dough matrix. These nuclei then serve as starters for the crumb evolution during proofing and baking.

The main task of kneaders in dough mixing is the transfer of a given amount of mechanical energy into the material to transform and develop it to the optimum consistency [6].

For the incorporation of the crucial energy input into the gluten network in wheat dough, different types of kneaders are available. For all kneaders, the transferred mechanical energy comprises tension, compression and shear to the forming dough [7]. Depending on the kneader geometry, rigid body rotation also takes place during dough movement in the kneaders. It is difficult to accurately define the crucial energy for the optimum development point of dough in general for all kneader types. Moreover, it is not clear what type of deformation is responsible for the ongoing crosslinking processes in the matrix. In industrial dough production, the rotating blades of high-speed mixers transfer energy predominantly by shear [7], whereas spiral hook kneaders incorporate crucial energy into a dough mostly by tension and compression [8,9]. According to Peighambardoust et al. [10], a total energy input of approximately 30 kJ/kg is required to produce an optimum wheat dough, but depending on the flour type and the mixer used, the required energy input can reach up to 100 kJ/kg. As suggested by Anderssen et al. [11], the evolving rheological behavior of the kneaded material will not be determined only by its current configuration, but also by the type, magnitude and duration of the applied forces to which it is subjected. With respect to these circumstances, the kneading process is difficult to reproduce, because it is so highly complex that as its composed of several force effects (as described above). As a result, it is hardly possible to completely trace the development of the dough matrix analytically. With regard to these barriers, a measuring device is missing that develops a dough matrix and additionally offers the possibility of in-line analysis. A farinograph or DoughLAB provides this approach, but with the major limitation of complex kneader geometry. In addition to the complexity limitations, interruption and sample transfer of the kneaded matter into additional equipment (e.g., rheometer, Kieffer rig) is mandatory to evaluate the dough matrix at different stages of development. Therefore, the possibility to measure the network formation in-line through a shear rheological approach in relation to the process parameters (energy input, temperature) and ingredients composition (flour type, water addition, other ingredients) would overcome this analytical gap. With this objective, several shear techniques have already been used to investigate and explain the rheological evolution of dough under shear load and their effects and changes during the development and processing of dough. As a result of this approach, [12] first succeeded in shear mixing dough in a special apparatus other than an extruder. Tietze et al. [13] introduced a shear mixing method directly in a conventional rheometer with shear in one direction. In the literature, it is found that shearing in one direction can produce dough-like structures but at the same time causes a separation of gluten and starch in the geometric cross-section [10,14–16]. The previous works applied simple one-directional shear at various speeds. A determinationof optimum development of the dough matrix in these works was difficult, as the process was affected by the separation processes. With the application of multiple stress–relaxation steps, this method was improved in Tietze et [7], as the phase separation was suppressed. The determination of the dough development in the previous work of Tietze et al. [7] was evaluated from mechanical relaxation spectra calculated from multiple stress–relaxation steps. The distribution of relaxation times then provided information about dough development. This dough development evaluation was an important step towards in-line rheological analysis systems. However, these spectra required a large computational effort, and the system could not be fully rheologically validated in-line. In other works, a multiwave frequency sweep method was evaluated on dough, which resulted in immense time savings in measurements [17,18]. This method allows the frequency sweep measurement to be performed during short interruptions in kneading or during the relaxation phases. With the possibility of additional in-line rheological frequency sweep testing in an improved shear kneading setup, the informative value could be expanded significantly.

The aim of this study was to develop an optimized shear kneading technique that produces an optimally developed wheat dough matrix in a rheometer and to evaluate its mechanical behavior compared to conventional kneaded dough in-line. Without a sample transfer to other devices, the system provides all necessary data to determine the stage of

dough development without high computational effort. The developed method contains consecutive stress–relaxations steps to incorporate the crucial mechanical energy to form a dough, as well as time-efficient multiwave frequency sweeps. With the multiwave frequency sweep testing as an additional evaluation of the stress–relaxation results, a distinct insight into the network evolution processes and changes in the dough matrix should be enabled. The implemented in-line frequency testing gives a direct comparison to the different stages of network development of conventional kneaded dough. Without sample transfer or interruption of the kneading process, a gain of knowledge regarding energy-dependent dough matrix development is achieved. With this aim, the new method is capable of producing wheat flour doughs in a rheometer for further rheological investigations at very small scales. Resulting from this, the shear kneading technique is capable of analyzing the smallest batches and sample sizes as are usual in breeding or flour blending applications.

2. Materials and Methods

2.1. Development of the Wheat Dough Matrix

German commercial wheat flour type 550 with 14.34 ± 0.24 g moisture per 100 g flour (AACCi 44-01), a protein content of 11.78 ± 0.05 g per 100 g dry flour (AACCi 46-16, N × 5.7), 0.53 ± 0.01 g ash per 100 g dry flour (ICC 104/1) and 26.18 ± 0.12 g wet gluten per 100 g flour was used in this study. Dough resistance and water absorption were measured in a Z-kneader DoughLAB (Perten Instruments AB, Hägersten, Sewden) according to AACCi 54–70.01 to determine the required dough development time (DDT).

2.2. Fundamental Shear Rheology

All rheological measurements were carried out in a MCR502 rheometer (Anton Paar, Ostfildern, Germany) with parallel plate geometries. To maintain constant humidity and temperature, a CTD 180 Humidity Ready (Anton Paar, Ostfildern, Germany) chamber was set to 30 °C and 80% relative humidity for all trials.

2.2.1. Dough Produced in a Z-Kneader

For the shear rheological measurements of classical kneaded dough, 50.2 g flour (corrected to 14% moisture) and 28.10 mL demineralized water were kneaded at 63 rpm using a Z-kneader equipped with a 50 g mixing bowl. After kneading, 4 g of dough was placed centered between cross-hatched parallel rheometer plates (25 mm diameter). Once reaching the constant gap of 2 mm, all excess dough was removed, and the cut surface was coated with paraffin oil to prevent dehydration.

2.2.2. Shear-Kneaded Dough

To produce shear-kneaded dough in the rheometer, a plane plate–cylinder geometry setup with 25 mm diameter of the upper plate geometry and 25.1 mm inner diameter of the cylinder was used. For all experiments, 192 mg of flour and 108 µL of demineralized water, to match the water dosage of the Z-kneader, were kneaded in the rheometer according to the following procedure. The gap was set at a height of 650 µm to obtain a completely sample-filled geometry. The deformation was applied in an oscillatory matter with an asymmetric deflection angle back and forth. After each 90° forward deformation, a 4 s relaxation step was implemented (see Figure 1), followed by a 45° reverse deformation.

2.3. Evaluation of the Dough Matrix Development

2.3.1. Frequency Sweep Testing

After the resting of the Z-kneaded dough in the measuring gap, a frequency sweep was performed in a range from 0.1 to 50 Hz at a constant deformation of 0.05%. The obtained complex shear modulus (G^*) data were fitted according to Gabriele et al. [19] with a power law equation (c.f. Equation (1)).

$$|G^*| = A_f \times \omega^{\frac{1}{z}} \quad (1)$$

where ω is the angular frequency (s^{-1}), A_f refers to the network strength (Pa $s^{1/z}$) and z refers to the network connectivity (–) [19–22].

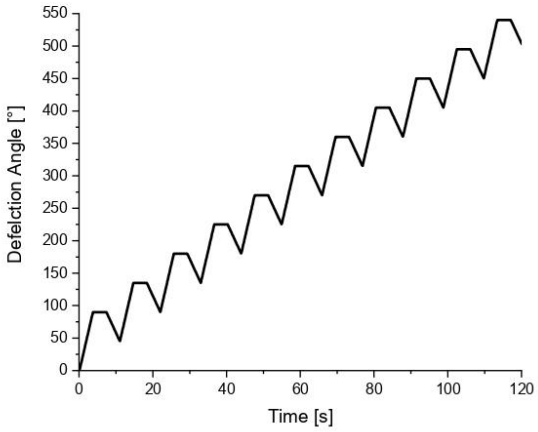

Figure 1. Increase in deflection for the kneading procedure with implemented relaxation phases.

2.3.2. Shear Kneading Setup

One approach to evaluating the ongoing network evolution was a fitting of the exponential decrease in the linear relaxation modulus $G(t)$. The relaxation modulus is calculated from the applied shear stress at time t, $\sigma(t)$ and the shear strain at the beginning of the relaxation γ_0. The fit calculates for each relaxation step as follows [23] (c.f. Equation (2)):

$$G(t) = \frac{\sigma(t)}{\gamma_0} = S \times t^{-r} \tag{2}$$

where S refers to the stiffness of the matter and r is the relaxation exponent.

As $G(t)$ is dependent on deformation/shear strain γ, another approach to evaluating the network evolution and kneading performance of the shear kneading setup focusing only on geometric properties and measured torque was considered. In fitting the shear stress decrease during the relaxation step, the behavior of the matter was evaluated according to Bhattacharya or Peleg [24,25]. This was achieved with a linearization of the exponential decreasing shear stress (c.f. Equation (3)).

$$\frac{\sigma_0 \times t}{\sigma_0 - \sigma(t)} = k_1 + k_2 \times t \tag{3}$$

In this normalization, σ_0 is the shear stress at the beginning of the relaxation step and $\sigma(t)$ the respective shear stress at time t. Calculated from the linearization parameters k_1 and k_2, it follows that $1/k_1$ is the initial decay rate and $1/k_2$ denotes the asymptotic value of the relaxed portion of the initial stress. It is stated that $1/k_2$ goes towards 0 for an elastic solid and $1/k_2$ goes towards ∞ for a liquid [25].

2.3.3. Multiwave Frequency Sweep Testing of Shear-Kneaded Dough

In an additional shear kneading setup, multiwave frequency sweeps were performed after the relaxation steps at specific setpoints of the shear kneading process to characterize the state of the dough. The framework of multiwave instead of standard frequency sweeps was chosen due to the shorter measuring time. The results from the multiwave tests on dough were carefully compared with the standard frequency tests to guarantee a correct rheological characterization of the dough's state.

The fundamental frequency was ω_0 = 1 Hz with an amplitude of 0.05%. The harmonic frequencies are each a multiple of the fundamental frequency, namely ω_1 = 3 Hz, ω_2 = 4 Hz, ω_3 = 5 Hz, ω_4 = 6 Hz, ω_5 = 7 Hz, ω_6 = 8 Hz, ω_7 = 9 Hz and ω_8 = 10 Hz, each with an amplitude of 0.05%. The resulting peak amplitude was 0.115%. The amplitude and harmonics were chosen to be in the range of linear viscoelasticity of dough networks.

The obtained sweep data were fitted accordingly to 2.3.1 with the power law equation from [19].

2.3.4. Energy Consumption

To evaluate the specific mechanical energy (SME) needed to develop the dough matrix at specific kneading times, it was calculated from the measured torque according to [14] (cf. Equation (4)):

$$SME = \frac{\omega}{m} \times \int M_d dt \quad (4)$$

In this equation, ω (s $-$ 1) is the rotational speed, m (kg) is the mass of the kneaded material and M_d is the torque (N m) measured on the kneading arm. The *SME* (kJ/kg) was calculated between t = 0 and the respective kneading time td.

2.3.5. Statistical Analysis

All measurements were performed in triplicates. The standard deviation accounts for the deviation between these triplicates. Mathematical and statistical evaluation was performed using MATLAB (R2018a, MathWorks Inc., Natick, MA, USA) and Origin (2018b, OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Standard Mixing Procedure in a Torque-Recording Z-Kneader

A torque-recording Z-kneader was used to determine the time to maximum resistance of the evolving dough matrix against the applied deformations during the kneading process. The kneading process can be divided into hydration and distribution, crosslinking and network development and, after a certain point of applied load, network breakdown. In the beginning of the kneading process, the protein gets hydrated, and the contained glutenins unfold and then interconnect [26]. Within this polymeric glutenin network, a fibrillary gliadin network emerges with ongoing kneading, which behaves as anisotropic elastomers with an elastic modulus that can be the same as that of fibrous elastin (depending on the degree of hydration) [27]. This gives the dough matrix its unique viscoelastic attributes. The backbone of the gluten network consists of covalent disulfide bonds, which contribute to the plasticity of dough. On the other hand, it contains elasticity-determining non-covalent interactions, especially intra- and intermolecular hydrogen bonds, which lead to the formation of loops and trains in the network structure, as proposed by [28,29]. The typical torque vs. time curve in Figure 2 shows the prominent peak value of the recorded torque after 150 $\pm$ 7.9 s (n = 3) kneading time (dough development time, DDT). After reaching the DDT, a short stability phase can be observed, followed by a decrease in the measured torque due to an ongoing network breakdown. After reaching the maximum, a wheat dough is overmixed, and a higher degree of rupture of the protein matrix can be observed [30]. This overmixed phase was observed with ongoing kneading over 225 s.

Besides the DDT and the following short stability phase up to 190 s, additional setpoints for rheological investigations were chosen to evaluate the network development throughout the kneading process. The first point sets the lower kneading time limit at which homogeneous matter is first reached, and with this achieved, the rheological investigation is reliable. In addition to 50, 100 and 150% DDT (see Table 1), the end of the kneading stability at 190 s was chosen. To get insights into overmixed dough behavior as well, two stages at 660 s and 1200 s were also chosen.

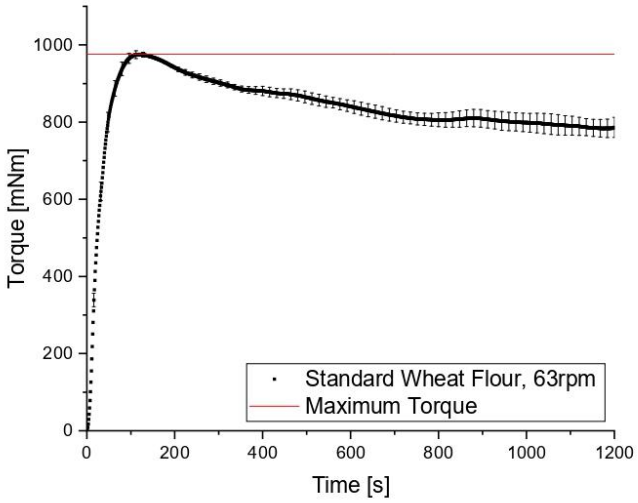

Figure 2. Kneading curve for standard wheat flour measured in a torque recording Z-Kneader (DoughLAB). Maximum peak resistance is marked at 976 mNm. Means are shown with standard deviation (n = 3).

Table 1. Selected kneading times to evaluate the network evolution along the ongoing kneading process of standard wheat flour water dough.

Reaching 400 FU	50% DDT	100% DDT	End of Stability	150% DDT	Over-Kneaded 1	Over-Kneaded 2
45 s	75 s	150 s	190 s	225 s	660 s	1200 s

To quantify the strength of short-range interactions as they occur in starch–gluten or starch–starch polymers, a frequency sweep in a rheometer with a low deformation within the linear viscoelastic region was used [26]. Along the dough matrix evolution, the strength of the network-specific short-range interactions was quantified considering the network strength A_f and network connectivity z. To obtain these coefficients, the power law equation was used to fit the frequency dependency of the complex shear modulus G^* [19,31–34]. For this purpose, a dough sample was taken after each step and analyzed using a frequency sweep. As shown in Figure 3, the rheological behavior of wheat flour–water dough follows the trend of the kneading curve mentioned before. At 100% DDT, both parameters reach a peak value, which drops when the dough gets over-kneaded and the network starts to break down. After reaching the end of the stability region (190 s), a drop in the network strength is clearly visible and even more pronounced than the drop in the connectivity. This drop could be explained by the breaking of disulfide bonds and a release of free water into the matrix [35]. Due to the broken covalent bonds, the non-covalent bonds are more pronounced, which explains the slight connectivity decrease, as they are weaker. The more connected but weaker bonds of smaller polymer compounds cause a slow decrease in the parameter z (-). As mentioned in Don et al. [36], the quantity of large protein clusters, but not the protein concentration itself, changes, and so the network configuration changes to a fibrillary appearance with less strong but highly connected protein strands.

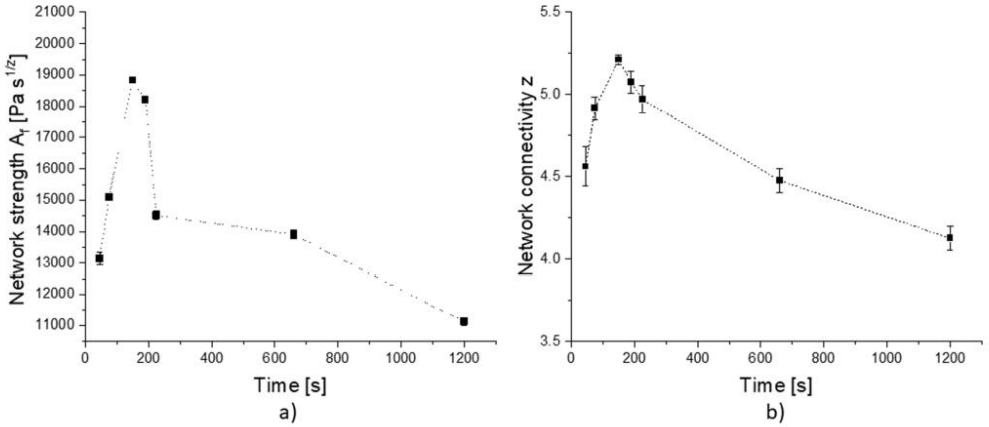

Figure 3. (**a**) Fitting parameter Af and (**b**) parameter z from power-law fitting the frequency sweep results of dough produced in a Z-Kneader according to Gabriele et al. [19]. Means are shown with standard deviation (n = 3).

3.2. Shear Kneading with Increasing Deformation

To produce dough in the rheometer, the shear kneading setup (see Section 2.2.2) was used. With the ongoing deformation/kneading in the rheometer gap, a dough matrix was able to be developed. In Figure 4, the decrease in the relaxation modulus after each positive 90° deflection is shown. The level of relaxation varies with ongoing kneading time. For each relaxation step, another magnitude of the decreasing $G(t)$ value is measured. In addition to that, differences in the relaxation speed of the dough matrix can be observed. This relaxation step is important for network formation, as it enables interconnection during recoil and gluten strand relaxation. With this relaxation, the material is able to reduce the inner shear-induced tension, and protein recoil takes place [37]. These kneading pauses, together with the gradually increasing deformation due to the asymmetric deflection of the rheometer geometry, enhance the interconnection and relaxation processes in the dough matrix and support crosslinking throughout the whole sample cross-section. This varying relaxation behavior was utilized to evaluate the dough matrix development according to Equation (2). The 'gel strength' parameter (S) from the power law gel model is an appropriate descriptor for both the linear and non-linear viscoelasticity of wheat flour doughs generated using a wide range of formulations. The calculated relaxation exponent n gives no deeper insight into dough matrix properties, as it shows no correlations between linear and non-linear dough matrix properties [22].

The 'gel strength' $\boldsymbol{S}$ depends on the mobility of the chain segments and is determined by the persistency length and crosslink density [38]. The relaxation exponent $\boldsymbol{r}$ may have values in the range $0 < r < 1$. The calculated results ranged between 0.262 ± 0.003 and 0.176 ± 0.002, which was in good agreement with the literature. Sun et al. [22] stated that for the recovery phase, very good fits with the power law were obtained, except at long relaxation times. Therefore, the approach of fitting the short-time relaxation in the hold phase is very promising, as it is sufficient to describe the dough matrix properties even when the relaxation exponent is not at a plateau value at the end of the relaxation step. As shown in Figure 5, at first, a decrease in the values of $\boldsymbol{S}$ around 4 Pa $\times$ S^r can be observed. Before reaching this state, mainly flour particle–particle interactions are dominant in the matter and stand in a competitive way with the evolving network, which is the reason for the higher first two start values [39]. These high values with high standard deviations at the beginning of the kneading can be explained by visual observed inhomogeneities of the sample (data not shown) and the before-mentioned particle–particle interactions.

After reaching the DDT from the DoughLAB around 150 s and after reaching farinograph stability (around 190 s), a decrease to a low level, around 2 Pa × S^r, was observed in the gel strength. This observation covers the assumption that at the DDT, the dough behaves like a weak physical gel [19,22]. With the implemented shear kneading technique, a dough matrix development in a time region similar to the DoughLAB DDT was reached. This curve shows an optimum state at the desired DDT when the network interactions become stronger than the particle–particle interactions, indicating the presence of a well-developed network structure. Stress–relaxation measurements at small strain amplitudes (0.1%) carried out for different doughs with different strengths from Safari-Adri et al. [40] showed that doughs with different strengths showed no difference in their relaxation behavior. However, at a range of large strains > 29%, the relaxation behavior was better correlated with the strength of dough [41]. Thus, the large deformations applied in the used rheo-kneading setup should be sufficient to determine the dough strength with respect to evaluating the optimum development stage of the matrix. Therefore, the results obtained from the stress–relaxation kneading, with a set strain in the process of 650%, are in good agreement with the necessary large stains.

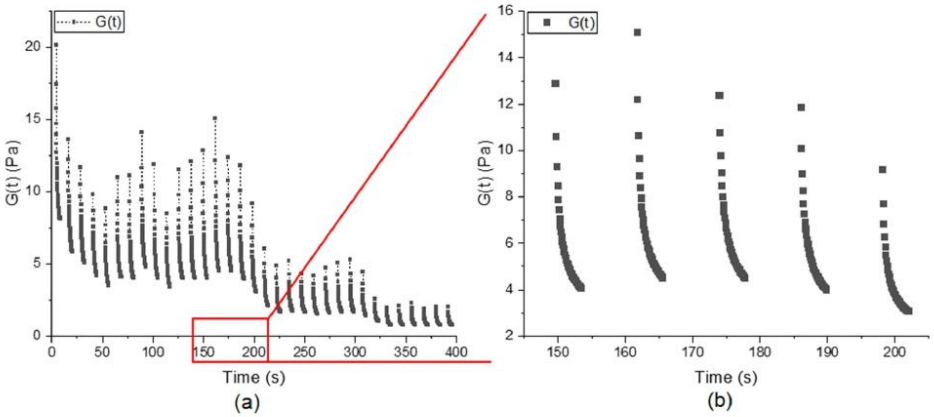

Figure 4. (**a**) Decrease in $G(t)$ during the relaxation steps for standard wheat flour and water [n = 1] as an example of the shear kneading process over the whole 400 s kneading process. (**b**) Zoom in on the exponential decrease in values for the relaxation steps from 150 s to 200 s kneading time.

Following another data evaluation approach with the normalization of the measured shear stress curves for each relaxation step during the rheo-kneading with Equation (3) [24,25], Figure 6 shows the calculated parameter $1/k_2$. The graph shows the increase in the reciprocal value k_2 over the kneading time. The horizontal red dashed line is the standard value for 100% DDT dough from a Z-kneader. To obtain this reference value of 0.81, the optimally developed dough was transferred to the rheometer and shear-kneaded for three consecutive shear kneading steps in a PP-smooth geometry. Since dough behaves as both a viscoelastic liquid and solid, this standard value is in good agreement with the behavior of optimally developed dough [42]. As shown in the increase in the values of $1/k_2$ in the first sample homogenization and hydration, the network's evolution and stabilization take place until approximately 200 s. During hydration, glutenin proteins unfold and interconnect to a continuous network. Within these interconnected protein strands, the globular gliadins form a fibrillary network [26] which can be observed with the increasing viscoelastic behavior of the matter. Starch gets embedded in the system and, together with the gliadins, viscosify the sample's flow behavior after homogenization and hydration. After reaching the over-kneaded state, the matter becomes more viscous and loses its viscoelastic properties. This over-kneaded state is reached at the vertical red dashed line.

The increasing values after reaching the over-kneaded stage indicate a network breakdown and the viscosization of the dough matrix. With the values close to 1, the behavior of the dough approaches a more liquid state. After reaching the over-kneaded state, a non-constant material response causes the high standard deviation (SD) values after 200 s (as shown in Figure 6, the SD goes up for over-kneaded dough). The calculation of the consumed mechanical energy in the shear kneading reaches 10.27 ± 0.81 kJ/kg until 150 s kneading time (at 400 s, *SME* equals 27.95 ± 6.79 kJ/kg). For classical Z-kneaded and extruded doughs, a range from 30 to 100 kJ/kg to produce a fully developed dough matrix is customary [13,43–45]. The low value of the *SME* can be explained by the incorporation of only shear deformations and thus a lower amount of dissipating energy through other deformations than shear within the kneading process.

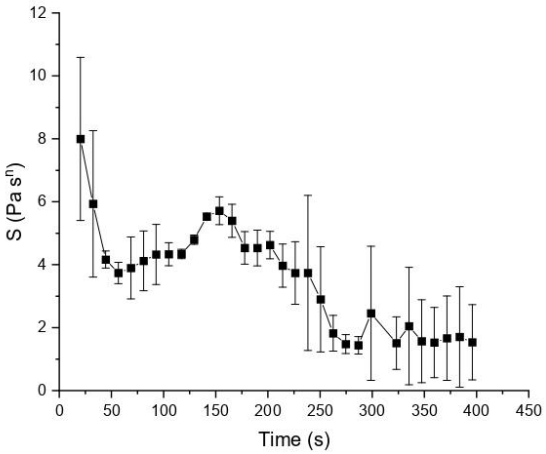

Figure 5. Gel strength *S* (fit of the relaxation modulus according to Bhattcharya et al. [24]) calculated from the relaxation steps during the shear kneading process of standard wheat flour and water. Means are shown with standard deviation (n = 3).

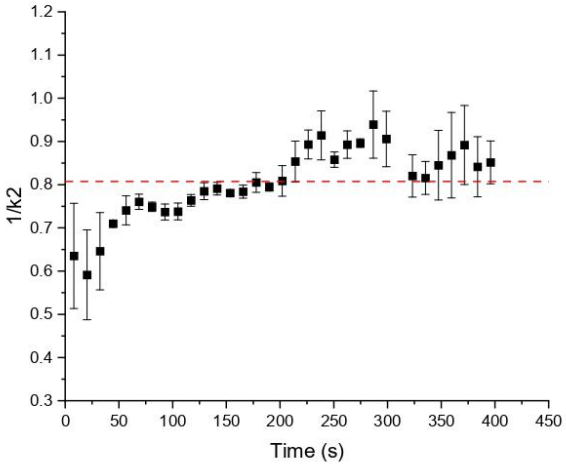

Figure 6. Parameter $1/k_2$ calculated from normalizing the shear stress curves of the shear kneading process of standard wheat flour and water. Means are shown with standard deviation (n = 3).

3.3. Multiwave Evaluation of the Shear Kneading Setup with Increasing Deformation

For further evaluation of the kneading process and dough matrix development, multiwave frequency testing was implemented in the shear kneading process.

In Figure 7, the network connectivity z is obtained from power-law fitting the G^* values of the multiwave measurements with the reference region obtained from measuring Z-kneaded dough at the predetermined DDT. Before the hydration and homogenization of the matter, the measured partly hydrated flour particles show a higher degree of the calculated network connectivity. These values can be classified as initial noise due to visual observed inhomogeneity of the sample, as mentioned before (data not shown). This behavior is explained by findings from Fröhlich et al. [39] for polymer networks with filler particles comparable to dough (containing the continuous gluten phase and starch particles as types of fillers). For the partially unhydrated flour particles in the early kneading stage and the contained starch granules, the filler–filler and filler–polymer interaction is competitive in a certain way. After adding the filler, at low strains, the modulus rises more than the high-strain modulus, resulting in a non-linear viscoelastic behavior, known as the Payne effect. The stiffer behavior at the unhydrated stage of the dough at the beginning of the kneading is explained by the fact that the filler cannot be deformed. In this early stage, partially unhydrated flour particles and starch are in the rigid filler phase. From the second multiwave sweep, the stress softening after homogenization and hydration is attributed to the breakdown of the inter-aggregate association respective to the breakdown of the filler network. In this case, the breakdown of the flour particle–particle and starch–starch interactions, as well as the evolution of a continuous polymeric network after homogenization, takes place. This equals approx. 120 s kneading time with a 900° deflection and therefore 2.5 revolutions of the upper geometry. According to the critical gel theory of Winter and Chambon [46] as basis for the fitting of $|G^*|$ and the assumption of Gabriele et al. [19] that no changes in the network nodes or strands take place during the frequency sweep, the first measured value can be seen as the earlier-mentioned noise, but it also shows the successful evolution of the dough in the ongoing kneading process. After over-kneading, at approx. 200 s, the values for the network connectivity z (-) drop below 5 and show a destruction of the protein network. Due to the small sample size and the high shear forces within the geometry gap of the rheometer during kneading, the network shows inconsistent attributes between 300 s and 400 s kneading. After a nearly complete breakdown of the network after 500 s kneading time, the SD gets smaller, as the network properties then equalize.

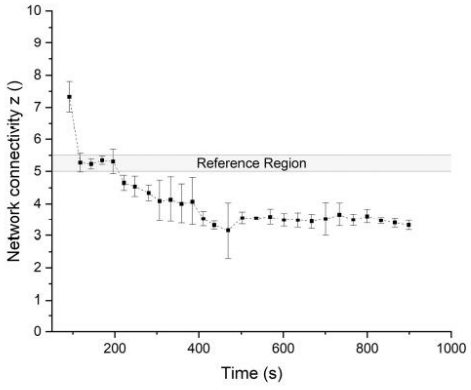

Figure 7. Network connectivity z from power-law fitting G^*. Measured in an additional test setup via multiwave frequency sweeps after certain relaxation steps during stepwise increases in shear kneading. The reference region (z = 5 to 5.5) shows the range of values of a Z-kneaded dough at its dough development time (150 s). Means are shown with standard deviation (n = 3).

4. Conclusions

To solve a major challenge in the evaluation of dough development, namely sample transfer from the kneaders to the measuring instruments, a shear kneading technique was investigated in a conventional rheometer. Based on fundamental rheology testing, the evolution of the continuous gluten phase in the developing dough matrix could be verified with in-line multiwave rheology tests. Additional to previous works on shear kneading techniques [7,10,13,14], the improved kneading process with the non-interrupting in-line rheology testing enabled the dough matrix evolution to be mapped from the very beginning of the kneading process. Therefore, the comparability of shear-kneaded to classically (in a conventional Z-kneader) produced wheat flour dough along the kneading process could be shown. The shear kneading setup in a conventional rheometer represents a useful tool to analyze structural formation reactions of the gluten phase, as well as a controlled energy input method to investigate the influence of deformation on network evolution processes.

In this study, the applicability of the applied shear stress–relaxation steps to develop a dough matrix was shown. The initial particle–particle interactions of the non-hydrated flour and starch granules were rheologically comprehensible in the early stage of dough development. For the evolving dough matrix, changes in flow and relaxation behavior, as well as gel strength increase due to ongoing hydration and homogenization, were observed. For the evolution of a continuous gluten phase, the applied shear forces were sufficient to develop the dough matrix over the whole sample cross section. Typical for kneaded wheat flour doughs, an optimum development stage was observed that was close to the externally (in a DoughLAB) determined one. Therefore, the identification of the crucial energy to develop the gluten phase during kneading became more comprehensible since having only one deformation type eased the calculation. As the results show, the energy consumption was comparable to other types of kneaders, except that no energy was wasted in the parallel plate rheometer due to the small sample size and large contact area of the kneading geometry. This step toward a fully comprehensible energy transfer to the kneaded sample is elementary for the understanding of microstructure formation. Since the results are carried out only for native wheat flour, the adaption of the kneading technique to other gluten-containing samples must be further investigated. Additionally, the limitation of the validity of the multiwave frequency sweep to the previous determined linear viscoelastic region for the kneaded sample sets a limit to the frequencies and amplitudes that can be measured simultaneously. Nevertheless, the developed kneading technique provides the basis for small-scale investigations of dough behavior during further processing, e.g., gas expansion during the proofing and baking steps.

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4.2.2 Microscopic analysis of gluten network development under shear load-combining confocal laser scanning microscopy with rheometry

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Microscopic analysis of gluten network development under shear load—combining confocal laser scanning microscopy with rheometry

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Abstract

A comprehensive in-situ analysis of the developing gluten network during kneading is still a gap in cereal science. With an in-line microscale shear kneading and measuring setup in a conventional rheometer, a first step was taken in previous works toward fully comprehensible gluten network development evaluation. In this work, this setup was extended by an in-situ optical analysis of the evolving gluten network. By connecting a laser scanning microscope with a conventional rheometer, the evaluation of the rheological and optical protein network evolution was possible. An image processing tool for analyzing the protein network was applied for evaluating the gluten network development in a wheat dough during the shear kneading process. This network evaluation was possible without interruption or invasive sample transfer comparing it to former approaches. The shear kneading system was able to produce a fully developed dough matrix within 125% of the reference dough development time in a classical kneader. The calculated network connectivity values from frequency testing ranged over all samples was in good agreement with traditional kneaded wheat dough just over peak consistency.

KEYWORDS
confocal laser scanning microscopy, gluten network, rheology, wheat flour dough

1 | INTRODUCTION

Wheat flour dough is characterized by a highly crosslinked and widespread protein network (Jekle & Becker, 2011a). The three-dimensional network enables the matrix to retain gas and is therefore, together with the heat-induced starch gelatinization, responsible for the achievement of high loaf volumes and appealing crumb structures after baking (Dowell et al., 2008; Hrušková et al., 2006; Wikstrom & Bohlin, 1996). This protein network evolves under hydration and input of mechanical energy (Jekle & Becker, 2015). The mechanical energy input in classical kneaders comprises tension, shear and/or compression (Connelly & Kokini, 2006, 2007; Tietze et al., 2017). The specific mechanical energy is crucial for the development of the desired network and is transferred from the kneading elements of the mixer to the material. The basis for most baked goods is the formation of an optimal developed gluten phase in the dough matrix. The optimum

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consistency of a dough is achieved when the dough best withstands the deformation caused by the kneading geometry and is therefore the peak of dough consistency (Parenti et al., 2021). At this stage of optimal dough development, the combination of covalent (disulfide) and non-covalent (e.g., H-bond, Van-der-Waals) bonds of the polymeric protein network is responsible for the specific mechanical behavior of the dough. The protein network consists mainly of two main fractions, glutenins and gliadins, the storage proteins of the grain which consist of high proportions of the amino acids glutamine (32%–53%) and proline (11%–29%) (Wieser et al., 2022). First, glutenins, represented by high and low molecular weight glutenin subunits (HMW-GS and LMW-GS), are polymeric. And second, gliadins are represented by α-, γ-, $\omega_{1,2}$-, and ω_5-gliadin, which are monomeric (Lindsay & Skerritt, 1999). The glutenins and gliadins show a wide variety of amino acid compositions and differ in their ability to bind other proteins and other flour constituents (Wieser et al., 2022). Besides these protein fractions, albumins and globulins are also present in wheat flour. These non-gluten proteins are mainly monomeric, but both albumins and globulins tend to form polymers by forming interchain disulfide bonds as well, but they make no significant contribution to the achievable loaf volume or baking quality of wheat flours (Tomić et al., 2015). At the beginning of the dough kneading process, the combination of mechanical energy input and the hydration of the flour particles causes glutenins and gliadins to crosslink and develop a continuous protein network. This network is known as the continuous gluten phase (Delcour & Hoseney, 2010; Jekle & Becker, 2011a; McCann & Day, 2013). Starch granules are also embedded in the gluten phase, which promote internal friction during kneading. Regardless of their shape and size, the surface functionality of the starch granules can be considered as a universal factor influencing the resulting network, since their surface contributes to the interface between the particle and the gluten phase (Brandner et al., 2021). Therefore, the resulting dough matrix depends both on the gluten attributes as well as on the starch protein interactions.

The kneading process is difficult to reproduce due to its complexity and the action of several forces. Therefore, it is hardly possible to completely trace the development of the dough matrix in-line analytically. In addition to the limitation due to complexity, an interruption of the process and sample transfer of the kneaded sample to other equipment (e.g., a microscope) is mandatory. These interruptions are the state-of-the-art to evaluate the dough matrix at different stages of development on a microstructural level. To isolate energy transfer from the complex kneading geometries of most commercial kneaders, Vidal et al. (2022) developed a microscale kneading process that uses only shear to develop a dough matrix. Analyzing the evolving protein network in gluten-starch systems and wheat flour doughs on a microscale using a confocal laser scanning microscope (CLSM) is already state-of-the-art in cereal science. The method established by Jekle and Becker (2011b) can be utilized as a tool for protein quantification. Additionally, the protein network analysis (PNA) developed by Bemklau et al. (2016) allows the identification of absolute morphological attributes such as total number of junctions (TNoJ) or the branching rate as well as end-point rate and lacunarity, and can precisely quantify and characterize the development stage of a gluten network. With the aforementioned tools for in-line production of dough in a rheometer and the possibility to precisely calculate the development stages of the network by means of microscopic analysis, the basis for a complete analysis of the network development would be given. Thus, by combining a rheometer with a microscale kneading technique and a CLSM in one unit, the development process of the resulting dough matrix could be studied in greater detail. As shown by Dutta et al. (2013) the inline measurement of network evolution in collagen polymerization with such a rheometer-CSLM coupling is possible and may be applicable for wheat dough network evaluation.

The aim of the study was to develop a combined kneading and microscopic analysis tool in a rheometer (Rheo/CLSM) and to evaluate the mechanical behavior simultaneously with the microstructural evolution of the emerging dough matrix. During kneading, the matrix development and the resulting rheological properties of the dough are influenced by the protein content and the gliadin and glutenin subunits of the flour. To gain more knowledge on these influences at the microstructural level, the tool should be able to precisely determine these intercorrelations from network attributes to flour composition. To investigate the influence of flour protein composition on the achievable network characteristics, a variation of five different wheat flours was analyzed. Without a sample transfer to other devices, the system provides all necessary data to determine the stage of dough development at the rheological and microscopic level and their dependency on the flour composition in-situ. As shown in previous works, the shear induced changes in polymeric matters can be further evaluated by the combination of rheology and microscopy in a combined analysis tool (Gagnon et al., 2020). With this aim, the new method can produce wheat flour doughs in a rheometer with additional microscopic analysis on a very small scale.

2 | MATERIALS AND METHODS

2.1 | Analysis of the wheat flour

For all experiments, commercial wheat flour type 0 and type 00 provided by Rieper AG, Vintl, Italy was used. The composition (see Table 1) has been previously analyzed (Vidal et al., 2023).

To determine the required dough development time (DDT) and water addition, the flours were analyzed according to AACCi 54–70.01 by Vidal et al. (2023) and the resulting specific kneading times derived from the DDT are listed in Table 2. The specific kneading times for further analysis were chosen to get a good overview of the different development stages of the emerging dough matrix.

2.1.1 | Analysis of flour protein composition

Flour proteins were analyzed according to Wieser et al. (1998). Albumins and globulins, gliadins and glutenins were extracted from flour in three steps using a Na_2HPO_4/KH_2PO_4 buffer (0.67 mol/L, pH = 7.6)

Flour	S1	S2	S3	S4	S5
Protein [g/100 g]	14.86 ± 0.06	14.29 ± 0.03	14.22 ± 0.01	11.79 ± 0.03	11.77 ± 0.05

TABLE 1 Flour samples and their protein content.

Flour	50% DDT [s]	100% DDT [s]	125% DDT [s]	150% DDT [s]	600 s
S1	151	302	377	453	600
S2	86	172	215	258	600
S3	137	274	342	411	600
S4	57	114	142	171	600
S5	64	128	160	192	600

Note: Specific analysis points derived from the DDT. A reference kneading time of 600 s for undocked comparison of kneading influence of the network evolution from DDT.

TABLE 2 Determined dough development times (DDT) in a DoughLAB for all flours with optimum water dosage.

containing 0.4 mol/L NaCl, 60% aqueous ethanol (v/v) and a mixture of TRIS/HCl buffer (0.1 mol/L, pH = 7.6) and 1-propanol (50/50; v/v) containing 2 mol/L urea and 10 mg/mL dithiothreitol. RP-HPLC analysis was performed on a Jasco XLC HPLC (Jasco Deutschland GmbH, Pfungstadt, Germany) as described by Schuster et al. (2022) of the injection volume was 20 µL for albumins and globulins, 10 µL for gliadins and 20 µL for glutenins. All determinations were performed in triplicate.

2.2 | Standard wheat dough

For the shear rheological measurements of classically kneaded dough, the specific amount for each flour (corrected to 14% moisture) and demineralized water were kneaded at 63 rpm using a z kneader DoughLAB (Perten Instruments AB, Hägersten, Sewden), equipped with a 50 g mixing bowl. 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 triplicate. After kneading, a small piece of dough was placed in the CLSM and analyzed according to Section 2.2.2.

2.2.1 | Small-scale deformation analysis

To analyze the rheological property, a frequency sweep within the linear viscoelastic limit was performed on a rheometer type MCR502 (Anton Paar, Osterfildern, Germany). For the frequency sweep, the measurement frequency was varied from 0.1 to 50 Hz at a deformation of 0.05%. The complex modulus G^* was analyzed according to the power law equation (Gabriele et al., 2001):

$$G^*(\omega) = A_f * \omega^{\frac{1}{z}},$$

where A_f (Pa $s^{1/z}$) describes the gel strength and z (-) describes the network elements' connectivity.

2.2.2 | Microstructural analysis

For optical analysis, the dough samples were stained by adding rhodamine B dye into the bulk water to visualize the contained proteins. After kneading, 2 g of the dough sample of each flour was 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, the samples were analyzed by an eclipse Ti-U inverted microscope with an e-C1 plus confocal system (Nikon GmbH, Düsseldorf, Germany) using a laser with a wavelength of 543 nm for excitation, the emission was detected at 590 nm with a 50 nm bypass filter. Four different images were taken of each dough sample with a resolution of 1024 × 1024 pixel and a size of 686 × 686 µm (for 20× magnification). The dough samples were produced in triplicate; therefore, 12 images were analyzed for one flour type. The analysis was performed according to Bernklau et al. (2016).

2.2.3 | Image processing and analysis

The software-based analysis of CLSM images was performed by AngioTool64 version 0.6a (National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA) (Zudaire et al., 2011). The AngioTool was applied on CLSM images of gluten network according to the method of Bernklau et al. (2016) (see Figure 1).

2.3 | Shear-kneading dough production

To produce shear kneaded dough in the MCR502 rheometer (Anton Paar, Ostfildern, Germany), a plane plate-cylinder geometry setup with 25 mm diameter of the upper plate geometry and 25.1 mm inner diameter of the cylinder was used. For all experiments, 192 mg of flour and demineralized water stained with rhodamine B (Merck KGaA, Darmstadt, Germany), 0.01 g/100 mL water were kneaded in the rheometer according to Vidal et al. (2022). The sample preparation is shown in Figure 2. After setting up the application cylinder, the

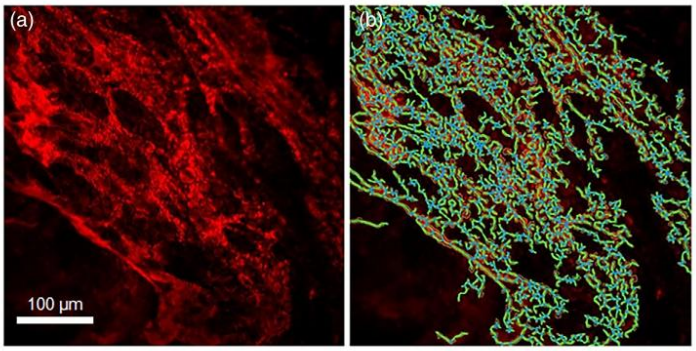

FIGURE 1 (a) Confocal laser scanning microscope (CLSM) image (1024 × 1024 pixels, 686 × 686 µm) of dough from flour S3 after a kneading time of 411 s (150% DDT) using Rheo/CLSM coupling; (b) AngioTool evaluation of image (a) with protein strands (green) and linkage points (blue) drawn in.

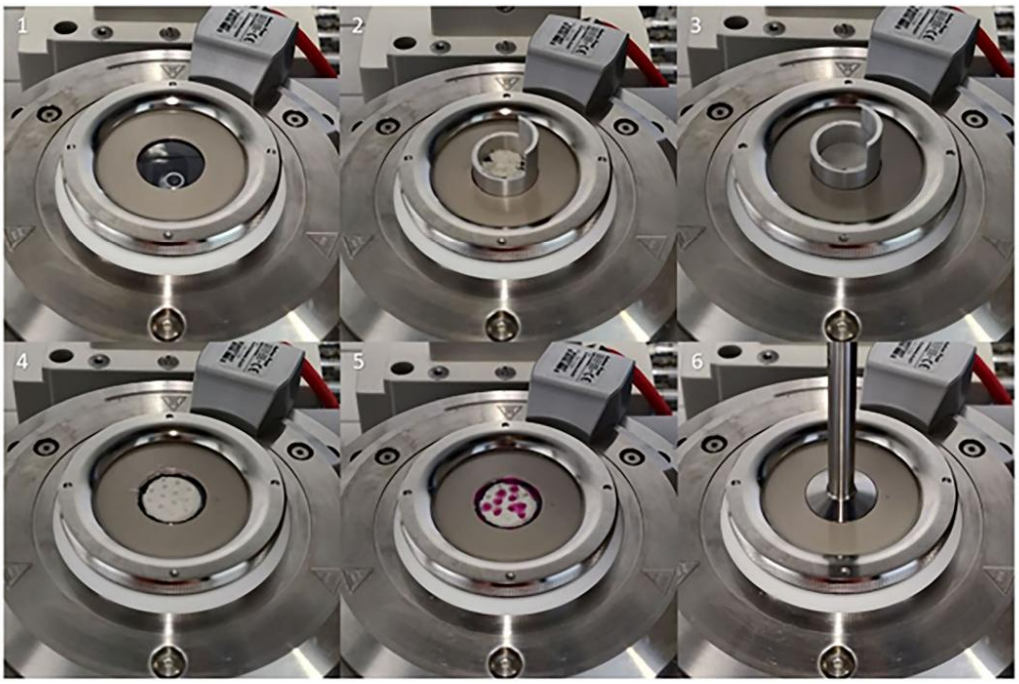

FIGURE 2 Setup of the coupling between rheometer and confocal laser scanning microscope. Process of sampling and setting the upper rheometer geometry with flour S3.

sample was added and smoothed with a smooth stamp. Subsequently, 12 wells were formed with a customized stamp, into which the rhodamine B solution was added dropwise. After adding the stained water, the kneading process started. At specific times, the kneading process was interrupted to capture images with the CLSM (see Table 2). The images were then analyzed according to Section 2.2.2.

2.4 | Statistical analysis

Results were evaluated statistically with Origin(Pro), Version Version 2022. (OriginLab Corporation, Northampton, MA, USA), with Pearson Correlation coefficient calculation. All values are represented with the standard error of the mean (SEM).

3 | RESULTS AND DISCUSSION

3.1 | Composition of the wheat flours

The results of the HPLC analysis in Figure 3 showed that with a lower total protein content of the wheat flour samples, higher quantities of albumin/globulin fractions occurred simultaneously. Tomić et al. detected the content of albumin fractions also in a range from 2.64% to 17.50% of total protein (Tomić et al., 2015). Taking into account, that the albumin and globulin fractions were determined together the observations are in good agreement with the literature (DuPont et al., 2005; Singh & Skerritt, 2001; Tomić et al., 2015). The results also show that the glutenin content for the analyzed flours increases with the total amount of protein measured. Only S4 does not fit these results and has the lowest value for glutenin from HPLC analysis with only 24.0%. The achievable protein composition during breeding is connected to fertilization and varying weather conditions and therefore such differences are not unusual (Edwards et al., 2007). Since the connection of achievable dough volume and total protein content is also linked to protein composition (Skerritt et al., 1999), the flour samples give a good overview of the actual processed flours in the baking industry. To evaluate the network development and rheological behavior of the kneaded dough samples in-situ with a combination of rheology and laser scanning microscopy, the results of the HPLC analysis give the possibility to elucidate these processes precisely. In Table 3, the difference between the analyzed flour is even more pronounced especially for the LMW-GS. As stated by Sisson et al. and Edwards et al., the variation in gluten strength found among LMW-GS and HMW-GS groupings suggest that the presence of specific allelic patterns can, but do not guarantee, a specific level of baking performance (Edwards et al., 2007; Sissons et al., 2005). The variation in total protein and gluten protein subunits gives a good basis for the microstructural evaluation of the network forming processes.

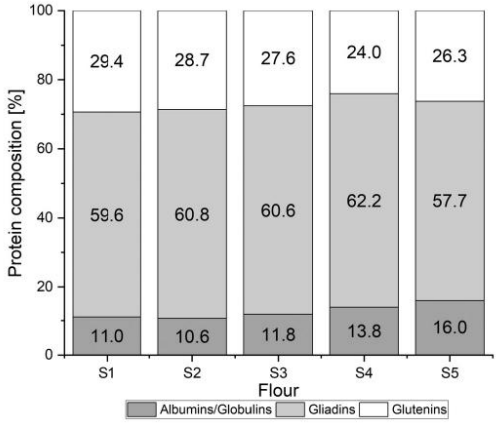

FIGURE 3 Protein composition of the flours divided into albumins/globulins, gliadins and glutenins, given as percentages relative to the sum of total extractable protein (modified Osborne fractionation and RP-HPLC analysis).

3.2 | Microscopic network analysis

Figure 4 shows CLSM images taken during the ongoing kneading process and the network evolution for both classical and shear-kneading processes. As a result of hydration and crosslinking processes in combination with mechanical energy input, the protein strands and nodes emerge from clusters of non-connected flour aggregates to interconnected protein filaments (Schiedt et al., 2013). The network develops from not connected flour particle aggregates at 137 s, consisting of starch, fiber, and protein, to an interconnected protein network at 342 s, with embedded starch and other components. The emerging network consists of the mostly elastic polymeric glutenin network and gliadins are embedded within this matrix. The combination of these two interacting protein fractions gives the dough matrix its unique viscoelastic attributes. The gluten network consists, on the one hand, of covalent disulfide bonds, which contribute to the plasticity of dough. On the other hand, it contains non covalent interactions, especially intra- and intermolecular hydrogen bonds, which are mainly responsible for determining the elasticity (Belton, 1999; Brandner et al., 2018). The resulting network development continues up to a certain point after which the applied load causes a network breakdown. As can be seen in the classically kneaded dough from 342 to 411 s and further on in Figure 4, the network configuration changes to a fibrillary appearance, which results in less strong but highly connected protein strands. This breakdown is caused mainly by three different processes, disentanglement, chain orientation, and bond rupture (Singh & MacRitchie, 2001). With ongoing kneading and network breakdown, the quantity of bigger protein clusters changes (Don et al., 2003). At the end of the performed kneading trials, the over-kneaded sample at 600 clearly shows destroyed and rough strands, where bigger aggregates are separated due to the network breakdown. Looking at

Flour	ω_5-gliadin	$\omega_{1,2}$-gliadin	α-gliadin	γ-gliadin	HMW-GS	LMW-GS
S1	3.9	3.8	31.2	20.8	8.6	19.9
S2	3.6	3.8	33.0	20.4	8.6	19.3
S3	3.9	4.0	32.2	20.6	8.1	18.7
S4	3.9	3.9	32.3	22.1	5.9	17.5
S5	3.3	3.5	29.6	21.4	6.6	19.0

TABLE 3 Differentiation of gliadin and glutenin subunits given as percentages relative to the sum of total extractable protein (modified Osborne fractionation and RP-HPLC analysis).

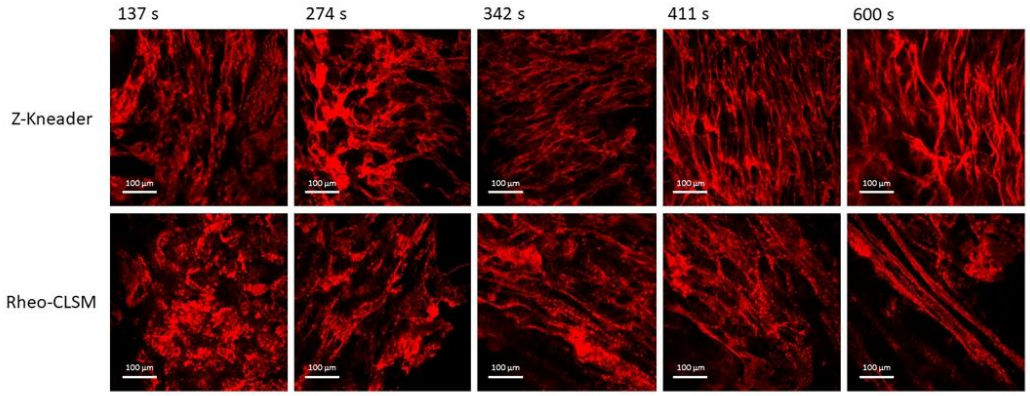

FIGURE 4 Confocal laser scanning microscope (CLSM) images of the evolving protein network of wheat flour S3 at the specific kneading times. Comparison of classically produced dough externally analyzed with a CLSM with shear-kneaded in-line microscopy images during the dough network evolution.

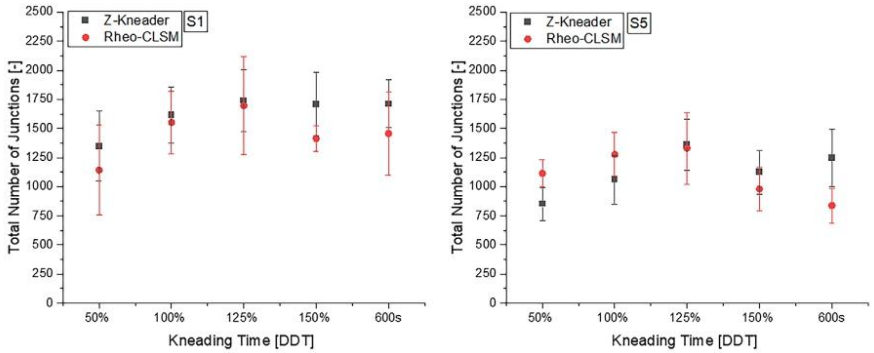

FIGURE 5 Total number of junctions for flour S1 (left) and S5 (right) from the protein network analysis of the confocal laser scanning microscope images taken from standard kneaded wheat dough and shear-kneaded samples. For each sample, three doughs were kneaded and for each time, three images were taken and analyzed.

the shear-kneaded sample, a similar behavior can be observed. The shear-induced destruction of the protein network and the development of bigger separated strands is visible at 600 s kneading time in the rheometer. To further investigate the network evolution, the PNA was performed.

A distinct increase in interconnections as well as protein network breakdown can be seen in Figure 5 by the detected number of nodes when evaluating the CLSM recordings for both flours. The standard deviation derives from threefold dough sample preparation with three images per sample taken for each time, which results in nine images per analysis time. The peak TNoJ indicates the maximum interconnected state of the polymeric matrix in the dough which is influenced by protein content and kneading time (Bernklau et al., 2016; Jekle & Becker, 2015). For both wheat dough samples, representing the highest (S1) and lowest (S5) protein contents of the used wheat flour, the peak can be found at by 125% DDT which can be explained by the ongoing overmixing and therefore changes in the structural appearance of the network (Schiedt et al., 2013). The shear-kneaded samples also showed the optimum development stage close to the DDT determined by the torque recording mixer. S5, with lower protein content, also results in lower TNoJ which is in good agreement for glutenin macro polymer (GMP) formation in the literature (Don et al., 2003; Wang et al., 2019). The less GMP formation, which is built during kneading, can therefore be explained due to lower gliadin and glutenin content (Lindsay & Skerritt, 1999). The breakdown behavior depends on the strength of the protein–protein interactions in the dough and has shown the potential to give an indication on protein content and composition characteristics in wheat (Pritchard &

Brock, 1994). The observed extent of network degradation is different for all the flours studied, since the observed increase in viscosity during mixing, followed by a decrease during overmixing, is caused by the increase in the molecular weight of the polymer as influenced by the gluten content (Skerritt et al., 1999). The gluten content-dependent behavior could also be observed in the CLSM results of this study. Especially the HMW-GS showed a strong Pearson correlation of 0.94 ($p < .05$) with the resulting TNoJ. Within gliadins, the γ-gliadins showed a negative correlation with the TNoJ with −0.87 ($p < .05$). These two gluten protein types also showed strong correlations to the DDTs of the flour samples. Pritchard and Brock (1994) found that the weight of gel protein in flour, and therefore total protein content, was poorly correlated with loaf volume and that correlations between breakdown rate and loaf volume were somewhat better: low loaf volumes were associated with higher rates of breakdown (Skerritt et al., 1999). Loaf volume for the flours was investigated in Vidal et al., underlining these findings for the less strong flours used (Vidal et al., 2023).

3.3 | Rheological network analysis

As shown in Figure 6, the results from standard analysis and in-situ inline testing of the kneaded dough samples were in good agreement and the rheological behavior was comparable. This kneading behavior was previously investigated by Vidal et al. (2022). The reduction in the number of connections due to the collapse of the network (as seen in Figure 4) also reduces the connectivity number z for all samples, which is due to the extended kneading times. Therefore, the value of z as the number of rheological units correlated with one another in the three-dimensional structure drops after at 125% DDT to lower values for all flour samples. This drop could be explained by the breaking of disulfide bonds and a release of free water into the matrix (Haraszi et al., 2008). The lower protein flour S5 shows lower z values for the shear-kneaded sample. The fitted z values strongly correlate with the LMW-GS with a Pearson coefficient of .89 ($p < .05$). The influence of LMW-GS groupings, in this case shown by the connectivity z, can but must not guarantee a higher level of gluten strength (Edwards et al., 2007). Gliadins showed a medium strong negative correlation coefficient of −.77 ($p < .5$) with the z values. Since gliadins are not polymeric, a higher content results in lower glutenin content and therefore less available network building proteins (Costa et al., 2013). During kneading, the quantity of large protein clusters changes (Don et al., 2003). Thus, the network configuration changes to a fibrillary appearance with less strong but highly connected protein strands as shown in the PNA and CLSM images (Appendix).

4 | CONCLUSION

With the developed setup the comparability of shear-kneaded to classically (in a conventional Z-kneader) produced wheat flour dough along the kneading process could be demonstrated. The shear kneading and microscopic setup in a conventional rheometer represents a useful tool to analyze structural formation reactions of the gluten phase. Furthermore, the system provides a controlled energy input method to investigate the influence of deformation on network evolution processes in gluten starch matrices and wheat flour doughs in-situ.

By implementing the above-mentioned combination of rheometer and CLSM the rheological and microscopic comparability with classical z-kneaded dough was shown and proved the comparability of the micro system with classical analysis. For the developing dough matrix, an increase in interconnected gluten network at the microscopic level (TNoJs) and network connectivity z at the rheological level was shown and could be directly linked. For the evolution of the continuous gluten phase, the applied shear forces were sufficient to develop the dough matrix over the whole sample cross section. In agreement with

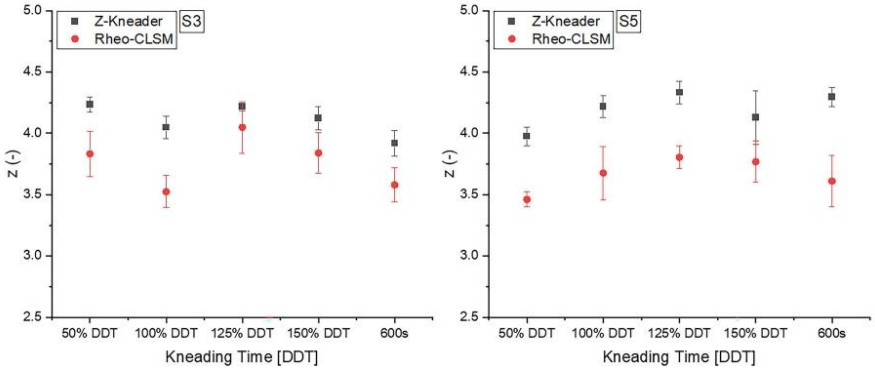

FIGURE 6 Network connectivity z with standard deviations resulting from power-law fitting of the complex modulus measured via frequency sweep with 0.1–50 Hz at 0.05% deformation for wheat flour samples S3 and S5 ($n = 3$).

classically kneaded wheat flour doughs, an optimum development stage was observed which was close to the externally determined stage in a DoughLab. As a result of investigating flours with different a composition, the influence of protein composition on the achievable network attributes (TNoJ, connectivity *z*) was also shown. With a Pearson coefficient of .94 ($p < .05$), the HMW-GS had the greatest impact on the achievable TNoJs within the network. For the rheological units correlated with one another as described by *z*, the LMW-GS showed strong correlations with a Pearson coefficient of .89 ($p < .05$). Since the results were obtained only for native wheat flour, the applicability of the combined shear-kneading-CLSM system must be further investigated for adapting the kneading technique to other gluten-containing samples. The developed method represents a new step toward a fully comprehensible investigation of dough and gluten network development without sample transfer and process interruption for microscopic and rheological analysis.

AUTHOR CONTRIBUTIONS

Leonhard Maria Vidal: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; formal analysis; project administration. **Hans Ewigmann**: Investigation; methodology. **Clemens Schuster**: Investigation. **Thekla Alpers**: Conceptualization; writing – review & editing; project administration. **Katharina Anne Scherf**: Writing – review & editing. **Mario Jekle**: Writing – review & editing; project administration; conceptualization. **Thomas Becker**: Writing – review & editing; supervision; project administration; funding acquisition.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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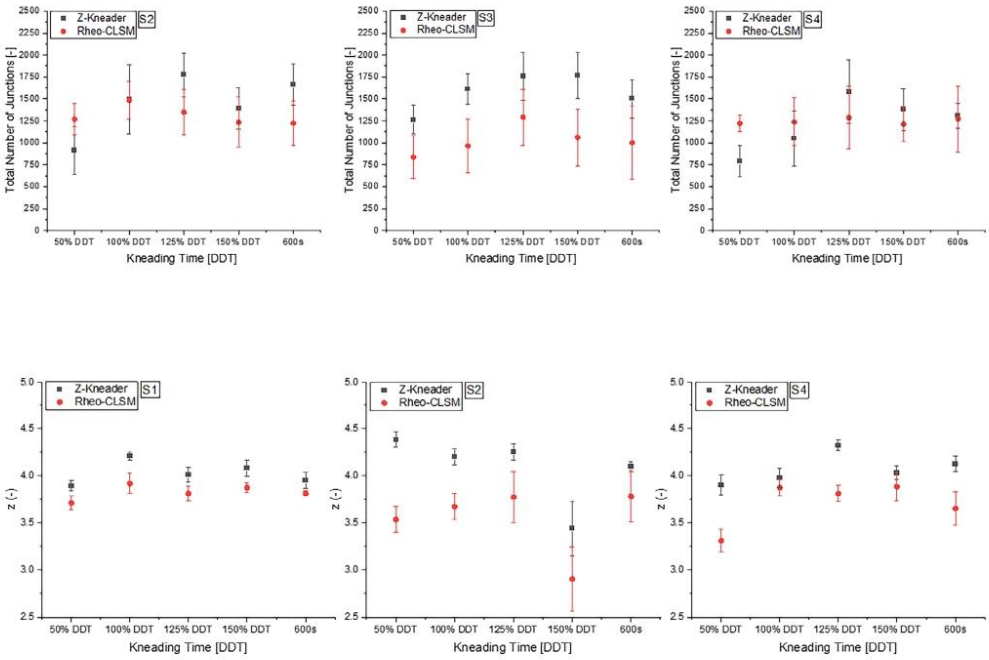
APPENDIX



4.2.3 A dynamic micro-scale dough foaming and baking analysis – Comparison of dough inflation based on different leavening agents

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A dynamic micro-scale dough foaming and baking analysis – Comparison of dough inflation based on different leavening agents

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Wheat flour dough
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ABSTRACT

Leavening agents play a pivotal role in the production of baked goods. Through gas production the inner structure of the product gets its typical foam structure and textural appearance. Baking trials are thereby a common way to determine the achievable loaf volume, crumb structure and other product specific properties. The required material input for these classic baking trials is high, as well as specific baking skills are required to obtain comparable and reliable results. To minimize the previously mentioned challenges, an in-line kneading, proofing, and baking process in a conventional rheometer was used and a microscale method was developed to determine both flour-specific baking performance and leavening-dependent volume increase without sample transfer. The results show a direct comparability of standard baking tests and the microscale method with yeast. In a second step the influence of the commercial used acidifying agent in baking powder D-(+)-Glucono-1,5-lactone (GDL) was compared to L-galactono-1,4-lactone (GGL), an alternative that has the potential to be biotechnologically produced from pectin-rich plant biomass residues. The results showed that GGL produced carbon dioxide slower then GDL and could therefore be interesting for frozen or slow rising products especially for protein rich flours.

1. Introduction

The most common baked good is wheat flour bread which can be subdivided in leavened and unleavened types (Németh et al., 2018). For leavened breads biological, chemical, and physical rising agents are necessary. All rising agents cause a foaming of the hydrated cereal biopolymers within the dough matrix in order to lighten and soften the structure (Brodie & Godber, 2007; Neeharika et al., 2020). The foaming can be subdivided in different processing steps: during **mixing**, the matrix is aerated by the incorporation of air bubbles. These bubbles serve as important initial nuclei for the other gases that form during **foaming**, into which they can diffuse and expand. As the temperature then increases during **baking**, the encapsulated gas in these bubbles expands and increases the bubble size due to the gas expansion and evaporation processes caused by the heating. The size as well as the number of these expanded gas bubbles is crucial for the achievable crumb and product appearance. The crumb itself, at a macroscopic level, is comprised of a fluid (gas) and a solid (gas cell wall material) phase (Scanlon & Zghal, 2001). During the expansion of the bubbles in the dough, the walls of the gas cells (dough matrix) are stretched tangentially in two directions and compressed uniaxially in the radial direction, forming lamellae around the enclosed gas cells (Alpers et al., 2020). Looking at the cross-section of a baked crumb, on the other hand, shows that this solid lamellar phase is completely crosslinked after baking (Torquato, 2000). The final product quality is strongly determined by the foaming capability of the dough. Here, the ability to maintain volume is directly related to foam stability during baking. This foam stability depends on the material properties of the bread dough, which depends mostly on the protein (in wheat dough the gluten) composition of the used wheat flour. Thereby, the gluten quality and functionality is mainly described by the strength and extensibility of the dough (Lásztity, 2002).

From the point of view of bubble mechanics, it is imperative that bubble growth during foaming originates from the trapped gas nuclei.

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Table 1
Results of the analysis according to AACC and ICC standards of the wheat flours used. (n = 3).

Flour	S1	S2	S3	S4	S5
Protein [g/100 g]	14.86 ± 0.06	14.29 ± 0.03	14.22 ± 0.01	11.79 ± 0.03	11.77 ± 0.05
Ash [mg/100 g]	411.86 ± 37.99	394.59 ± 76.92	479.17 ± 61.4	410.24 ± 48.88	524.23 ± 54.46
Moisture [%]	13.48 ± 0.05	13.86 ± 0.06	13.48 ± 0.4	13.49 ± 0.5	12.97 ± 0.7
Wet Gluten [%]	33.47 ± 0.63	31.85 ± 0.55	31.18 ± 1.31	25.88 ± 0.39	22.87 ± 1.62
Dough Development Time (DDT)[s]	302 ± 21	172 ± 13	274 ± 27	128 ± 7	114 ± 11

These bubble mechanics state that: $P = 2\gamma/r$ (with the inner pressure of a bubble P, the interfacial tension γ and the radius of the bubble r). This indicates that for a dough/mass with constant interfacial tension, the pressure required to form a new bubble becomes infinite when the radius of the forming bubble starts at zero. To expanse the bubbles in the matter carbon dioxide is mostly responsible for this process during foaming and baking. Carbon dioxide can be produced biologically by bacteria or yeast by fermenting carbohydrates or chemically. During yeast fermentation, sugars extracted from grain are converted by the cells into carbon dioxide and other metabolic products. To achieve consistent product quality, baking trials have been critical to select suitable yeast strains with specific phenotypic characteristics to achieve optimal fermentation rates depending on the dough type and desired end product (Struyf et al., 2017). On the other hand, the most used chemical leavening agent in fine bakery products is sodium bicarbonate in combination with an acid in the wet or dry state (Neeharika et al., 2020). The chemical leavening agents release carbon dioxide gas into the dough through an acid-base reaction or thermally induced decomposition, which causes the nuclei to expand during baking, creating the final texture of the product (Diez-Sánchez et al., 2020). In the case of acid-activated decomposition the following chemical equation for the acid base reaction applies:

$$NaHCO3 + H^+ \rightarrow Na^+ + CO_2 + H_2O$$

Sodium bicarbonate alone only partially decomposes when heated above 80 °C and releases less than half of the available carbon dioxide as follows (Neeharika et al., 2020):

$$2\ NaHCO_3 + heat = Na_2CO_3 + CO_2 + H_2O$$

To release all of the carbon dioxide the leavening acid is required (Miller, 2015). The acidifying agent used determines the speed of the gas release. Most of the common acids react instantly with the baking soda and liberate all the available carbon dioxide within a minute (Brodie & Godber, 2007). However, a limitation of the usage of baking soda is the production of the neutral salt during the reaction which can influence the rheological behavior of the dough, making it stiffer and less elastic, as well as it can have an impact on the sensorial appearance of the final product (Miller, 2015).

To evaluate the influence of the leavening agent, baking trials are essential to evaluate the achievable crumb properties. Several standards and established methods for evaluating and predicting baking performance have been developed so that the time-consuming and material-intensive baking tests can be at least partially substituted. These methods have been reviewed in several publications (Hadnadev et al., 2011; Németh et al., 2018; Zaidel et al., 2010). However, as Németh (Németh et al., 2018) noted, developing reliable baking processes on a small or microscopic scale is a major challenge, and a reliable and robust system has not yet been developed.

Against the background of the limited sample size in conventional baking tests, the focus of this work is on the development of a microscale baking process in a conventional rheometer with minimal sample size in order to overcome the problems mentioned before. For this purpose, dough production, foaming and thermally induced gas expansion are carried out in a well-controlled and closed system in the rheometer gap. Based on a rheo-kneading technique (Vidal et al., 2022) a possibility to produce dough within a rheometer, and therefore a highly controllable volume, is utilized to scale down the baking trials to micro-scale. In a first step, the performance of the system is evaluated by comparison of standard baking trials with yeast as leavening agent in comparison to the achievable volume and crumb evolution in the rheo-kneading and rheo-baking system. In a second step, the system is used to compare the performance of the established acidifying agent D-(+)-Glucono-1,5-lactone (GDL) with a possible alternative L-Galactono-1,4-lactone (GGL), that could be obtained by (spontaneous) lactonization from L-galactonate, which was shown to be producible biotechnologically via fungal-mediated biotransformation from galacturonic acid, a sugar that is abundant in pectin-rich plant biomass, such as apple pomace, orange peels or sugar beet pulp(Benz et al., 2014; Schmitz et al., 2019).

2. Materials and methods

2.1. Wheat flours

Commercial wheat flours type 0 and type 00 provided by Rieper AG, Vintl, Italy were used with protein contents stated by the producer as shown in Table 1. Dough resistance and water absorption were measured in a Z-kneader doughLAB (Perten Instruments AB, Hägersten, Sewden) according to AACCi 54–70.01 to determine the required dough development time (DDT) and water addition. The moisture per 100 g flour (AACCi 44-01) as well as the ash mg per 100 g dry flour (ICC 104/1) and the wet gluten content (AACC 38-12A) were measured. Also, the Protein content according to the Kjeldahl method from AACC 46-16 was measured.

2.2. Wheat dough production

2.2.1. Spiral kneader

For baking trials, 1 kg flour was first kneaded at 100 rpm for 60 s and further 320 s at 200 rpm with a laboratory spiral kneader at 30 °C (Diosna Dierks & Söhne, Osnabrück, Germany). The desired leavening agent was added to the sample as mentioned in 2.4. The trials were performed in threefold for each batch of dough.

2.2.2. Rheometer

To produce shear-kneaded dough in a rheometer (MCR 502, Anton Paar, Ostfildern, Germany), a plane plate-cylinder geometry setup with 25 mm diameter of the upper plate geometry and 25.1 mm inner diameter of the cylinder with a 1 mm gap was used. For all experiments, 384 mg of flour and the previous calculated amount of demineralized water, to match the water dosage of the Z-kneader, were developed (shear-kneaded) in the rheometer according to Vidal et al. (Vidal et al., 2022). The yeast was added with the bulk water as a suspension with the specific concentration generated for each sample to reach 1 % (w/w), the specific amount of acidifying agent was diluted in the bulk water as well.. The sodium bicarbonate was added as grinded powder to the flour sample in the rheometer sample holder. To maintain constant humidity and temperature a CTD180 Humidity Ready (Anton Paar, Ostfildern, Germany) chamber was set to 30 °C and 80 % relative humidity (RH) for all trials during kneading. The volume of the samples during the kneading, foaming and baking process in the rheometer gap is calculated

according to the volume of an cylinder $V = \pi \cdot r^2 \cdot h$.

2.3. Leavening agents

In this work, biological and chemical leavening agents were used, with dried *Saccharomyces cerevisiae* as the biological leavening agent. On the chemical side, sodium bicarbonate was added with two different acidifying agents.

2.3.1. Saccharomyces cerevisiae

For all trials with yeast, Fermipan Red dry yeast (*Saccharomyces cerevisiae*) produced by Casteggio Lieviti, Italy, was used. The yeast was stored in a sealed container at 4 °C until usage. Before use, the yeast was suspended with water and then added to the sample as a yeast solution. For all trials 1 % (w/w) yeast calculated on the used dry flour was added to the samples.

2.3.2. Sodium bicarbonate

For all trials with sodium bicarbonate, CAS-No 144-55-8 produced by Merck KGaA, Darmstadt Germany, was used.

2.3.3. Leavening acids

In this work two leavening acids are used. Glucono-Delta-Lactone (GDL) as an established leavening acid with similar foaming abilities to *S. cerevisiae* and Galactono-Gamma-Lactone (GGL) as a biological alternative.

2.3.4. Glucono-Delta-Lactone

Glucono-delta-lactone is an inner ester of gluconic acid. In a water solution, GDL slowly hydrates to become acidic and thus acts as a leavening acid. Hydrolysis is slow in the cold, but is accelerated by heat, making GDL ideal for refrigerated or frozen dough products (Brodie & Godber, 2007).

GDL produced by ThermoFisher (Kandel) GmbH, Kandel Germany, with a purity of 99 % was used.

2.3.5. Galactono-Gamma-Lactone

GGL produced by Merck (Sigma-Aldrich), Germany, with a purity of ≥95.0 % (GC) was used (Sigma 05313).

2.4. Standard baking trials

After kneading the dough was divided in 300 g portions. All dough samples containing *S. cerevisiae* were stored at 30 °C and 80 %RH in a Koma SunRiser^{AT} proofing chamber for 30 min. After fermentation, the baking trays were placed in a deck oven preheated to 220 °C. All baking tests were performed with 0.5 L of steam injection into the oven. The loaves were baked for 20 min. The dough density was evaluated by measuring the volume of a known weight of dough against the displacement of mineral oil. The test specimen was weighed in air and its mass was being recorded. It then was immersed in silicone oil and its apparent mass upon immersion was recorded. From these measurements the specific gravity (mass ratio) and the density (mass ratio × the density of the liquid) was calculated. From these measurements the dough volume was calculated. After baking the volume of the loaf was analyzed with a TexVol Instruments BVM -L370. The baked crumb structure was analyzed using a Basler acA2500-60ue camera and a MATLAB code to process the pictures and calculate the pore size distribution. The parameters gained from the pore size distribution are the x_{50} and the x_{90} values, representing the diameters of the pores where 50 % (90 %) of the distribution has smaller and 50 % (10 %) has larger pore size.

Table 2
Results of the optical crumb analysis of classic baking trials for all flours. The x50 and x90 pore diameters are given with the standard deviation. Each flour was baked three times and 4 slices were evaluated with image processing and analysis for eac.

	Flour				
	S1	S2	S3	S4	S5
x50	0.13 ± 0.012	0.11 ± 0.021	0.12 ± 0.016	0.11 ± 0.024	0.10 ± 0.016
x90	1.23 ± 0.014	1.29 ± 0.024	1.27 ± 0.021	1.19 ± 0.047	1.14 ± 0.074

2.5. Micro-Foaming and baking method

For the shear-kneaded dough (according to 2.2.2) containing *S. cerevisiae* a subsequent foaming/fermentation step was implemented in the rheometer program sequence. The samples were held at 30 °C at 80 %RH for 30 min with an applied normal force of 1 N. The normal force was necessary to standardise the load and to calculate the volume correctly. The start of the foaming phase was set at the Temperature where the measured hight of the gap firstly increased. After the foaming/fermentation step, the temperature was increased with 4 °C/min according to the temperature rise in a baked product in a deck oven until reaching 95 °C (Chhanwal & Anandharamakrishnan, 2014). All samples containing sodium bicarbonate were directly heated after kneading without the additional foaming step.

2.6. Statistical analysis

All measurements were performed in triplicates. The standard deviation accounts for the deviation between these triplicates. Mathematical and statistical evaluation was performed using Matlab (R2018a, MathWorks Inc., Natick, MA, USA) and Origin (2018b, OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Flour analysis

To gain deeper insight on the influence of the flour compositions on the specific baking quality of wheat flours a variety of different purposed flours was used. The AACC and ICC Standards mentioned in 2.1 were utilized to analyse these flours and the results are shown in Table 1. The ash contents were in good agreement with the expected ash content of the Typo 0 and 00 flours. These analytical results cover a wide range of flour compositions found in commercial wheat flours for different purposes. Several attempts to enrich flours with fibre or protein by improved cultivation processes can be found in the literature, and contours of these available nutrient contents can be presented with the chosen flour selection (Gao et al., 2012; Shi et al., 2010; Svečnjak et al., 2013; Wu et al., 2014). The wet gluten content underlines the diversity of the samples and provides additional insight into the foaming properties of the dough in terms of protein content. With this variationthe in flour composition, its influence on the achievable volume/crumb can be displayed. With moisture content close to 14 %, all samples were in the expected range. The moisture content was the basis for determining the dough development time (DDT) and water absorption expected for an optimally developed dough. The DDT is not only depended on protein content, but also on the gluten quality. For the tested flours the DDT increased with higher protein content of the samples, only S2 deviates from these findings. But as the literature states, the DDT alone cannot predict the baking performance of a wheat flour (Dobraszczyk & Schofield, 2002). Based on the measured water uptake, the estimated amount of water in the samples ranged from 550 to 610 ml/kg.

3.2. Baking trials with Saccharomyces cerevisiae

3.2.1. Standard baking trials

Standard baking tests were carried out in a deck oven to determine

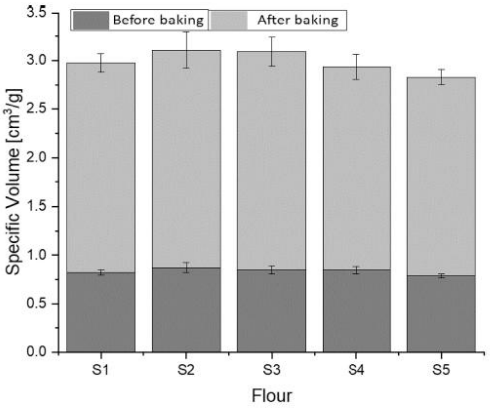

Fig. 1. Specific volume before and after baking in classic baking trials in a conventional deck oven with *S. cerevisiae* (n = 3).

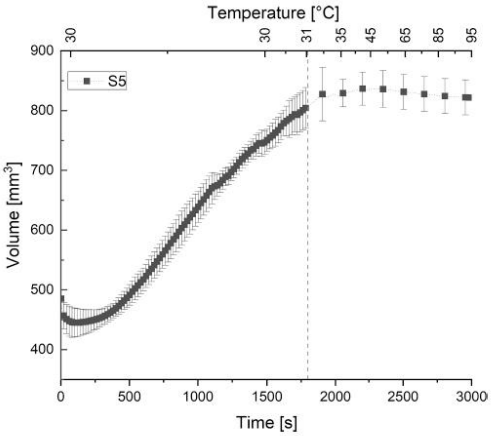

Fig. 2. Kinetics of dough volume increase in the measuring gap between upper and lower rheometer geometry during rheo-baking with dry bakers' yeast for S5. The dashed vertical line marks the beginning of the baking process. (n = 3).

the baking quality of the flours, and the specific volume of the dough and the baked loafs were compared. The results showed that the volume expansion in the baking tests was in good agreement with flours with similar composition in the literature (Beck et al., 2012; Chevallier et al., 2012; Hesso et al., 2015). Thereby, the influence of gluten content was clearly visible. A high gluten content resulted in a strong protein network which withstands the expansion of the gas bubbles and leads to a reduced volume increase. This observation is also reflected in the reduced x_{90} values of the pore size of the optical crumb analysis results in Table 2. The low gluten containing flours (S4 and S5) are not able to retain all the produced gas and result in lower total end volume with the smallest detected x_{90} values for the pore size distribution (see Table 2).

The influence of the gluten content and total flour composition on crumb structure was investigated by optical analysis of the baked crumb. In Table 2 the dependence of the achievable x_{50} and x_{90} values of the pore size on the composition of the flours is shown. At medium gluten content, the volume increase in Fig. 1 is in good agreement with the

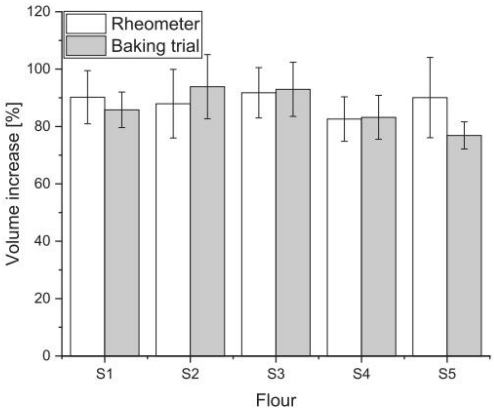

Fig. 3. Comparison of the total volume increase after baking the dough samples in a conventional deck oven and the rheo-baking procedure with 1 % (w/w) *S. cerevisiae* (n = 3).

higher pore sizes at the x_{90} values. In flours with low gluten content, the dough matrix cannot retain the gas produced, and both the volume and the achievable pore size are reduced.

3.2.2. Hight growth during micro-foaming and baking

For a better overview, the results of the micro-foaming and baking procedure are discussed for S5 only. The graphs for S1-S4 are shown in the supplementary data (see Fig. 7). In Fig. 2 the dough of S5 with *S. cerevisiae* shows a relaxation process immediately after the end of kneading and, therefore, the distance of the rheometer geometry decreases during the first approximately 200 s. This relaxation could be caused by the applied normal force and released gases from the dough as well as continuous reorientation and crosslinking processes of the gluten matrix immediately after kneading (Brandner et al., 2022). After the relaxation, the yeast begins to produce enough carbon dioxide to leaven the matrix until the baking process starts after 1800 s (vertical dashed line). This increase in the measurement gap over time showed a continuous expansion phase during foaming and only a slight increase in the gap during the subsequent heating. This could be due to a more rapid drying of the outer dough layers in the geometry gap with increasing temperature, comparable to the crust formation in the deck oven, and thus blocking of further expansion by the heat-induced gas expansion known from standard baking tests. Equal results are shown for the gap size increase of the samples S1-S4 in the Appendix in Fig. 7.

No significant differences ($p = 0.05$) were found in the direct comparison of the total volume increase between the standard baking tests in the deck oven and the micro-foaming and baking process as shown in Fig. 3. The trend of the volume development of the dough depending on gluten content could be confirmed in the micro-scale baking trials. Thereby, surrounding temperature influences dough viscosity as well as the yeast activity and is a crucial parameter (Bloksma, 1985; Fintan Walton & Pringle, 1980). Only S5 shows a higher volume increase in the rheo-baking method than in the deck oven. This could be due to the small gap between the upper and the outer cylinder geometry of the rheometer, which could hinder the escape of the generated gas, as observed in the standard baking experiments, due to the small free surface of the sample. With these findings, the volume increase for both systems is in good agreement with total growth for yeast leavened products and achievable loaf volume from literature (Rózylo & Laskowski, 2011). For doughs leavened with yeast, the reproducibility is depending on multiple process parameters. These parameters are good to control in the rheometer setup and give comparable results with the

Table 3
Foaming phase characteristics for glucono-delta-lactone and galactono-gamma-lactone samples over all flour types during heating (n = 15).

Acidifying agent	Start foaming phase [°C]	End foaming phase [°C]
D-(+)-Glucono-1,5-lactone	48.07 ± 3.01	80.55 ± 2.89
L-Galactono-1,4-lactone	48.67 ± 5.20	78.40 ± 2.67

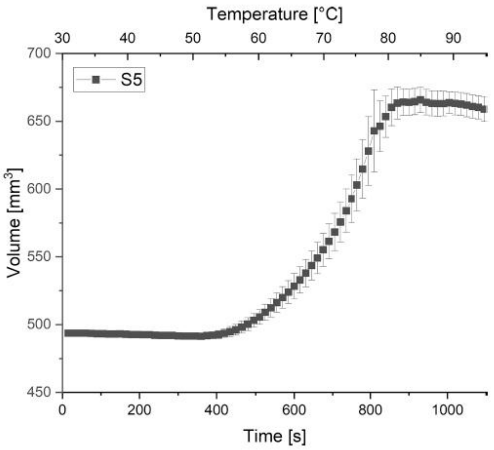

Fig. 4. Kinetics of dough volume increase in the measuring gap between upper and lower rheometer geometry during rheo-baking with sodium bicarbonate and glucono-delta-lactone (n = 3).

deck oven.

3.3. Micro-baking trials with sodium bicarbonate

In the samples with sodium bicarbonate, the initial reduction of the rheometer gap due to relaxation processes of the dough after kneading was only very slight. After reaching the critical temperature the heat induced carbon dioxide production at 48.07 °C (see Table 3) began, and a rapid foaming phase could be observed in Fig. 4 for the sample with GDL. The starting temperature for the GGL samples was very close with 48.67 °C (see Table 3). The volume increase then shows the acid induced release of the carbon dioxide within the range of 50 to 80 °C. At around 50–60 °C the gelatinization of wheat starch begins (Matsuki et al., 2003). Two opposing trends then can be observed. On the one hand, further expansion due to the released carbon dioxide and the heat-induced gas bubble expansion, on the other hand, the progressive starch gelatinization and protein denaturation, which limit further growth (Jekle et al., 2016). The rheo-baking trials showed that at 80 °C the gelatinization and denaturation was completed and the hight growth stopped (see Table 3). This starch gelatinization takes place due to the thermal impact of the heating. Thereby, the starch granules hydrate and increase their volume with the rising temperature (Jekle & Becker, 2012). This observation is in good agreement with the gelatinization temperatures for wheat starch found in the literature (He & Hoseney, 1991; Jakobi et al., 2018; Wehrli et al., 2021). Protein denaturation takes place in parallel to the gelatinization processes as water vaporizes or shifts towards the starch granules. After reaching the 80 °C the acid and thermal induced decomposition of the available $NaHCO_3$ is mostly completed and no more growth of the gap can be observed for both GDL and GGL (see Table 3). The thermal induced decomposition of NaHCO3 starts at temperatures over 65 °C to form Na_2CO_3, H_2O and CO_2. In this temperature range above 65 °C to 80 °C, the structure is almost

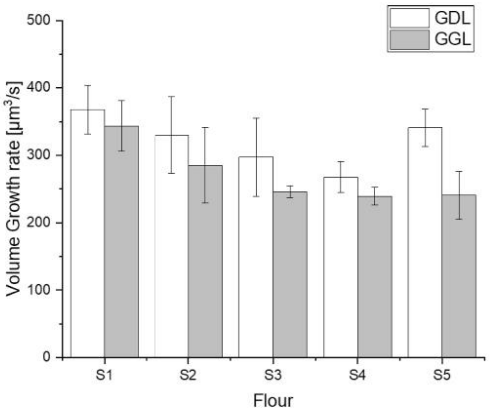

Fig. 5. Volume Growth rate of the rheometer measuring gap between upper and lower geometry of all wheat flour types for glucono-delta-lactone and galactono-gamma-lactone samples during the rheo-baking procedure (n = 3).

Table 4
Growth rate for both acidifying agents from start to end of the thermally induced growth phase.

Flour	Growth rate [µm/°C]	
	D-(+)-Glucono-1,5-lacton (GDL)	L-Galactono-1,4-lacton (GGL)
S1	10.59 ± 1.05	9.31 ± 1.02
S2	9.50 ± 1.64	7.72 ± 1.53
S3	8.56 ± 1.68	6.66 ± 0.24
S4	7.71 ± 0.66	6.48 ± 0.35
S5	9.83 ± 0.81	6.51 ± 0.96

completely solidified due to gelatinization of the starch and denaturation of the proteins, and the solid crumb also stops further thermal gas expansion from this point on. In classical baking experiments, the volume of a baked good decreases slightly after reaching a maximum due to water evaporation and shrinkage, which agrees well with the observed results (Shittu et al., 2007).

In Fig. 5 the growth rate of the rheometer gap for all flours with sodium bicarbonate and the two acidifying agents is shown. The growth rate showed for both acidifying agents a dependency of the protein content of the flour. Only S5 showed for the GDL a higher growth rate than expected. Statistical analysis showed no significant differences (p = 0.05) between the GGL and GDL samples, except for S5. The GGL in comparison to the GDL produced the carbon dioxide slower in terms of µm/s and therefore caused a slower growth of the dough during foaming and baking. The temperature-dependent growth rate was also higher in the GDL samples. (See growth rates in Table 4).

For gluten rich flours the achievable volume increase for GGL was not significant deviating (p = 0.05) from GDL (S1 and S2). For S3, S4 and S5 the GGL showed significant (p = 0.05) lower results from the GDL samples. The lower gluten containing flours may not be able to hold the very slow produced carbon dioxide and it mostly seems to diffuse to bubbles near the edges of the sample and then get released to the surroundings. GDL is reviewed in literature as one of the slowest acidifying agents in commercial use (Brodie & Godber, 2007; Chung, 2000; Neeharika et al., 2020; Vetter, 2003).The overall volume increase does not fit well with results from the yeast experiments because the heating rate of 4°/min (from conventional baking methods) may be too fast for the slow-reacting acidulants GDL (Hui & Sherkat, 2005; Vetter, 2003) and, as seen in the experiments, also GGL. The difference to deck oven experiments could also be explained by the more direct and fast heat

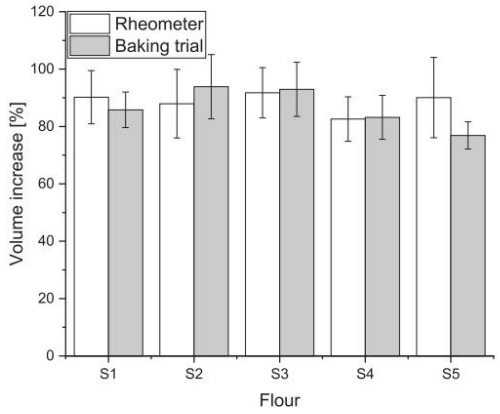

Fig. 6. Comparison of the total volume increase after baking the dough samples in a conventional deck oven and the rheo-baking procedure with Glucono-Delta-Lactone (n = 3).

transfer from the heating chamber to the sample (see Fig. 6).

The study showed that with some restrictions the GGL is a potential acidifying agent for leavening of baking goods. with regard to the slow reaction speed, it could be a possible application for cakes, donuts or chilled and preserved biscuit doughs, for which GDL is also used for (Miller, 2015). Additionally, a use in long leavening and longer dough resting processes like in other frozen baked goods could be a possible application.

4. Conclusion

To determine the baking quality of the used leavening agents a micro-scale baking trial in a conventional rheometer was investigated. Based on the rheo-kneading technique from Vidal et al. (2022) the crumb evolution and volume expansion of the dough produced with different leavening agents was examined. In comparison to established small scale kneading and baking methods, the used amount of flour and leavening agent was considerably reduced. Through direct comparison of standard baking trials with the results from the new micro-scale method, the method showed a high comparability. The rheo-kneading and rheo-baking procedure represents a useful tool to analyse the baking performance of flour as well as the functionality of additives such as leavening agents with a material expenditure of only 384 mg wheat flour.

The results of this study demonstrate that a reduction in the material used and a reduction in volume have no effect on the baking performance of *Saccharomyces cerevisiae*. The achieved volume of the baked dough was in the same manner for standard baking trials with 300 g loafs as wells as the small-scale trials with only 384 mg of flour in the

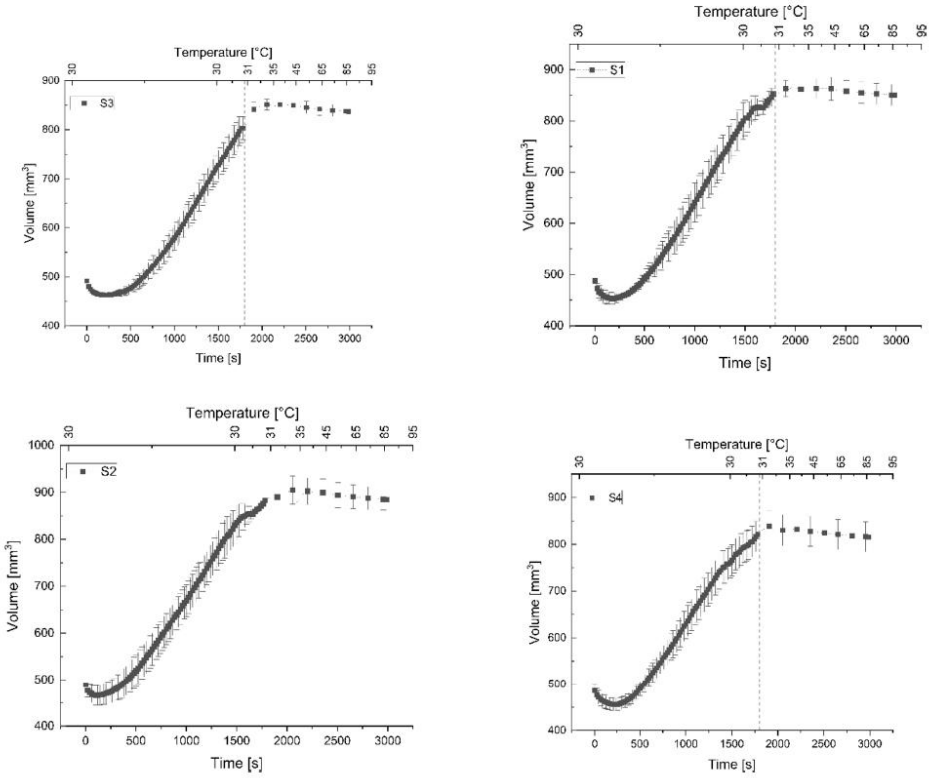

Fig. 7.

rheometer. The linear growth after an initial lag phase, comparable to results from literature (Chevallier et al., 2012; Hesso et al., 2015) could been observed. Also, the flour depending growth of the standard baking trials, based on the composition the flour, was in good correlation with the achieved volume expansion of the rheometer experiments. In the second part, the influence of two different acidifying agents of chemical leavened samples showed good comparability within each other. L-Galactono-1,4-Lactone (GGL), which was used here for the first time for this application, showed a volume expansion comparable to D-(+)-Glucono-1,5-Lactone (GDL), which is used in baking powders in the industry. Thereby, a dependency of the baking performance on the flour composition was more pronounced for flours with a lower gluten content then for the gluten rich samples. A slower growth rate was observed for flours low in gluten as well as a lower achievable total volume after baking. These observations lead to the conclusion that the amount of acidifying agent may need to be adapted to thegluten content of the used flour when the GGL is used. The microscale kneading and baking technique developed has shown that downscaling and baking in a rheometer can evaluate the baking performance of flours and investigating the influence of new leavening agents on a very small sample size.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

CRediT authorship contribution statement

Leonhard Maria Vidal: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Theresa Wittenkamp:** Investigation. **J. Philipp Benz:** Writing – review & editing. **Mario Jekle:** Conceptualization, Writing – review & editing. **Thomas Becker:** Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

None.

Appendix A

See Fig. 7.

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4.2.4 Structure Strengthening Phenomena of Gluten Matrices under Different Stress Types

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Structure Strengthening Phenomena of Gluten Matrices under Different Stress Types

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Abstract: To predict the achievable product volume with respect to the gas retention capacity of the gluten matrix in wheat flour doughs, strain hardening evaluation is crucial. But assessing these structure hardening phenomena in wheat flour dough systems is still a challenging task. In this work, a simple shear method applied to kneaded dough samples was tested and compared to biaxial extension tests performed with a lubricated squeezing flow method. The comparability of shear-induced structure hardening with biaxial extension tests was shown. Structure hardening and breakdown after overload were visualized using shear flow and a comparison of the obtained shear flow over Hencky strain curve peaks. To predict the behavior of the analyzed flours according to their composition, a correlation analysis of the flour and dough properties was performed. The influence of the HMW glutenin subunits on the sensitivity of the dough matrix according to the applied shear speed (0.1 and 1.0 mm/s) could be shown with a correlation coefficient of 0.94. The LMW glutenin subunits, on the other hand, showed a high correlation coefficient of 0.89 with the achievable network connectivity parameter z [-] gained from frequency sweep testing.

Keywords: strain hardening; dough; wheat flour; baking performance; rheology; dough; rheology; strain hardening; gluten network; wheat flour

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1. Introduction

Strain hardening is a complex phenomenon that occurs in wheat flour dough as it is subjected to mechanical forces, such as mixing or kneading. The physical and chemical changes that take place during this process are critical to the development of the dough's structure, as well as its ability to retain gas and water. Mixing wheat flour or gluten–starch blends with water leads to a unique viscoelastic matter. The evolving dough owes these viscoelastic properties to its crosslinked protein network and enables a variety of food applications [1]. In the beginning of the dough kneading procedure, the mechanical energy input together with the hydration of the flour/gluten particles causes the crosslinking of glutenins and gliadins into a continuous protein network with sizes between 150 kDa and 1500 kDa [2]. One key reaction is the oxidation of sulfhydryl groups on the gluten proteins, which leads to the formation of disulfide bonds between adjacent protein strands. These bonds contribute to the dough's strength and elasticity, as they support the protein network interconnections. This network is known as the continuous gluten phase [3,4]. Both during kneading and further processing of the dough, strain hardening can be observed when the dough is subjected to a constant load. Strain hardening occurs if a sample is subjected to an increasing strain at a constant strain rate. This phenomenon is defined as when the stress required to deform a material increases to more than the resulting strain at constant deformation [5]. Since it was found that strain hardening in dough is strongly affected by the gluten network and responsible for the gas retention capacity, extensional or biaxial compression tests were implemented to evaluate these effects for dough systems [6–9]. Regarding the time- and load-dependent behavior of wheat flour

doughs, an increasing resistance to shear stress can also be observed. If we consider the structural changes in the dough under a given load, several changes could be responsible for strain hardening: Firstly, the entangled polymer strands are orientated in load direction and accumulate closer together [10]. This results in more hydrogen and van der Waals-type bonds between the strands. Secondly, the agglomerated gliadins break down to primary particles, which become entrapped in the crosslinked glutenin strands. Interaction with the strands creates a more interconnected network. Additionally, the starch–starch and starch–gluten interactions are slightly deformed and store some of the applied load [11]. Until the maximum achievable particle deformation is reached, the strain can increase, and with higher loads, the resistance of the dough also increases. Meerts et al. stated that as the load increases, the short-range gluten–starch and starch–starch interactions in the dough begin to break down to the point where eventually only the long-range gluten–gluten interactions remain to provide structural integrity to the material [12,13]. Especially when considering the reversible entropy–elastic behavior of entangled polymers, wheat flour dough can be compared with other polymeric networks when a load is applied, and strain hardening occurs [14]. The breaking, or the shift, of molecular bonds under load is a widespread polymeric behavior. This process results in an increase in the material's shear modulus and tensile strength, as well as a decrease in its extensibility at break. Regarding this assumption of comparability, a definition of a strain hardening index (SHI) similar to an equation for polypropylene blends was defined for extensional stress and was experimentally validated [15]:

$$SHI = \frac{\eta_e^+(\varepsilon_{max})}{\eta_{e0}^+(\varepsilon_{max})} \quad (1)$$

where the maximum strain is ε_{max} and $\eta_e^+(\varepsilon_{max})$ represents the actual value of the transient extensional viscosity at the maximum strain, and $\eta_{e0}^+(\varepsilon_{max})$ is the value of the extrapolated LVE at maximum strain. This index reaches a maximum for kneaded doughs close to the optimum development stage [13]. At the optimum development stage, the network best withstands the applied deformations of the kneader geometry [16]. Since the kneading process is not only based on extension, shear forces also play a decisive role in network development during dough kneading [17]. To bridge the gap between strain hardening evaluation and shear-induced matrix hardening, shear flow and stress–strain curve evaluation were investigated for their predictive quality in the hardening and baking performance of wheat flours. In addition to the evaluation of the predictive capabilities of the calculated strain hardening indices, flour composition in the form of Osborne fractions and dough properties such as dough development time or the microscopically analyzed total number of protein junctions in the matrix are considered.

The aim of this study was to evaluate the value of a shear-only structure hardening test to describe gluten-phase strengthening under different load types. Also, a principal component analysis of flour, dough and bread properties was conducted to determine the accuracy of shear testing and classical analysis of the network attributes. The evaluated shear-stress-induced structure strengthening testing method could be an additional tool to evaluate flour and dough properties in line with a conventional rheometer. With respect to the previous work of Vidal et al. [16,18,19], microstructural evolution and flour properties were linked with dough processing behavior. Taking a broad overview of baking-performance-determining attributes into account, correlations between these flour and dough properties were revealed. The evaluated shear-stress-induced structure strengthening testing method provides a precise tool with which to evaluate flour and dough properties in line with a conventional rheometer.

2. Materials and Methods

For all experiments, commercial wheat flours type 0 and type 00 provided by Rieper AG, Vintl, Italy were used. The composition (see Table 1) was previously analyzed [16,18,19].

Table 1. Flour samples and their protein content [18,19].

Flour	S1	S2	S3	S4	S5
Protein (g/100g)	14.86 ± 0.06	14.29 ± 0.03	14.22 ± 0.01	11.79 ± 0.03	11.77 ± 0.05
ω5-Gliadin	3.9	3.6	3.9	3.9	3.3
ω1,2-Gliadin	3.8	3.8	4	3.9	3.5
α-Gliadin	31.2	33	32.2	32.3	29.6
γ-Gliadin	20.8	20.4	20.6	22.1	21.4
HMW-GS	8.6	8.6	8.1	5.9	6.6
LMW-GS	19.9	19.3	18.7	17.5	19

For the shear rheological measurements of dough in the MCR502 (Anton Paar, Ostfildern, Germany), a specific amount of each flour (corrected to 14% moisture) and demineralized water were kneaded at 63 rpm using a z kneader, DoughLAB (Perten Instruments AB, Hägersten, Sweden) equipped with a 50 g mixing bowl [18]. For the lubricated squeezing flow (LSF) measurements on a TA.XT.Plus with a 50 kg load cell (Stable Microsystems, Godalming, UK), a 300 g mixing bowl with adapted flour weight and the maximum water dosage was used. In the MCR502, a 4 g sample piece was compressed to a 2 mm gap size according to the lower and upper rheometer geometry (serrated plates, Ø 25 mm), the dough overhang was removed with a scalpel and the edges were covered with paraffin oil to prevent drying. The measuring environment during the shear rheological experiments was set to 30 °C and 80% RH in the MCR502. For the LSF measurements, the temperature was set to 30 °C with no humidity control.

2.1. Strain Hardening Index from Biaxial Extension

In order to calculate the biaxial strain ε_b (1) and biaxial strain rate $\dot{\varepsilon}_b$ (2) according to Chatraei et al. [20], the dough samples were placed between two plates 45 mm in diameter in a texture analyzer (TA.XT.Plus, Stable Microsystems, Godalming, UK) equipped with a 50 kg load cell.

$$\varepsilon_b = -\frac{1}{2} \cdot ln\left(\frac{h(t)}{h_0}\right) \tag{2}$$

$$\dot{\varepsilon}_b = \frac{v}{2h(t)} \tag{3}$$

In (1) and (2), h_0 and h_t are the initial sample thickness and the thickness at time t with v as the compression speed. The samples, having the same diameter as the two plates, were compressed from their initial height of 20 mm to 2 mm at displacement speeds of 0.1, 1, 2, 5 and 10 mm/s. To obtain the strain hardening index as proposed by [21–23], the force F, determined from the measurements, can be converted into the stress σ using the area given by the specimen geometry A (as $\pi * r^2$, where r is the diameter of the sample) and the following equation.

$$\sigma = \frac{F}{A} \tag{4}$$

For the given deformations of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0, the stress values were extracted for each measurement at the five displacement speeds. This was achieved by plotting these stress values against the biaxial strain rate on a double logarithmic scale. The deformation data were then fitted with a linear model. Using this regression model, stress values were calculated for specific values of $\dot{\varepsilon}_b$ (0.1, 1.0) and plotted against deformation on

a logarithmic scale. The slope of the linear fit of the plotted data is defined as the strain hardening index (SHI) expressed by Equation (4) [21,23]:

$$SHI = \left(\frac{\delta \ln(\sigma)}{\delta \varepsilon_h}\right)_{\dot{\varepsilon}_h = const.} \quad (5)$$

2.2. Strain Hardening from Shear

By fitting the stress–strain curve obtained from shear testing the dough samples in the MCR502 with shear speeds of 0.1 and 1.0 mm/s, the Equation (5) was utilized to calculate the strain hardening exponent [24]:

$$\sigma = K * exp(n * \varepsilon) \quad (6)$$

where n stands for the strain hardening exponent and K for the strength coefficient. Both can be calculated by fitting the empirical exponential equation, in which σ is the measured stress and ε the calculated Hencky strain. The Hencky strain from shear can be calculated with Equation (6), taking into account the shear rate $\dot{\gamma}$, which was set in additional experiments to fixed values of 0.1 and 1.0 s^{-1}, and test time t [25]:

$$\varepsilon_h = \frac{1}{2} \ln\left(1 + \frac{\dot{\gamma}^2 t^2}{2} + \dot{\gamma} t \sqrt{1 + \frac{1}{4}\dot{\gamma}^2 t^2}\right) \quad (7)$$

Following Tanner et al. [26], one can find the viscometrical functions for shearing with the shear rate $\dot{\gamma}$ beginning at $t = 0$, with the same form of shear rate and strain dependence as seen in elongation [27]:

$$\tau = \frac{G(1)}{1-p} \dot{\gamma}^p (\gamma)^{1-p} \quad (8)$$

where $\gamma = \dot{\gamma} * t$ and p is a constant. From oscillation measurements, it is also known that $G(t)$ is the relaxation function and $\dot{\gamma}$ is the shear rate. It is known that the form of $G(t)$ therefore corresponds to (8) with the constant p [28]:

$$G(t) = G(1) * t^{-p} \quad (9)$$

By calculating the shear flow (9) by fitting the stress curve with the calculated constant p and the strain rate, the determination of structure breakdown at the peak can be visualized:

$$Shear\ Flow = \frac{\tau}{\dot{\gamma}^p} \quad (10)$$

2.3. Statistical Analysis

Statistical evaluation was performed with OriginPro2022b (OriginLab, Northampton, MA, USA) using Pearson correlations and one-way and two-way analysis of variance (ANOVA) with Tukey's post hoc test at a level of significance of $p < 0.05$.

3. Results and Discussion

Strain hardening indices can be calculated using different methods. Different methods may lead to different results, as the prerequisites are not always fulfilled, and difficulties in the accurate determination of strain and stress, variable strain rates and the use of different equations might arise [5,24]. As the strain hardening index is dependent on the method used, we focused on the comparison of biaxial extension and shear flow for the Results section. The results were then analyzed according to their correlations with previously analyzed flour and dough properties.

3.1. Biaxial Extension

Under constant load, wheat flour dough exhibits strain hardening as well as strain rate hardening. Previous works underlined the influence of the strain rate, as it affects the behavior of the entangled coupling of large glutenin molecules at the molecular level [24,29]. The SHI is therefore dependent on the strain rate, which can be seen in Figure 1. The higher extension speed revealed that for faster deformation, the lower-protein-containing flours showed comparable hardening. No significant differences were found between S3 and S5 for the extension speed of 1 mm/s. For the slower extension speed, the influence of the protein content was stronger. Less strong and less interconnected protein strands (in the lower-protein-containing flours) were not able to align in the same manner as the higher-protein samples in the direction of the applied stress to form new bonds or to orientate along the direction of deformation. With respect to the protein contents in this work and considering the trend of a lower ratio of higher speed to lower speed Shi, the lower-protein-containing flours may lead to a reverse ratio between SHI for slow and higher biaxial extensions [30].

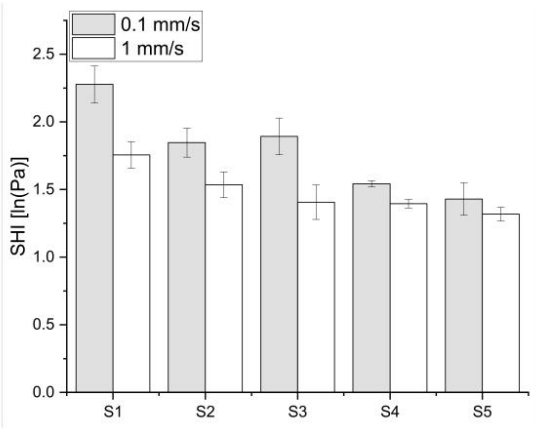

Figure 1. Strain hardening indices (SHIs) for five kneaded dough samples. The SHI is determined via compression tests and the resulting biaxial extension. Results are shown for the respective 0.1 and 1 mm/s elongation rate $\dot{\varepsilon}_b$. (n = 3).

3.2. Shear Experiments

3.2.1. Dependency of the Strain Hardening Exponent on Deformation Speed

As shown for biaxial extension in Figure 1, as well as for the shear load experiments in Figure 2, the lower deformation speed showed higher stiffening. This may be caused by the internal relaxation processes, which allow the material to adapt to the applied load and rearrange the protein strands during their deformation in the shear direction [31]. At a higher speed, the structure may become damaged even before the alignment can take place properly. For the shear load experiments, the higher-protein-containing flours (S1–S3) showed comparable behavior for the faster deformation rate. Therefore, the higher protein content enabled the dough matrix to withstand deformation better and led to a stronger network. The reason for this behavior may be that stronger flours and the resulting stronger doughs tend to contain more HMWs [19]. Therefore, the strength of the secondary forces is higher due to the larger chains, and these doughs need higher shearing to stretch the gluten molecules to the maximum and cause bond scission or the opening of entanglements [32].

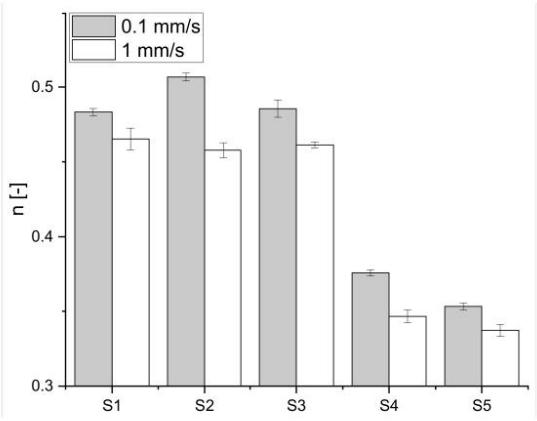

Figure 2. Strain hardening exponent, n, obtained via power-law-fitting the results of the stress–strain curves for five flour samples sheared in a rheometer at deformation speeds of 0.1 and 1 mm/s. (n = 3).

3.2.2. Increase in the Shear Flow Depending on Hencky Strain and Flour Composition

In Figure 3, the shear flow curves calculated from the simple shear load test showed a dependence on the flour composition as well as the applied shear rate. The peak of the curve, determining the structural breakdown of the sample, shifted to lower Hencky strains but higher shear flow values with decreasing protein content. This can be explained by the fact that with increasing strain, the short-range gluten–starch and starch–starch interactions in dough start to break down. This breakdown may happen to such an extent that only the longer-range gluten–gluten interactions remain to provide structural integrity [12,13]. On the other hand, for higher-protein-containing flours, the difference in the possibility of withstanding these deformations and the adaption to the applied load through structure strengthening is not as pronounced as for weaker flours. As mentioned before, the secondary forces interconnecting the protein strands may be more pronounced due to the higher HMW content and therefore larger chains. Comparing the two methods analyzed in this work, the shear method was able to identify the gas retention capability of the flour samples in the same manner as the biaxial extension. For the lower shear rate of 0.1 s^{-1}, the shear flow evaluation method was even more precise in connecting the achievable product volume with the protein content (see Table 2) with a correlation coefficient of 0.84.

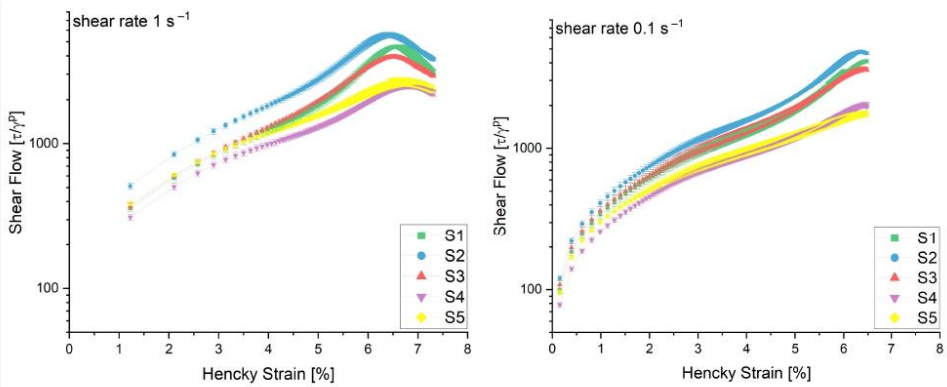

Figure 3. Calculated shear flow over the Hencky strain for five flour samples at a shear rate of 1.0 and 0.1 s^{-1}. (n = 3).

Table 2. Correlation coefficient of linear correlations between dough process parameters and Osborne fractions of the analyzed flours. Significant correlations are marked in red color ($p < 0.05$).

	DDT	Network Connectivity	TNoJ	Peak Shear Flow 0.1 1/s	Peak Shear Flow 1 1/s	K 0.1 mm/s	n 0.1 mm/s	K 1 mm/s	n 1 mm/s	SHI 0.1 mm/s	SHI 1 mm/s	Gliadine	Glutenine	Albumine /Globuline	Omega 5	Omega 1,2	Alpha	Gamma	Omega b	HMW	LMW	Volume Increase in Baking Trials
DDT	1																					
Network Connectivity	0.19	1																				
TNoJ	0.90	0.24	1																			
Peak Shear Flow 0.1 1/s	0.63	0.35	0.88	1																		
Peak Shear Flow 1 1/s	0.54	0.48	0.82	0.98	1																	
K 0.1 mm/s	−0.43	0.05	−0.71	−0.88	−0.82	1																
n 0.1 mm/s	0.77	0.18	0.85	0.86	0.79	−0.84	1															
K 1 mm/s	−0.94	−0.07	−0.94	−0.78	−0.68	0.70	−0.92	1														
n 1 mm/s	0.84	0.30	0.98	0.95	0.90	−0.80	0.91	−0.92	1													
SHI 0.1 mm/s	0.90	0.02	0.90	0.78	0.67	−0.75	0.95	−0.99	0.90	1												
SHI 1 mm/s	0.51	0.12	0.70	0.87	0.82	−0.94	0.94	−0.75	0.81	0.81	1											
Gliadine	−0.06	−0.77	0.09	0.21	0.09	−0.63	0.32	−0.23	0.14	0.32	0.49	1										
Glutenine	0.76	0.69	0.87	0.86	0.87	−0.53	0.75	−0.74	0.89	0.69	0.62	−0.30	1									
Albumine/Globuline	−0.67	−0.09	−0.88	−0.95	−0.89	0.96	−0.93	0.86	−0.94	−0.88	−0.94	−0.45	−0.72	1								
Omega 5	0.51	−0.60	0.49	0.39	0.23	−0.67	0.68	−0.70	0.49	0.76	0.65	0.80	0.08	−0.65	1							
Omega 1,2	0.48	−0.68	0.54	0.39	0.23	−0.61	0.48	−0.62	0.48	0.65	0.44	0.76	0.05	−0.61	0.87	1						
Alpha	0.10	−0.46	0.40	0.58	0.51	−0.87	0.51	−0.40	0.46	0.47	0.69	0.88	0.09	−0.72	0.69	0.75	1					
Gamma	−0.65	−0.50	−0.87	−0.88	−0.89	0.59	−0.60	0.65	−0.87	−0.59	−0.53	0.15	−0.90	0.74	−0.06	−0.25	−0.30	1				
Omega b	0.96	0.25	0.97	0.82	0.74	−0.64	0.89	−0.98	0.95	0.95	0.70	0.05	0.85	−0.84	0.54	0.50	0.29	−0.77	1			
HMW	0.78	0.52	0.94	0.94	0.94	−0.69	0.81	−0.81	0.97	0.78	0.72	−0.08	0.97	−0.85	0.24	0.27	0.31	−0.94	0.90	1		
LMW	0.61	0.89	0.64	0.63	0.69	−0.23	0.54	−0.52	0.67	0.46	0.39	−0.60	0.93	−0.44	−0.22	−0.30	−0.26	−0.74	0.66	0.81	1	
Volume Increase in Baking Trials	0.49	−0.13	0.78	0.84	0.77	−0.88	0.89	−0.66	0.65	0.66	0.70	0.53	0.52	−0.88	0.55	0.74	0.83	−0.75	0.65	0.71	0.18	1

3.3. Correlation Analysis of Flour and Dough Parameters

To take all the analyzed parameters of the flours used in this work along with the previous works of Vidal et al. into account, a correlation analysis was carried out. Table 2 lists the correlation coefficients. The red-marked coefficients correlated with a level of significance of $p < 0.05$. The results show that the dough development time (DDT) had a strong correlation of 0.90 with the total number of junctions (TNoJ) as determined via microscopic analysis. This can be explained by the fact that more contained protein, and in the case of the analyzed flours, also more high-molecular-weight subunits (HMWs) can lead to more intermolecular bonds between protein strands [33,34]. As a result, a high correlation of 0.94 between the HMW and the TNoJ could be observed. The TNoJ also had a strong correlation of 0.88 with the maximum peak of the shear flow curve occurring at the 0.1 mm/s shear speed. This peak represents the structure breakdown point of the matrix and is also strongly correlated with the HMWs for both shear speeds. In addition to the peak of shear flow, the TNoJ correlates strongly (0.90) with the SHI calculated for the slower biaxial extension speed of 0.1 mm/s. For the faster shear speed of 1.0 mm/s, the correlation with the TNoJ was even higher at 0.98. With respect to this, one can clearly see that the strongly crosslinked proteins play a pivotal role during structure strengthening as well as during network breakdown. On the other hand, the low-molecular-weight subunits (LMWs) showed a high correlation of 0.89 with the achievable network connectivity z [-] at the optimum development stage of the network evolution of the kneaded dough samples. Therefore, LMWs may be a crucial binding partner for HMWs within the network in which the matrix best withstands the deformation applied by the kneader geometry. Until the optimum development stage, HMWs do not play a significant role due to their lower molecular mobility, which is caused by bigger and more extensive intermolecular disulfide bonds [33]. These bonds reduce the mobility of the HMW, and therefore, the influence on network connectivity, z, is mainly based on the LMW at this stage of dough development. The Albumine and Globuline content showed strong negative correlations with the calculated exponent n at speeds of 0.1 and 1.0 mm/s. This can be explained by the viscosifying nature of these subunits due to their solubility in water and their ability to form polymeric connections with other proteins, entrapping water in the matrix [35,36]. The Omega b subunits showed strong positive correlations with the exponent n as well as the HMW at 1 mm/s. The influence of the HMW on the exponent n may be explained by the strong disulfide bindings of the gluten network and could be caused by the low molecular mobility of these molecules, as mentioned before. In a direct comparison of the biaxial extension and shear-dependent structure hardening phenomena, the SHI and the exponent n showed, at both deformation speeds, high correlations between 0.81 and 0.95. With these findings, the comparability of the shear-induced structure hardening evaluation with other testing methods was demonstrated, and the shear setup was determined to be even more sensitive to the protein contents of the flour samples. Compared to the volume increase in classical baking trials, the shear method using the slower shear rate of 0.1 s^{-1} showed strong correlations of 0.84 for the calculated shear flow peak and 0.89 for the strain hardening exponent n. The biaxial extension method was not able to determine the baking performance of the flour samples as precisely as the shear method and showed no such strong correlations.

4. Conclusions

By applying constant shear load on wheat flour dough samples with a rheometer, the strain hardening induced by shear load was directly comparable to biaxial extension. Based on fundamental rheology, structure hardening could be observed in the same manner as under the biaxial extension applied. Therefore, the shear flow calculation and the power law fitting of shear-induced stress–strain curves represent a useful method for determining the quality parameters of wheat flours. The controlled energy input together with the small sample size and limited sample handling provides a good alternative to lubricated squeezing flow methods. For the shear flow calculations, the dependency of

the protein content (especially HMWs), with a negative correlation of up to −0.95 with the albumin/globuline content of the peak of the curve achieved, and the suitability of the shear rheological measurement to obtain flour/dough strength under load could be demonstrated. With respect to previous works, the findings of this work show strong correlations between flour attributes such as dough development time (DDT) and total number of junctions (TNoJ). The developed dough matrix demonstrated the strongest correlation of structure strengthening under shear with the total number of junctions. For the biaxial extension, the same magnitude of correlation could be observed for TNoJ as well as for the DDT at a lower extension rate of 0.1 mm/s. In a direct comparison of simple shear (calculated as shear flow peak) to biaxial compression SHI, the 0.1 mm/s as well as the 1.0 mm/s shear and extension rates showed strong (0.87 and 0.82) and medium (0.78 and 0.67) correlations with the same deformation speeds. This underlines the applicability of the setup to further investigate dough strengthening under shear load. Since results were obtained only for a small batch of commercial wheat flours, the applicability of the shear-induced structure strengthening testing method must be further investigated, especially for lower-protein-containing flours. Since the total amount of protein in commercial flours may be trending downwards due to limitations in fertilization or precipitation, a precise and simple-to-use technique to predict the gas retention capacity of wheat flour doughs, and therefore the baking characteristics of the matrix, is needed to maintain high product quality and consumer acceptance of baked goods.

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5 Discussion and conclusion

The currently established methods for assessing dough development and evaluating the properties of the gluten network and matrix lack accurate prediction of these complex processes. Furthermore, they are based on highly unpredictable kneading movements and the resulting different energy input into the kneading mass. Therefore, there is a need to revise and further develop established and new methods to overcome these problems. In the first and second publication of this study, an inline shear-kneading and measuring system in a conventional rheometer is developed and compared with classical z-kneaders (doughLAB®). By combining CLSM and the shear kneading technique, the achievable rheological dough properties can be directly linked to the development and properties of the adhesive network. In the third part, the process line of classic baked goods, containing kneading, foaming, and baking, is reduced to the microscale of the shear-kneading system. Thereby, the foaming and baking behavior of different protein compositions in flours is elucidated. In the fourth part the strain hardening behavior of the dough matrix, responsible for dough development and gas retention, under different load types is discussed.

The development of gluten networks under simple shear load depends on the applied magnitude and duration of the load [12], [52], [74]. Regarding using simple shear to develop a gluten network and to knead a wheat flour water dough, the following should be noted: The literature and previously investigated simple shear methods without normal stress and shear direction change lead to the formation of a gluten network, but also to the orientation of the matrix in shear direction. With ongoing kneading, the dough matrix split up into a starch-rich and a protein-rich section in the measuring gap and no continuous uniform network developed. Additionally, centrifugal forces limited the applicable rotation speed, and a special rotating geometry in these setups needed to be developed [75] to prevent the processed matter from getting pushed out by centrifugal force. But plate-plate geometries were found preferrable, as they restrict the mobility of flour particles less compared to cone geometries in the rheometer [76]. It was also shown in literature that resting times are present in classical kneaders during rigid body motions [77], [78]. These resting times were determined to be crucial for dough development, and therefore, the rigid body motions in classical kneaders are key for successful and full network development. Thus, splitting up the kneading

process into kneading and resting periods would be more suitable, as this could lead to a fully developed network. To implement these kneading and resting periods, the previous works relied on stress-relaxation tests with alternating shear direction. The investigation of the dough development was then carried out by calculating relaxation spectra for each kneading step and comparing them to classical kneaded dough spectra, which are already known [52], [79]. But the application of stress-relaxation measurements in consecutive steps, and the evaluation of these spectra lead to a highly complex computation of these data. This led also to the demand for complex user knowledge to interpret the data resulting in limited applicability for general usage. To overcome this issue, the first research question of this thesis needed to be answered:

How can wheat flour and water be mixed and kneaded in a conventional rheometer to form a fully developed gluten matrix with a simple analysis of the network evolution?

For this purpose, the shear rate and duration of the load were applied stepwise as well as split up in positive and negative shear direction in a conventional rheometer. The influence of commercially available rheometer geometries was analyzed regarding their suitability to transmit the forces to the kneaded matter. The first assumption that crosshatched serrated plates would prevent the flour water slurry from slipping and maintaining contact with the rheometer geometry to obtain valid rheological data was given by Tietze et al. [76]. However, the experiments showed that the contact area in the geometric cavities was filled with flour, which then was not hydrated and, therefore, did not form a continuous dough matrix:

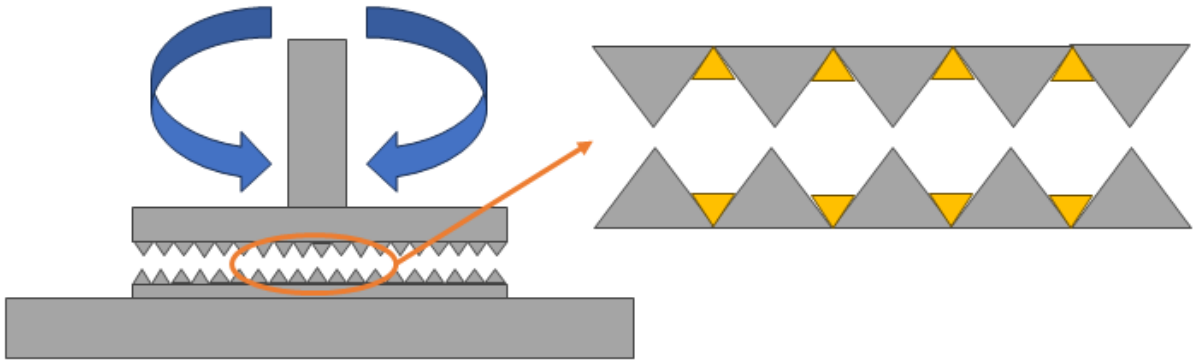

Figure 5: Flour deposited in the crosshatched serrated geometry cavities, which is not subjected to shear or other forces and not hydrated during the mixing and kneading process.

This finding led to the choice of smooth plate-plate geometry to obtain full hydration and mixing of the flour-water slurry in the measuring gap. Between the shear direction changes, the matter was allowed to rest for 4 s with no applied load to allow the gluten matrix to relax and the form new bonds after orientation and entanglement of the protein structures [80]. With an applied shear speed of 3.75 rpm in the positive movement the rotational speed was just underneath the determined speed limit at which the kneaded matter got pushed to the edges of the geometry by centrifugal force. Additionally, the measuring gap was set to 650 µm to hinder material transport by compression by the plates out of the measuring gap in the plate-plate setup of the rheometer. Since starch particles have sizes up to 40 µm [20], [21], [22] and the recommendation for setting the measuring gap between the plate-plate geometries should be ten times higher than the biggest contained particles, the chosen gap size fulfills this prerequisite. For rheological investigation of the network development in the dough matrix, frequency testing is an established and widely investigated tool. In this application, the applied amplitude must be within the limit of the visco-elastic region (LVR) to maintain the structural integrity of the matter [81]. With a base strain amplitude of 0.05 %, the deformation of the sample was within this range. A classical frequency test takes up several minutes to cover the necessary range for calculation of the material parameters. In the evaluation of the shear-kneading technique in the first publication [49] a so called multiwave frequency measurement was utilized. Thereby, multiwave rheology is an advanced technique in rheological analysis, involving the application of multiple oscillatory shear waves simultaneously to a sample. This approach allows for the study of complex viscoelastic properties with short measuring times. The basic principle is to superimpose several sinusoidal shear strains or stresses, each with different frequencies. The formula for a multiwave strain input, for instance, can be expressed as:

$$\gamma(t) = \sum_{i=1}^{n} \gamma_i \sin(\omega_i t + \phi_i) \tag{12}$$

where γ(t) is the total strain at time t, γ_i is the strain amplitude, ω_i is the angular frequency, and ϕ_i is the phase angle of each wave component. The response of the material, typically in the form of stress, is then analyzed to understand its complex behavior in response to the imposed strains. The stress response, σ(t), is similarly a sum of sinusoidal components:

$$\sigma(t) = \sum_{i=1}^{n} \sigma_i \sin(\omega_i t + \delta_i) \quad (13)$$

Here, σ_i is the stress amplitude, and δ_i is the phase difference for each frequency component.

In multiwave rheology, the complex viscoelastic parameters, such as storage modulus (G') and loss modulus (G''), are determined for each frequency. This method provided a more comprehensive understanding of the material's behavior under realistic, variable conditions it may encounter in practical applications. The technique is particularly useful in studying complex fluids like polymers, gels, and biological materials, where the viscoelastic properties are not adequately represented by simple linear models [82], [83], [84].

In the described experiments [49], the base frequency used was 1 Hz with 0.05 % amplitude. The harmonic frequencies, multiples of this fundamental frequency, ranged from 3 Hz to 10 Hz (ω_1 to ω_8), each maintaining the same amplitude of 0.05%. The cumulative peak amplitude reached was 0.115%. These frequencies and amplitudes were specifically selected to fall within the linear viscoelastic range suitable for analyzing dough networks [85], [86]. The evaluation of the dough development followed the application of the weak gel model for foods [87]:

$$|G^*| = A_f * \omega^{\frac{1}{z}} \quad (14)$$

where ω is the angular frequency [s^{-1}] and A_f refers to the network strength [Pa $s^{1/z}$]. In this calculation of the network parameters z refers to the network connectivity [–] [87], [88], [89], [90]. The results showed that the shear-kneaded sample also underwent the classical evolution of dough development:

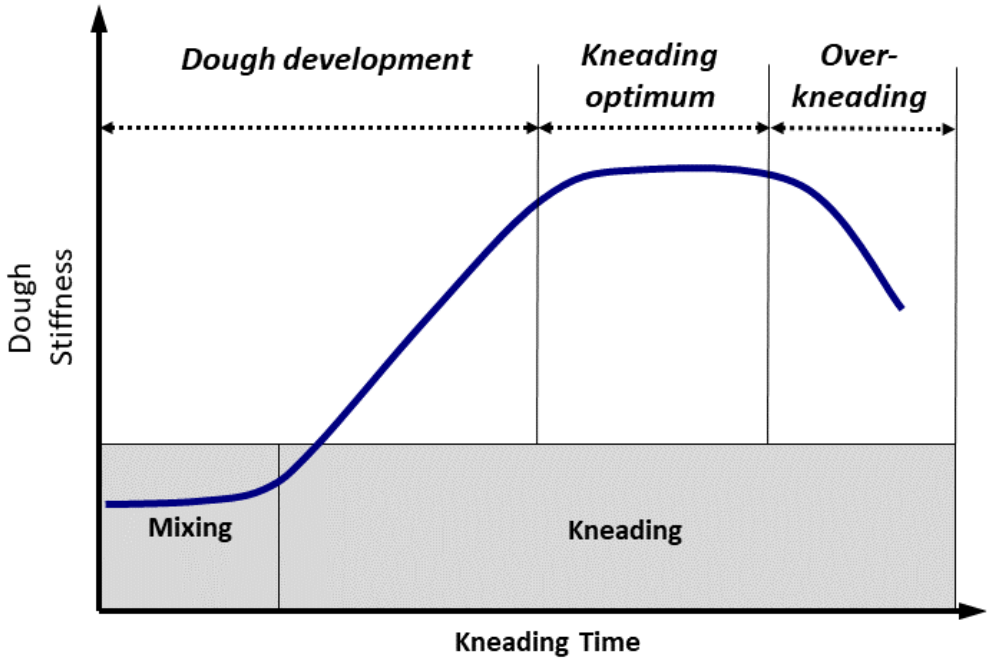

Figure 6: Repreentative mixograph curve to obtain the optimum stage of gluten network development.

The parameter z (see equation 13) showed an inverted progression compared to the classical mixograph curve of the used doughLAB® torque recording mixer. So, the network developed from an unconnected to a fully connected state. With ongoing shear-kneading the network structure got disrupted again, and the results showed a decrease of the parameter z. These findings lead to the second research question:
How can this network formation be inline validated on a rheological and optical basis?
To answer this question, a coupling device was developed together with Anton Paar, Ostfildern, Germany [73]. To monitor network development on a microscopic scale, literature gave the basis for the chosen setup [34], [91]. The staining of the contained proteins was achieved using a rhondamine B solution (Merck KGaA, Darmstadt, Germany, 0.01 g/100 mL water). However, offline analysis always had the limitation of sample transport and preparation before analysis. The mechanical load applied to the sample during the sample preparation might affect the results and cause manipulation of the network structure. In the coupled device of rheometer and CLSM, the samples were analyzed using a laser with a wavelength of 543 nm for excitation, the emission was detected at 590 nm with a 50 nm bypass filter inline during kneading. To further investigate the macroscopic behavior of kneaded dough samples the in-situ analysis gave deeper insight into mechanical driven dough development. Thereby, these macroscopic network properties are significantly influenced by the composition, spatial arrangements of the components, and types of bonds existing [92]. Implementing this

knowledge of macroscopic network properties, the developed setup gave the opportunity to directly link the rheological and microstructural characteristics of the dough at the same time. Besides the total number of junctions [73], regarding network development and the link to the network connectivity z, the branching rate of the samples showed a link between network formation on the rheological and microscopical side. The branching rate was calculated from the resulting data of AngioTool64 version 0.6a (National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA) as follows [93]:

$$Branching\ Rate = \frac{TNoJ}{A_{Protein}} \tag{15}$$

With the total number of Junctions (-) TNoJ, and the $A_{Protein}$ as the protein area (mm^2) in the CLSM picture. In Figure 7 the network evolution up to 125 % of the dough development time (DDT) is shown. Due to the buildup of the network, the linkage between the involved proteins raised with the ongoing energy input during classical (doughLAB®) and shear-kneading (Rheo-CLSM). As shown, the branching rate for the shear-kneaded samples was higher than the classical kneaded doughs, until they reached 125 % DDT. As mentioned above [73], the mechanical state of optimum development (100 % DDT) does not directly intersect with the highest crosslinked state of the network. At 100 % DDT, the interconnected protein strands best withstand the deformation of the kneader geometry applied to the sample. With ongoing kneading, these strands get thinned, and some interconnections break down to form a more fibrillary appearance. This breakdown is based on different processes, including disentanglement of the strands, chain orientation within the load direction, and bond rupture [94]. At this over-kneaded state, > 125 % DDT, the network breakdown increases the number of bigger protein clusters in the matrix [95]. Therefore, the branching rate goes down. With respect to the polymeric property of the dough matrix, changes during the whole kneading process from agglomerated flour and starch particles to a fully developed network and the destruction of it must be considered. During overkneading, an additional process occurs after reaching peak consistency. This process involves the formation of particle clusters/agglomerates due to bond breakdown. These clusters/agglomerates might enhance certain mechanical effects, such as the Payne effect, through their breakdown. [96]. Physically, the Payne effect

is due to alterations in the material's microstructure caused by deformation. Specifically, it involves the breaking and reforming of weak physical bonds that connect neighboring clusters of filler (starch) material [96], [97], [98]. The assumption that the polymeric structure of the dough network consists of the polymeric gluten phase and embedded starch particles as fillers and also shear-induced friction caused by these particles adds to the behavior of the matrix with ongoing kneading and disruption of the network.

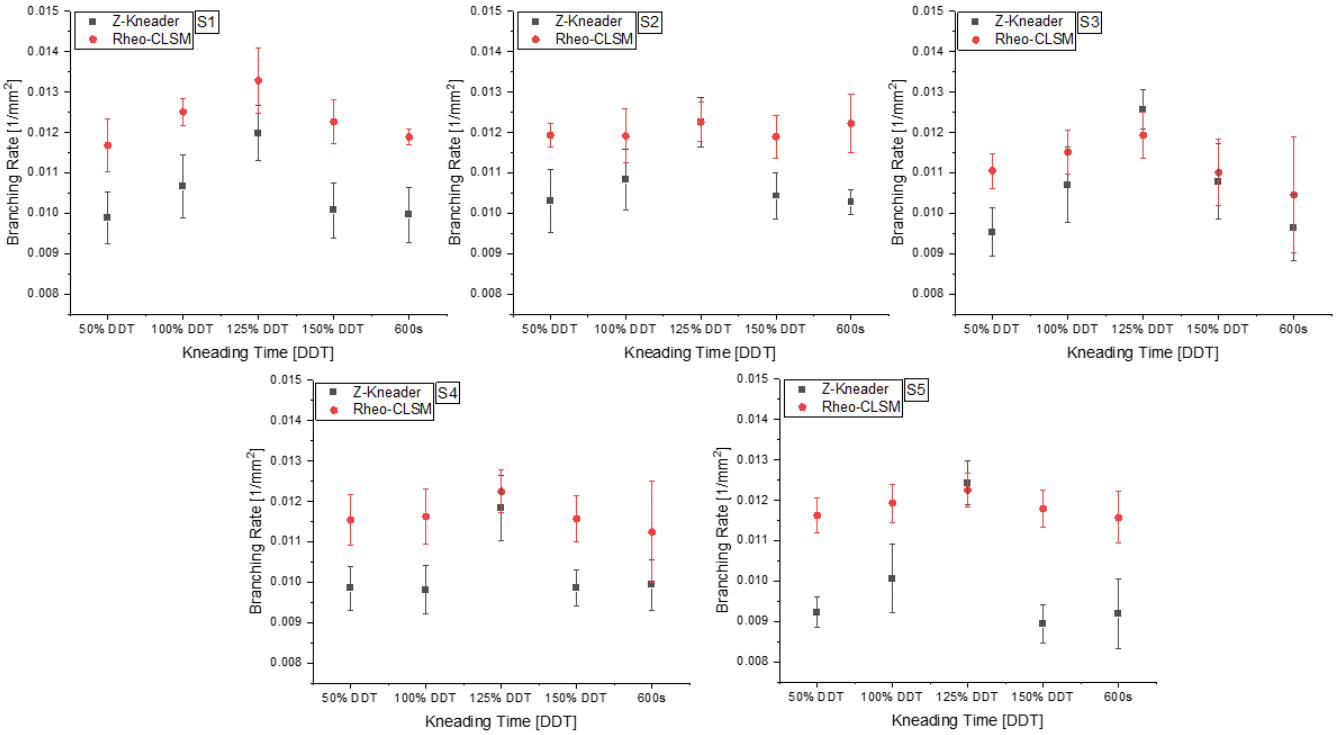

Figure 7: Branching rates for all flours S1 - S5 from the protein network analysis of the CLSM images taken from standard kneaded wheat dough and shear-kneaded samples. For each sample, three doughs were kneaded, and for each time, three images were taken and analyzed.

The direct linkage between the rheological behavior and the underlying microstructural properties of the dough network within one measuring device paves the way for further analyses. It gives the chance to include the impact of process conditions, like temperature or pH value, on naturally occurring and technically relevant network formation processes in the evolving dough matrix. This leads directly to a deeper understanding of wheat dough behavior. With the link between microstructural and mechanical properties of the shear-kneaded dough samples, further utilization of the developed setup led to the implementation of additional steps along the classical baking line. Therefore, the third scientific question arises:

Does the reduction of the sample size on a microscale influence the foaming and the baking properties of the kneaded samples?

State-of-the-art in baking trials to determine the achievable volume of the baked goods and to evaluate the gas retention capacity of the doughs classically rely on large amounts of flour and dough (300 – 1000 g). But there are also small-scale tests requiring a reduced sample size of 25 – 50 g of flour to be found in the literature [99], [100]. With a reduction of the sample size to 10 g based on AACC 10-52 [101] several studies showed the applicability of the small-scale trials for baking analysis of wheat flours [102], [103], [104], [105]. A further reduction to 2 g was investigated also by Beasley [106] and Uthayakumaran [107]. The results were reviewed by [108] with the conclusion that the micro-scale method established gave similar results to the standard methods, with the adaption of manual loaf shaping. In the third publication, even the prerequisite of manual interference within the dough processing to a baked good was overcome. Regarding achievable loaf volume and growth rates, the comparison of the micro-scale baking method was successful. The reduction of the sample size to the previously established 192 mg of flour [49] showed a good correlation between classical baking trials with 300 g dough samples and the micro-scale method. With the high impact of the surrounding environmental parameters on dough viscosity and yeast activity, the control of these is crucial for reproducibility during foaming and baking trials [109], [110]. With the implementation of these processing steps into the controlled environment of the climate chamber of the rheometer the volume growth showed a negative correlation parameter between the contained albumin/globulin content of the flours and the achievable volume of the baked samples. These proteins can inhibit the activity of certain enzymes involved in grain germination and sprouting, thereby helping to regulate the timing of seed germination and ensure optimal conditions for seedling growth and may vary in special breeds from seed producers to adapt the wheat plant to varying climate and growth conditions. Besides new breeds the method opened a wide field of possible research fields. With focus on the application of new leavening agents the micro-scale method opened the door for evaluation of the biological producible leavening acid Galactono-1,4-lactone (GGL). The production of it could be based on the spontaneous lactonization of biotechnologically produced L-galactonate by fungal-mediated biotransformation from galacturonic acid [111], [112]. GGL could be an alternative to D-(+)-Glucono-1,5-lactone (GDL) used in commercial baking powders [113]. As the composition of the flours varied not only on protein content but

also in the extractable Osborne fractions, a deeper dive into the mechanisms of gas retention capacity of the kneaded doughs was mandatory. Based on the mechanisms of strain-hardening within the gas cell surrounding dough matrix, the fourth scientific question of this work was formulated as follows:

How does the flour composition influence the dough properties, in particular the strain-hardening and gas holding capacity?

To understand the gas-holding capacity of wheat flour dough matrices, one needs to pay attention to the polymeric network properties of the matrix. The structure of the dough, therefore, contains the continuous gluten phase as the backbone, embedded starch particles as fillers, and small gas nuclei. While kneading the small gas nuclei, in form of entrapped air, are incorporated into the matrix. These nuclei are crucial for further bubble growth [114]. The bubble growth is limited by the magnitude of stress the gluten lamella around the bubble can withstand during expansion. Thereby, strain-hardening is a phenomenon that describes the strengthening of a metal or polymer by plastic deformation. Thereby, strain-hardening refers to the increased difficulty in deforming the material as the strain (or deformation) progresses while keeping the strain rate constant. This phenomenon is typically quantified using the relationship:

$$\frac{dln\,\sigma}{dln\,\varepsilon} > 1 \tag{16}$$

where σ represents the stress needed to deform the material and ε signifies the uniaxial strain. In this term, as material is stretched or strained, it becomes harder and requires more force to deform it further. For dough, the lamella separating two gas cells needs adequate extensibility to withstand the biaxial strain during expansion, thus preventing any tearing or breaking [115]. In the context of dough expansion, the dough surrounding a growing gas cell undergoes tangential stretching in two directions while being compressed radially. This type of deformation is known in rheology as biaxial extension. The biaxial strain, ε_b, which is the relative deformation experienced by the liquid dough adjacent to a gas cell, can be described accordingly [55], [116], [117]:

$$\varepsilon_b = \ln\left(\frac{r}{r_0}\right) \tag{17}$$

The equation involves r, representing the radius of the gas cell at a specific time t, and r_0, the initial radius. According to equation 17, the biaxial strain implies that with ongoing expansion, the strain constantly increases. To withstand these strains, the material needs to increase its stability over time and strengthen the matrix due to the underlying processes. In the fourth publication [56], these phenomena could not only

be shown in biaxial extension testing but also transferred to simple shear tests. With the calculation of the shear-flow, an equivalent measurement parameter was utilized to describe the structure strengthening of the matrix. For flours with lower protein content, the peak of the calculated shear flow, which indicates the beginning of structural breakdown of the sample, shifted towards lower Hencky strains but higher shear flow values. This shift can be attributed to the weakening of short-range gluten-starch and starch-starch interactions under increasing strain, leading to a breakdown of the matrix. Eventually, this process leaves only longer-range gluten-gluten interactions to maintain the dough's structural integrity [118], [119]. When comparing biaxial extension to shear-dependent structure hardening, both the Strain Hardening Index (SHI) and the strain hardening exponent n demonstrated high correlations, ranging from 0.81 to 0.95 ($p < 0.05$), in the experiments. These results established the effectiveness of using a shear setup for evaluating shear-induced structure hardening in comparison to other methods. Moreover, this approach proved to be more sensitive in detecting variations in the protein content of flour samples. The correlation analysis of the results from the publications one to four found strong correlations between various flour properties and dough behavior. Dough development time (DDT) showed a strong correlation with the total number of junctions (TNoJ) and high molecular weight subunits (HMWs). TNoJ also correlated with the maximum peak of the shear flow curve and strain hardening index (SHI) at different shear speeds. Low molecular weight subunits (LMWs) correlated with network connectivity. Negative correlations were observed with albumin/globulin content and the exponent n, reflecting the impact of protein subunits on dough properties [120], [121]. Thus, the shear-only investigation of flour and dough properties provides a realistic prediction of kneading, foaming, and baking behaviour. This approach can be regarded as an alternative to offline and multidevice flour and dough analysis, whose drawbacks were discussed previously. In addition, results are obtained faster and decoupled from manual intervention, in this way allowing for better reproducibility and independence from environmental factors. However, it is important to acknowledge the limitations of this approach. The reliance solely on shear-based metrics, while effective in specific contexts, may oversimplify the intricate physical and chemical processes occurring in the dough matrix. These interactions, which are critical to dough quality and performance, may require complementary analytical methods to ensure a holistic understanding. Additionally, the predictive capability of the shear-based investigation remains untested across a

broader range of flour types, dough formulations, and baking environments, raising questions about its generalizability and scalability for industrial applications. The integration of confocal laser scanning microscopy (CLSM) into this workflow, although a promising innovation, also demands further scrutiny. Its successful implementation in a real-time, inline setting will depend heavily on resolving potential technical challenges, such as ensuring consistent image quality in dynamic and heterogeneous dough systems, as well as managing the complexity and cost of such advanced equipment in routine production environments.

Building on the significant advancements presented in this study, the outlook for future research and applications in dough development and analysis is both promising and expansive. The development of an inline shear-kneading and measuring system, along with the integration of CLSM for real-time analysis, marks a pivotal step forward in the field. However, future work should focus on addressing these limitations by expanding the method's scope, incorporating additional complementary techniques, and validating its applicability under diverse and practical conditions to maximize its industrial relevance and reliability. Exploring advanced real-time monitoring technologies beyond CLSM could provide deeper insights into the physicochemical changes occurring during dough development. Techniques such as Raman spectroscopy and X-ray diffraction offer potential for comprehensive analysis of molecular and crystallographic changes in the dough, complementing existing methodologies. The implementation of machine learning algorithms to analyze the vast amounts of data generated from these advanced monitoring techniques could significantly enhance predictive capabilities for dough development and baking outcomes. Identifying patterns and correlations between specific kneading actions, dough properties, and final product characteristics through machine learning could lead to optimized recipes and processes. With a growing emphasis on sustainability and dietary diversity, future research could explore the application of the developed methodologies to alternative flours and ingredients, such as those derived from legumes, ancient grains, or insect protein. Understanding the rheological and baking properties of these alternative ingredients is crucial for developing sustainable and nutritious baked products. The insights gained from this study could lead to the development of more customizable and adaptive baking processes. By understanding the specific impacts of kneading and resting periods on dough development, bakers could tailor processes to different types of flour or desired product characteristics,

enhancing efficiency and product quality. Translating the findings from microscale and laboratory-scale studies to industrial applications presents a challenge but also a significant opportunity.

Beyond immediate applications in research and industry, these findings have the potential to inspire further exploration into the mechanisms underlying dough development and functionality. They provide opportunities for interdisciplinary collaboration, especially with material science and computational modeling, to simulate and optimize dough properties at both the molecular and macrostructural levels. The pursuit of such approaches may yield deeper insights into the molecular interactions within the dough matrix, helping to bridge the gap between theoretical understanding and practical application.

Finally, these innovations hold broader implications for addressing global challenges. By enhancing the efficiency and sustainability of dough and baking processes, this research contributes to reducing resource consumption, minimizing waste, and improving product quality. Moreover, optimizing the nutritional properties of baked goods aligns with efforts to promote public health and address global food security. Looking ahead, continued advancements in this field will be critical not only for the progress of food science but also for tackling broader societal challenges related to sustainability, nutrition, and food system resilience.

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7 Appendix

7.1 Non-peer reviewed publications

- Vidal, L.M., Wittkamp, T., Alpers, T., Jekle, M., Becker, T.: Kleine Probe, großes Wissen – Mehlanalyse im Kleinstmaßstab. Brot+Backwaren 4 (2023), 58-61

7.2 Conference Contributions

- Vidal, L.; Jekle, M.; Becker, T.: IQ-Back, Freising, Germany, 2019-05-17
- Vidal, L.; Jekle, M.; Becker, T.: ISBP2021, online, 2021-07-01
- Vidal, L.; Benz. P.; Jekle, M.; Becker, T.: 11. WIG-Getreidetagung, Freising, Germany, 2022-03-28
- Vidal, L.; Alpers, T.; Benz. P.; Jekle, M.; Becker, T. 22. VGMS-Getreidetagung, Freising, Germany, 2023-06-21