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Wheat dough formation - Impact of mechanical starch modification

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„Der Beginn aller Wissenschaften ist das Erstaunen,
dass die Dinge sind, wie sie sind.“

Aristoteles (384–322 v. Chr.)

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Preface

Stefanie Hackenberg
M. Sc.

The results of the publications of this thesis were developed at the Technical University of Munich, Chair of Brewing and Beverage Technology, Research Group Cereal Technology and Process Engineering from 2013 to 2017.

Publications

The following **peer-reviewed publications** (presented in chronological order) were generated during the period of this work:

1. Hackenberg, S., Verheyen, C., Jekle, M., Becker, T.: Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. Journal of European Food Research and Technology 243 (2017), 287–296. https://doi.org/10.1007/s00217-016-2743-8

2. Hackenberg, S., Leitner, T., Jekle, M., Becker, T.: Maltose formation in wheat dough depending on mechanical starch modification and dough hydration. Carbohydrate Polymers 185 (2018), 153–158. https://doi.org/10.1016/j.carbpol.2017.12.064

3. Hackenberg, S., Jekle, M., Becker, T.: Mechanical wheat flour modification and its effect on protein network structure and dough rheology. Food Chemistry 248 (2018), 296–303. https://doi.org/10.1016/j.foodchem.2017.12.054

4. Hackenberg, S., Vogel, C., Scherf, K. A., Jekle, M., Becker, T.: Impact of altered starch functionality on wheat dough microstructure and its elongation behavior. Food Chemistry 290 (2019), 64–71. https://doi.org/10.1016/j.foodchem.2019.03.016

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Abbreviations

Abbreviation	**Meaning**
AACC	American Association of Cereal Chemists
$AgNO_3$	Silver nitrate
Aw	Water activity
CLSM	Confocal laser scanning microscopy
CO_2	Carbon dioxide
Da	Dalton
DMTA	Dynamic mechanical thermal analysis
Ext_{max}	Maximum extensibility
G*	Complex shear modulus
G`	Storage modulus
G``	Loss modulus
Hm	Maximum dough height
HPAEC-ED	High-performance anion-exchange chromatography with electrochemical detection
MSM	Mechanical starch modification
$Peak_{time}$	Dough development time according to the AACC International Method 54-21.02
PNA	Protein network analysis
R_{max}	Maximum resistance to extension
RP-HPLC	Reversed-phase high-performance liquid chromatography
rpm	Revolutions per minute
SEM	Scanning electron microscopy
T_0	Onset temperature (DSC)
TPA	Texture profile analysis
T_x	Time of porosity
WHC	Water-holding capacity
ΔH	Enthalpy of gelatinization (DSC)

Summary

Starch and gluten properties strongly determine the technological behavior of wheat dough. The mechanical treatment of wheat flour in a mill modifies the starch structure and functionality. This mechanical flour modification is commonly defined by the mechanical starch modification (MSM) level, formerly known as starch damage.

Although the use of mechanically modified wheat flours negatively influences the final wheat bread quality, the effect of the altered structure and functional properties of starch during the different processing steps of bread making has not yet been sufficiently explored. The non-heating steps of dough processing are mainly process-determining; therefore, the influence of MSM on the dough structure formation during kneading and the gas-holding properties of the dough during proofing is clarified in detail in this thesis.

High MSM strongly impacted dough development during kneading. The dough produced from high-MSM wheat flour exhibits a poorly developed protein microstructure at $\text{Peak}_{\text{time}}$ (dough development time according to AACC International Method 54-21.02) characterized by a reduced protein branching rate, a high protein end-point rate, and high values of lacunarity. Due to the increased swelling of highly mechanically modified wheat starch, its granular volume increased, which might have led to stronger adhesion forces between starch particles caused by the formation of capillary bridges. Due to this effect, more kneading energy might be required to separate these accumulated starch granules from one another, particularly at the beginning of the kneading phase. It is assumed that the protein component in dough therefore receives proportionally less energy. This could be confirmed by adding more energy in the form of kneading by prolonging the kneading time, resulting in a homogenously, closely meshed gluten network with high connectivity comparable to the standard dough microstructure. With this increased protein network development, the dough rheological properties, particularly the maximum resistance to extension (R_{max}), increased. However, the network coherence of dough produced from high-MSM flour was even lower despite the kneading time adaption compared with the standard. In summary, the comparable protein networks had the same structural properties but differed in rheology. Due to reduced interactions between protein and starch in addition

to the increased gap formation within the protein network, both of which are caused by starch swelling, the cohesion of the protein network might be poor in dough produced from high-MSM flour compared with dough produced from low-MSM flour. Nevertheless, the volume of wheat bread produced from high-MSM flour could be significantly increased. This was achieved by forming a highly branched gluten network by adapting the mechanical energy input during kneading.

Furthermore, the maximum dough height (Hm) during proofing and therefore the baking volume strongly depends on the availability of fermentable carbohydrates, which are needed by yeast for gas production. The release of fermentable carbohydrates, particularly maltose, increased during grinding in a ball mill due to improved accessibility of mechanically modified wheat starch by amylolytic enzymes. High MSM levels combined with low dough hydration increased maltose release primarily during kneading. These heightened maltose concentrations in the dough, however, were not utilized by the yeast during proofing. The results led to the conclusion that high concentrations of fermentable carbohydrates obtained from MSM should not have a relevant effect on gas formation during proofing. This was confirmed by examining the gas production curve of the standard compared with those of dough produced from high-MSM flours during a fermentation cycle of 180 min. Moreover, the gas-holding properties were comparable between the standard dough and the dough produced from high-MSM flour. Thus, the gas production and gas-holding properties during proofing did not cause the different dough heights between the standard and the dough produced from high-MSM flour. Rather, the dough height limiting factor is clearly the kneading step.

Having distinct knowledge about the effects of MSM during the various steps of bread-making facilitates control and improves the product quality of the wheat bread produced from flour with high MSM levels. High-MSM flours with defined functionalities could then be used to improve the volume, shelf life, or flavor attributes of wheat bread, for example. Thus, more “clean label” baking will be possible in the future.

Zusammenfassung

Die technologischen Eigenschaften eines Weizenteiges hängen stark von den Stärke- und Gluteneigenschaften ab. Die mechanische Zerkleinerung von Weizenmehl mittels einer Kugelmühle bewirkt eine Modifizierung der Stärke und beeinflusst maßgeblich deren Struktur- und Funktionseigenschaften. Die mechanische Mehlmodifikation wird im Allgemeinen über den Grad der mechanischen Stärkemodifikation definiert. In der Literatur wird in diesem Zusammenhang auch oft der Begriff „Stärkebeschädigung" verwendet. Obwohl die Verwendung von stark mechanisch modifizierten Weizenmehlen einen negativen Einfluss auf die Endproduktqualität hat, wurde noch nicht hinreichend untersucht, welche Rolle hierbei die veränderten Struktur- bzw. Funktionseigenschaften der Stärke bei den unterschiedlichen Prozessschritten der Weizenbrotherstellung spielen; insbesondere beim Knetprozess und bei der Gare.

Der Stärkemodifikationsgrad beeinflusste maßgeblich die Bildung der Teigmatrix während dem Kneten. Bei Verwendung von Weizenmehl mit einen hohen Stärkemodifikationsgrad war das Proteinnetzwerk am Viskositätsoptimum (Peak$_{time;}$ Teigentwicklungszeit gemäß der AACC Internationalen Methode 54-21.02) des Teiges nur schwach entwickelt, was sich in einer verringerten Proteinverzweigungsrate, in einer hohen Anzahl an offenen Protein-Enden und in einer starken Lückenhaftigkeit (Lakunarität) des Netzwerkes wiederspiegelte. Die erhöhte Quellung der mechanisch beanspruchten Weizenstärke führte zu einer Volumenzunahme der Granula. Dies könnte zu einer Zunahme der Adhäsionskräfte zwischen den Stärkepartikeln geführt haben, bedingt durch die Ausbildung von Kapillarbrücken. Es wird angenommen, dass durch die Zunahme der Haftkräfte zwischen den Stärkepartikeln insbesondere zu Beginn der Knetphase mehr Energie benötigt wird, um die sich aneinander angelagerten Stärkegranula voneinander zu trennen. Infolgedessen steht der Proteinkomponente im Teig proportional weniger Knetenergie zur Verfügung. Diese Annahme konnte bestätigt werden, da infolge einer höheren Energiezufuhr, in Form von einer verlängerten Knetzeit, ein homogenes Glutennetzwerk mit einer hohen Konnektivität erzeugt werden konnte; die Proteinverzweigungsrate und die Gesamtproteinlänge nahmen zu, während die Lakunarität abnahm. Die Struktur des Proteinnetzwerkes war nach der Knetzeitanpassung vergleichbar mit der

Proteinmikrostruktur des Standardteiges. Mit der Ausbildung des Proteinnetzwerkes erhöhte sich auch der Dehnwiderstand der Teige. Das Proteinnetzwerk der Teige, welche aus Weizenmehl mit stark mechanisch modifizierter Stärke hergestellt wurde, zeigte dennoch einen schwächeren Netzwerkzusammenhalt im Vergleich zum Standard. Damit wiesen diese beiden Proteinnetzwerke die gleichen strukturellen Eigenschaften auf, unterschieden sich jedoch maßgeblich bezüglich ihrer Rheologie. Dies könnte auf verminderte Interaktionen zwischen Protein und Stärke als auch auf die Bildung von größeren Lücken im Proteinnetzwerk zurückzuführen sein; beide Effekte werden verursacht durch die Quellung und damit durch die Volumenzunahme der mechanisch modifizierten Stärke. Nichtsdestotrotz konnte durch die Anpassung des mechanischen Energieeintrags während dem Kneten die Proteinnetzwerkausbildung soweit verbessert werden, dass sich das spezifische Volumen der Brote, welche aus Weizenmehl mit stark mechanisch modifizierter Stärke hergestellt wurden, signifikant erhöhte.

Die Teighöhe während der Gare und das spezifische Brotvolumen hängen zudem stark von der Verfügbarkeit fermentierbarer Kohlenhydrate ab. Diese werden von der Hefe metabolisert und infolgedessen bildet diese CO_2. Durch die verbesserte Zugänglichkeit der mechanisch modifizierten Stärkegranula für amylolytische Enzyme wurden mit steigendem Stärkemodifikationsgrad bereits während der Mehlbeschädigung in der Kugelmühle vermehrt Zucker, insbesondere Maltose generiert. Weiter wirkte sich ein hoher Stärkemodifikationsgrad in Kombination mit einer reduzierten Teighydratation positiv auf die Maltosebildung, vorwiegend während der Knetung aus. Die erhöhten Maltosekonzentrationen im Teig wurden jedoch während der Gare durch die Hefe nicht umgesetzt. Die hohen Maltosemengen hatten auch keinen wesentlichen Einfluss auf die Gasbildung während der 180-minütigen Gare. Die Gashaltefähigkeit der Teige, welche aus Weizenmehl mit stark mechanisch modifizierter Stärke hergestellt wurden, war ebenfalls vergleichbar mit dem Standard. Aus den Ergebnissen lässt sich schlussfolgern, dass der limitierende Prozessschritt für die Teighöhe der Knetvorgang ist.

1 Introduction

Mechanical flour treatment in the form of grinding affects the biopolymers of flour and thus influences its structural and functional properties. The level of mechanical wheat flour modification is generally defined by the mechanical modification of wheat starch. Approved methods for quantifying mechanical starch modification (MSM) in wheat flour are the enzymatic method (AACC International Method 76-31.01) and the amperometric method (AACC International Method 76-33.01). Thereby, the enzymatic method determines the MSM level through the hydrolysis of starch that is susceptible to fungal α-amylase. By contrast, the amperometric method measures the extent of MSM by the absorption kinetics of iodine. Through this, the iodine absorption is proportional to the MSM level. McAllister et al. (2008) found a high correlation ($R^2 = 0.94$) between the enzymatic and amperometric methods.

Since wheat dough functionality primarily depends on gluten and on starch properties, a combined investigation of the starch modification level and the technological functionality of wheat flour, or rather the dough produced from it, is of great importance. In the following sections, changes in the starch structure as a consequence of mechanical treatment during grinding and the impact on its functional properties are discussed. Furthermore, the effect of native starch, or rather the expected effect of mechanically modified starch during kneading and proofing, is argued. Then, based on the findings, the thesis outline and working hypotheses are developed.

1.1 Effect of mechanical treatment on the different structural levels of wheat starch

Starch is commonly used in the development of food products (Ball et al. 1996) and is influencing the structure of food matrices (Parker and Ring 2001). By modifying starch structures, the interaction between starch and other components in food materials can be affected and thus alters its physicochemical behavior.

Starch properties, such as solubility, swelling, pasting, and gelatinization, are altered by mechanical treatment during grinding (Li et al. 2014). Therefore, mechanical starch modification is a critical factor that influences the final quality of food products.

According to Dona et al. (2010) and Tran et al. (2011), the complex starch structure can be categorized into six structural levels simplified illustrated in Fig. 1.

The cereal grain represents the highest-level structure (level 6), in which starch granules (level 5) are embedded in the endosperm surrounded by gluten (Lindhauer 2017). On the starch granule surface and inside the granule proteins are contained (Baldwin 2001; Rahman et al. 1995). Friabilin is one of these starch granule surface proteins, whose main components are puroindolines (Lösche 2017b). Furthermore, starch granules contain internal and surface lipids. In the inner part of wheat starch granules are primarily located monoacyl lipids, such as lysophospholipids. On the surface are mainly free fatty acids from the endosperm non-starch lipids (Morrison 1988). The wheat endosperm typically contains two types of starch granules: the large A-granules of approximately 10 to 34 µm in diameter (Peng et al. 1999) having a disk or lenticular shape (Schirmer et al. 2013), in addition to the small B-granules (< 10 µm diameter) (Brocklehurst and Evers 1977) having a ellipsoidal or spherical form (Schirmer et al. 2013). Their size distribution is bimodal (Peng et al. 1999; Schirmer et al. 2013). Individual starch granules are synthesized in alternating crystalline and amorphous shells, termed as a growth ring structure (level 4) (Ball et al. 1996; Tran et al. 2011). Thereby, the crystalline structures primarily consist of clusters of shorter portions of amylopectin branches, whereas amylose is mainly contained in the amorphous layers (Dona et al. 2010). This lamellar, or rather semi-crystalline structure represent level 3 of the starch granule.

Gallant et al. (1997) revealed a further concept of starch granule organization between the growth ring structure and the lamellar structure. Thereby, hard crystalline and soft semi-crystalline shells alternate within the granule. These shells are organized into spherical blocklets (Gallant et al. 1997).

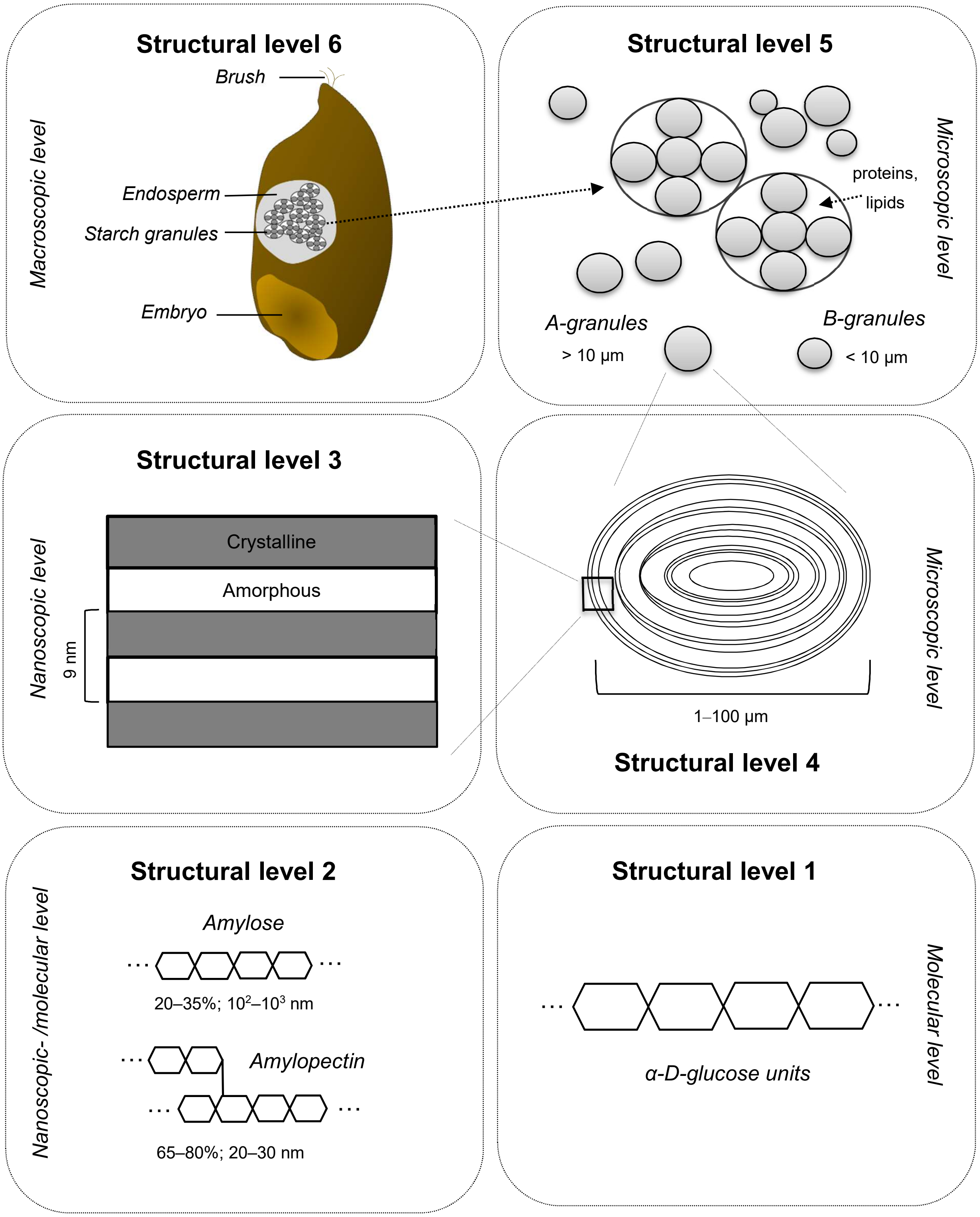


Fig. 1. Structural organization of starch in wheat grains adapted from Dona et al. (2010) and Tran et al. (2011). The complex starch structure can be subdivided into different structural levels from the macroscopic to the molecular scale: level 6, wheat grain; level 5, starch granule, A- and B-types; level 4, growth rings; level 3, semi-crystalline structure; level 2, branched starch structure; and level 1, individual branches. Data from Schneider (2017), Brocklehurst and Evers (1977), Ball et al. (1996), Dona et al. (2010), Peng et al. (1999), and Tester et al. (2004).

The whole starch molecule is described by structural level 2. The amylose and amylopectin molecules are buildups of α-D-glucose molecules (level 1). Most starches consist of approximately 20–35% amylose (Tester et al. 2004). The structure of amylose is primarily linear and forms a helical structure based on α-1,4 linked monomers of α-D-glucose (Parker and Ring 2001; Schneider 2017). The standard analysis AACC International Method 76-33.01 takes advantage of this structure. Thereby, the iodine is embedded in the amylose helix of starch. According to Ball et al. (1996) amylose contains less than 1% α-1,6 branching points. The molecular weight of amylose is between 10^5 and 10^6 Da and is therefore much smaller than amylopectin, which has molecular weights ranging from 10^7 and 10^9 Da (Ball et al. 1996). In contrast to amylose, amylopectin is highly branched and consists of, on average, 20–25 α-1,4 linked D-glucose molecules, which are interlinked by α-1,6 glycosidic bonds (Manners 1989).

During grinding, the wheat kernel is separated into endosperm, embryo, and shell components. Endosperm contains the following components: protein particles (< 18 µm), starch (1–50 µm), flour particles without cell walls (5–80 µm) as well as endosperm cells, endosperm fragments, endosperm cell walls or cell bonds (30–160 µm) (Lösche 2017b). In general, roller mills are used for industrial wheat flour production. For research purposes, several grinders are used for dry grinding, such as hammer mills, ultra-centrifugal mills, attrition mills, ball mills, roller mills, impact mills, and cryogenic mills. Depending on the grinding process, different forces act on the starch granules in wheat flour, such as pressure, resiliencies, compressive forces, shear forces, and frictional forces. The effects of the grinding technologies on the different structural levels of starch are explained in the following.

In their native state, starch granules of wheat, rice, maize, and potato have a relatively smooth surface (Barrera et al. 2013b; Chen et al. 2003; Dhital et al. 2010; Liu et al. 2011). After severe disk milling, wheat starch granules have a rough and flaky surface with cavities of different forms and sizes (Barrera et al. 2013b). Chen et al. (2003) observed that rice starch granules lose their smoothness with increased grinding time in a ball mill (Chen et al. 2003). In addition, the surface of maize starch becomes rough after ball milling visualized with scanning electron microscopy (SEM). The granules are fissured, and the irregular surface structure of the granules increases with the milling time. Furthermore, the outer layer of maize starch granules is partially chipped due to mechanical treatment (Liu et al. 2011). Mechanical treatment in a grinder influences starch particle size, as well. Thus, Martínez-Bustos et al. (2007) observed a partial fragmentation of starch granules after high-energy treatment in a ball mill. After ball milling, the number of broken granules of carrot and cassava starch also increases (Moraes et al. 2013).

Mechanical treatment of native maize starch in a planetary ball mill for 3 h with a milling speed of 500 rpm implies that starch granules break apart and lose their alternate growth rings (Liu et al. 2011). Mechanical treatment in a ball mill reduces the structural integrity of starch (Huang et al. 2008; Liu et al. 2011; Martínez-Bustos et al. 2007; Moraes et al. 2013; Morrison et al. 1994). Martínez-Bustos et al. (2007) revealed that the crystallinity of cassava and jicama starches ground with high moisture contents decreases with prolonged grinding time. Furthermore, the crystallinity of cassava and maize starch mechanically treated in a stirring ball mill decreases from polycrystalline to amorphous (Huang et al. 2008). Moreover, mechanical treatment of maize starch in a planetary ball mill at 500 rpm for 2 h reduces its crystallinity (Liu et al. 2011). Moraes et al. (2013) observed that the relative crystallinity of carrot and cassava starch significantly decreases after treatment in a ball mill at 108 rpm for 32 h, resulting in an increase of amorphous areas (Moraes et al. 2013). It was thus determined that ball milling leads to a decreased crystallinity. The question becomes whether this results from mechanical energy input or possible heating during the grinding process. Martínez-Bustos et al. (2007) suppose that local heat during ball milling treatment causes the crystalline starch structure to be lost (Martínez-Bustos et al. 2007). How ball milling affect temperature development during processing is described in the following.

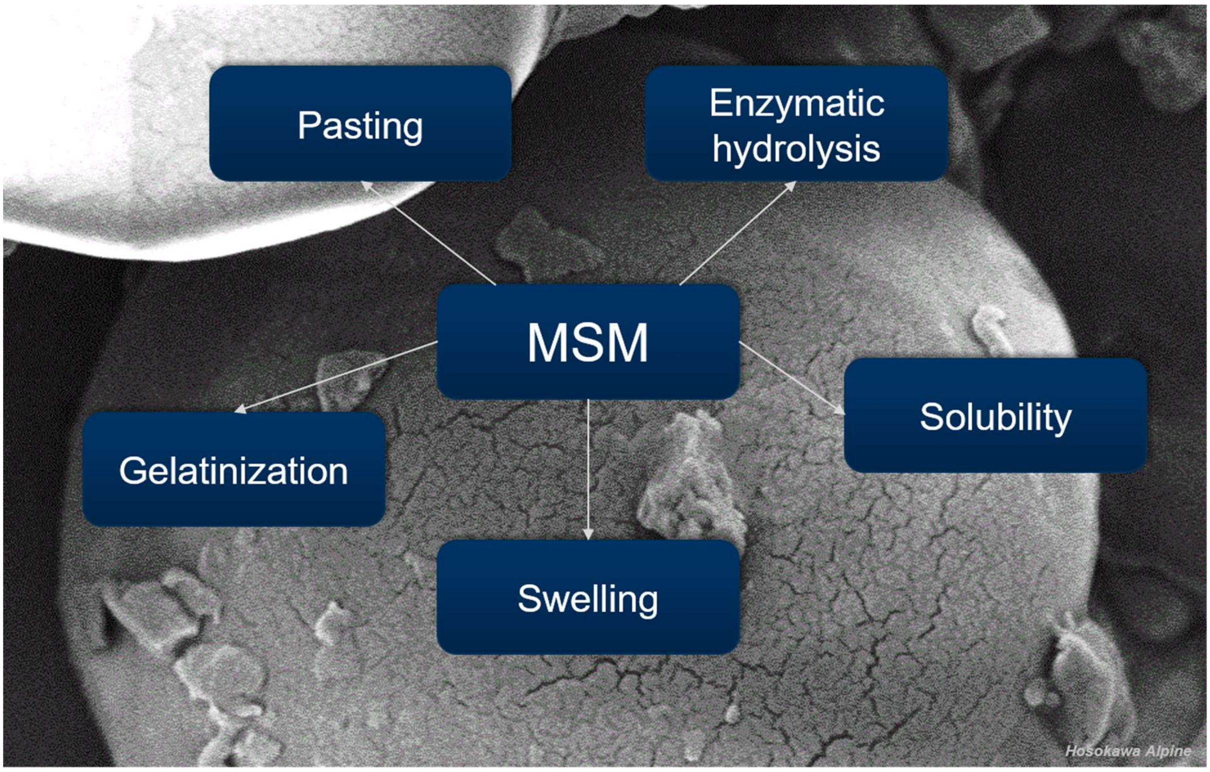
The temperature inside the milling beakers of a ball mill depends on parameters such as the operating frequency, the size of the milling beaker, the milling ball filling degree, and the diameter of the balls (Schmidt et al. 2016). Vogel (2019) investigated the temperature progression in wheat flour in a planetary ball mill and an ultra-centrifugal mill based on rotational speed and the number and diameter of balls and the sieve mesh size, respectively. The temperature of wheat flour steadily increases with increasing rotational speed and grinding time in a ball mill. Thereby, the temperature increases up to 100 °C when using a rotational speed of 600 rpm, whereas the variation of balls (8 x Ø 30 mm and 4 x Ø 40 mm) does not affect the temperature development in the wheat flour measured directly after grinding by means of an infrared thermometer. Furthermore, the author detected a continuous temperature increase in wheat flour with increasing rotational speed and reduced sieve mesh size when using an ultra-centrifugal mill. The temperature of wheat flour is approximately 117 °C when using a rotational speed of 18,000 rpm and a sieve mesh size of 80 µm (Vogel 2019). The ball temperatures range from approximately 50 °C to 75 °C after a milling time of 30 min in a high-energy ball mill (Takacs and McHenry 2006). In planetary ball mills, the temperature of the balls can increase up to 600 °C (Kwon et al. 2002). Based on the findings, it can be concluded that the destruction of crystalline starch structures during grinding in a ball mill could indeed be the result of thermal effects.

However, mechanical treatment in a cryo mill also decreases the crystalline structure of starch. During this process, however, starch is ground at cryogenic temperatures, preventing starch granules from heat (Dhital et al. 2011). It can therefore be excluded that heat is the only cause for the degradation of crystalline structures. According to Retsch GmbH (2020), samples become brittle due to the use of liquid nitrogen during cryo milling (Retsch GmbH 2020). It appears clear that mechanical forces acting during cryo milling can disrupt starch structures caused by increased brittleness of the granules.

Furthermore, there are indications that mechanical treatment in a grinder leads to the molecular degradation of starch. In that respect, Morrison and Tester (1994) investigated the molecular weight and molecular size of amylose and amylopectin of wheat starch ground in a ball mill for 24 h. Amylose is influenced only after severe grinding, which leads to slight depolymerization. By contrast, amylopectin is converted to low molecular weight fragments with less mechanical treatment in the mill. Molecular degradation tends to increase with prolonged grinding time (Morrison and Tester 1994).

1.2 Effect of mechanical treatment on wheat starch functionality

Alterations of the starch structure as a consequence of grinding effect starch functionality in different ways are illustrated in Fig. 2. These altered starch functionalities might strongly influence the production process of wheat-based baking goods and therefore their end-product quality.



SEM Image © Hosokawa Alpine

Fig. 2. Effect of mechanical starch modification (MSM) on starch functionalities.

A survey concerning functional alterations of wheat starch induced by different grinding technologies and their relation to structural changes of starch is presented in Table 1.

Table 1. Overview of functional alterations of starch as a consequence of mechanical treatment and their relation to structural changes of starch.

Functionality attribute and its alteration as a function of mechanical treatment	**Possible reasons for the respective functional alteration**
Swelling ↑ 1.), 6.), 7.)	Improved hydration of mechanically modified starch granules due to changes in the granular surface, such as flake-off[1.)]; Improved water penetration of mechanically treated starch caused by the destruction of crystalline regions[6.)]; Fragmentation and thus a reduced flour particle size facilitates water diffusion[10.)]
Solubility ↑ 2.), 3.), 6.), 7.), 8.), 11.)	Molecular leaching in cold and hot water could be favored due to increased surface area or rather a loosely organized inner structure of starch granules with an increased MSM level[2.)]; A higher number of free hydroxyl groups, which are free to bind water, are becoming available due to the breaking of hydrogen bonds of double helices; therefore, the solubility of starch increases[11.)]; Greater water ingress into the granule caused by the fracturing of starch crystalline structures[6.)]; It is presumed that particularly the fragmentation of the amylopectin fraction facilitates starch solubility[8.)]
Enzymatic hydrolysis ↑ 4.)	A reduction in the starch granule size is related to improved enzymatic digestibility due to a larger surface area[12.)]; Structural changes of mechanically modified starch (cracks, fissures[13.)]) may improve enzymatic accessibility; The presence of highly accessible pores and channels facilitate enzymatic hydrolysis[4.)]
Pasting *Temperature* 5.), 8.), 11.) *Peak viscosity* 5.), 8.), 11.) ↓	Pasting temperature decreases due to the loss of crystalline structures resulting in an increased granule water absorption capacity[8.)]; Fragmentation of flour particles increases the heat transfer and the water diffusion and therefore decreases the pasting temperature[10.)]
Gelatinization ΔH 5.), 6.), 9.), 11.) T_0 5.), 6.), 11.) ↓	Less ordered starch structures, such as reduced crystallinity and reduced double helix content, might favor a low enthalpie of gelatinization (*ΔH*)[9.),10.)]; Particle size is strongly related to gelatinization temperature. Thus, smaller flour particles have better thermal conductivity[10.)]

[1.)] Barrera et al. (2013a) *using a disk mill*
[2.)] *Hasjim et al. (2012) using a hammer- or rather a cryo mill*
[3.)] *Morrison and Tester (1994) using a ball mill*
[4.)] *Dhital et al. (2010) using a cryo mill*
[5.)] *Chen et al. (2003) using a ball mill*
[6.)] *Huang et al. (2007) using a ball mill*
[7.)] *Tester and Morrison (1994) using a ball mil*
[8.)] *Devi et al. (2009) using a cryo mill*
[9.)] *Dhital et al. (2011) using a cryo mill*
[10.)] *Hasjim et al. (2013) using a hammer- or rather a cryo mill*
[11.)] *Moraes et al. (2013) using a ball mill*
[12.)] *Noda et al. (2008)*
[13.)] *Liu et al. (2011) using a ball mill*

During kneading, the gluten network develops (Letang et al. 1999; Van der Mijnsbrugge et al. 2016) and gas cells are incorporated in the dough (Shehzad et al. 2010). The porous dough structure is developed during the biological dough leavening. During this process, gas cells are filled with CO_2, which is produced by the yeast. Thermal solidification of the pore structure occurs during the baking step (Lösche 2017b). Since the non-heating steps of dough processing (kneading and proofing) are mainly process-determining, the focus of this thesis is on dough structure formation during kneading and gas-holding properties of the dough during proofing.

1.3 Function of (mechanically modified) starch during the non-heating steps of bread making

As presented in Section 1.1, mechanical starch modification causes various changes in starch structures from the macroscopic to the molecular level. Starch functionality is consequently influenced in manifold ways (see Section 1.2, Table 1), which might affect the bread-making process differently. In the following, the influence of starch and the possible effect of MSM during the non-heating steps of bread making are described and discussed.

1.3.1 Starch and its effect during dough microstructure formation

Wheat dough formation occurs by the addition of water and energy in the form of kneading to flour enriched with ingredients, such as salt and yeast.

Without energy addition, the gluten network reveals a non-regular connected structure with a reduced coherence. Starch granules are only partially integrated into gluten aggregates (Unbehend et al. 2004). Through the addition of mechanical energy in the form of kneading, this irregular protein structure is converted into a viscoelastic gluten network (Letang et al. 1999; Van der Mijnsbrugge et al. 2016). This network is stabilized by different bonds, such as disulfide crosslinks (Letang et al. 1999). At optimum kneading time, the gluten matrix in wheat dough is evenly interconnected and surrounds most of the starch granules (Unbehend et al. 2004).

Mechanically modified wheat starch has an increased water absorption and swelling capacity compared with native wheat starch in water at room temperature (Barrera et al. 2013a). Furthermore, it has improved water retention capacity (Jakobi et al. 2018).

During dough preparation, starch absorbs approximately 50% of the added water. The other half is absorbed by proteins and pentosans (Lösche 2017a). Interactions of protein and water are necessary for developing the specific properties of the proteins (Lösche 2017b). Increased water absorption of mechanically modified starch could cause that starch and protein compete for free water during dough processing if water is not adapted accordingly. Such a competitive situation might affect the protein microstructure formation in wheat dough during kneading.

Since starch also serves as a filler particle in the protein network, it can be supposed that gluten structure formation might be influenced by an increased filler particle size caused by the increased swelling capacity of the granules. In general, the size of fillers can influence the reinforcement of a material. Thus, the reinforcement decreases with the increasing diameter of the filler particles (Yuan and Mark 1999). The effect of MSM on the reinforcement of wheat dough remains unknown.

Furthermore, it is supposed that protein-starch interactions determine the rheological properties of dough. Thus, a low storage modulus (G`) and low loss modulus (G``) of dough might be explained by decreased protein-starch interactions (Petrofsky and Hoseney 1995). In a viscoelastic, cohesive gluten network, starch is linked to gluten by glycolipids (Lösche 2017b). Due to a possible volume increase of starch granules caused by swelling, the relative surface of the granules decreases, and therefore, protein-starch interactions might decrease. The effect of possible altered protein-starch interactions on dough development during kneading and on dough rheological behavior has not yet been investigated.

Starch-starch interactions might be affected, as well, caused by an increased volume of swollen starch granules. When a liquid is present, liquid bridges can be formed between particles (Schubert 1973). With the increasing diameter of a spherical particle, the theoretical adhesion force proportionally increases (Borho et al. 1991). An approximately linear increase in capillary forces using particles on the microscopic scale is observed by Dörmann and Schmid (2015). Whether there is a relationship between possible increased adhesion forces between swollen starch particles and the microstructure formation in dough is not known.

1.3.2 Starch and its effect during proofing

During biological dough leavening, yeast (e.g., *Saccharomyces cerevisiae*) produces gas in the form of CO_2, which accumulates in the gas cells in the dough. During this process, gas cells expand, flow together, and branch out resulting in a porous dough structure (Lösche 2017b). The dough height during proofing and the final spongy bread structure are strongly influenced by the gas production by yeast during fermentation. For gas formation, yeast utilizes fermentable carbohydrates.

The endosperm of cereal grains contains mainly carbohydrates in the form of starch (Lindhauer 2017). Further carbon sources are contained in wheat flour, such as sucrose, maltose, fructose, and glucose; thereby, sucrose is the predominant sugar (MacArthur and D'Appolonia 1979). Starch is hydrolyzed by amylolytic enzymes into shorter-chained carbon sources, such as dextrins, maltose, or single glucose molecules (Lüttge et al. 2005; Tegge 2004). Yeast metabolizes sugars in a specific order. First, glucose and fructose are consumed, followed by other mono-, di-, and trisaccharides (Broach 2012). Yeast can produce CO_2 under anaerobic conditions, which are equal to the conditions during proofing (Lösche 2017a). The anaerobic glucose catabolism provides two molecules of ethanol, two of CO_2, and two ATP (Lösche 2017a; Lüttge et al. 2005).

As presented in Section 1.2, Table 1, structural alterations of mechanically modified starch facilitate its hydrolysis through amylolytic enzymes. However, the enzymatic activity strongly depends on the hydration level of the reaction medium (Rezaei et al. 2007). The water absorption capacity of highly mechanically modified starch is increased (Barrera et al. 2013a) and therefore the dough hydration is altered, which influences the dough viscosity. The extent to which the changed dough hydration and thus altered diffusion conditions in dough influences enzymatic starch hydrolysis and thus the formation of fermentable carbohydrates for yeast has not yet been sufficiently investigated.

1.4 Thesis Outline

Changed starch functionality as a consequence of mechanical treatment negatively influences the baking volume. Various explanatory approaches for this observation are discussed in the following.

It is assumed that the low specific bread volume was caused by the decreased gas retention capacity of the dough (Barrera et al. 2007; Brütsch et al. 2017). Barrera et al. (2007) presume that reduced gas retention capacity might be caused by reduced dough consistency. Moreover, they suppose that the reduced dough consistency is caused by the increased initial water absorption of the flour due to mechanically modified starch and its subsequent hydrolysis by amylolytic enzymes (Barrera et al. 2007). Therefore, water addition is optimally adapted according to the applied method (farinograph method). However, water is released from mechanically modified granules caused by enzymatic degradation. Brütsch et al. (2017) assume that decreased gas retention capacity is caused by a poor gluten network. A further explanation for the reduced baking quality might be that mechanically modified starch and protein compete for water, resulting in an incomplete formation of the gluten structure during kneading (Barrera et al. 2007).

To summarize, these effects of mechanically modified starch on dough or rather its properties are only assumptions. Existing research has primarily focused on the structural and functional changes of starch as a function of botanical sources and grinding technology reviewed in detail by Li et al. (2014) but not on its technological effects on dough during the various stages of bread making. The knowledge of the cause-effect relationship of MSM during dough processing would create the potential to control and conceivably improve the quality of bread produced from wheat flour with a high MSM level. Therefore, the mechanistic relationships between high MSM and a low volume of wheat bread, which are associated with poor quality, must be clarified.

In this thesis, the focus lies particularly on the non-heating steps of dough processing. Thus, the possible effects of MSM during kneading and proofing are illustrated in Fig. 3.

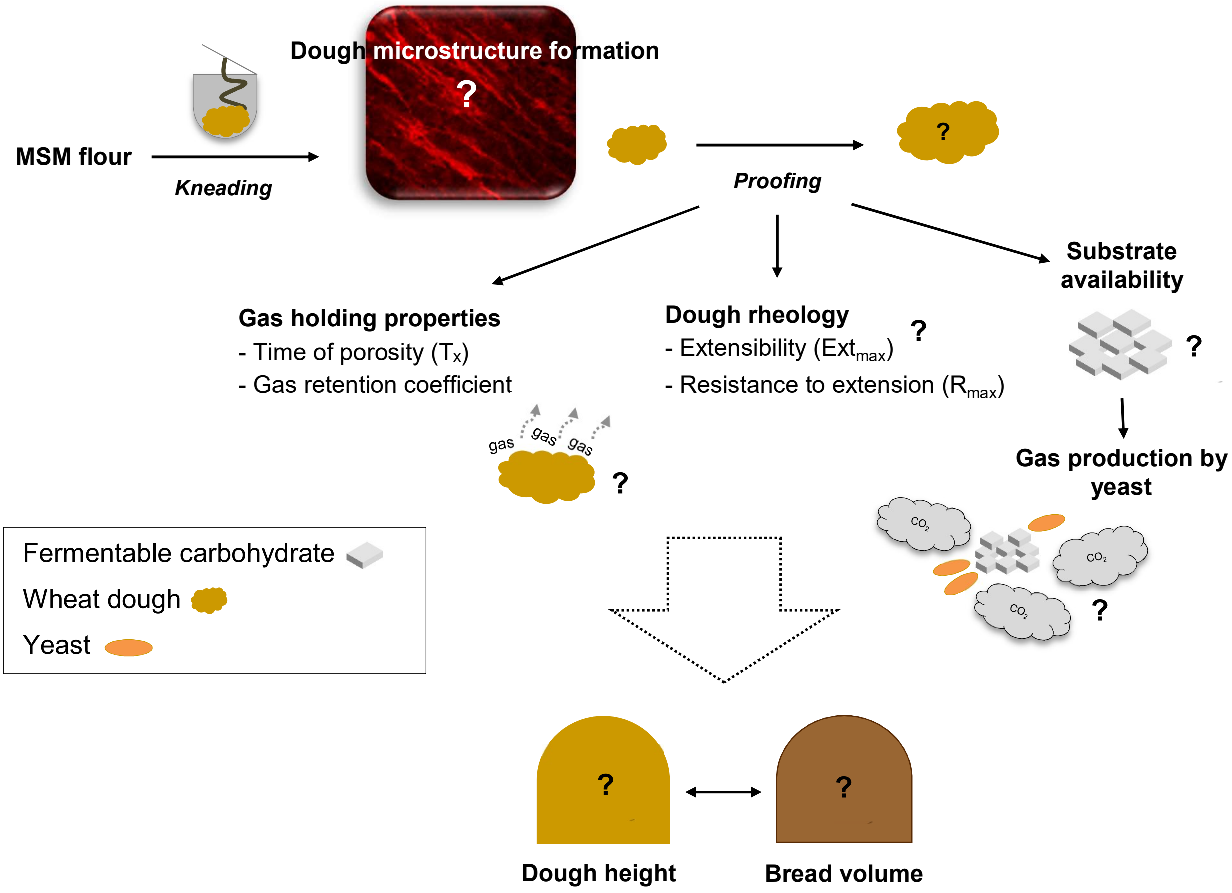


Fig. 3. Possible effects of MSM during the non-heating steps of wheat dough processing.

In particular, the effect of MSM on dough microstructure development, or rather gluten network formation, during kneading has not yet been clarified. Furthermore, the relationship between MSM, amylolytic hydrolysis, the consumption of fermentable carbohydrates by yeast, and the maximum dough height during proofing is not known. The gas-holding properties of wheat dough, such as the porosity (the time when the dough is losing gas, T_x) and the gas retention coefficient as a function of MSM and its effect on dough height during proofing are unknown, as well. The effect of MSM on the dough elongation behavior during proofing, which is a critical factor regarding the dough height, has also not been sufficiently investigated until now.

Based on the overview provided in the previous sections and in Fig. 3, the following working hypotheses have been developed:

Effect of MSM during kneading

- *Poor gas-holding properties of MSM wheat dough are caused by an altered dough elongation behavior, determined by a modified protein microstructure development during kneading.* Rheological dough properties are strongly related to the protein microstructure of wheat dough. Changes in the starch structure by MSM cause an altered starch functionality, such as increased water absorption and swelling. This leads to an altered protein microstructure formation during kneading. As a consequence, dough rheological properties, such as the maximum extensibility (Ext_{max}) and the maximum resistance to extension (R_{max}), are decreased. Thus, the gas retention capacity of dough is negatively influenced during proofing.

Effect of MSM during proofing

- *MSM leads to an influence of dough hydration, which determines the gas production in wheat dough.* The amount of water required for the wheat dough preparation must be adapted in the form of a bulk water increase caused by the increased water absorption capacity of mechanically modified starch. It is hypothesized that an increased dough hydration (through a standardized viscosity) decreases the interaction between mechanically modified starch and amylolytic enzymes. It is assumed that this effect predominates the effect of high substrate availability resulting from MSM; therefore, the gas production by yeast is negatively influenced during proofing.

With more knowledge about the effects of MSM during the various steps of bread making, the product quality of wheat bread can conceivably be controlled and improved. The beneficial properties of MSM flours could then be used to positively influence the bread quality (storage properties, flavor, and crust browning). In the future, more “clean label” baking will be possible.

2 Methods

The primary methods employed in this thesis are summarized in the following.

2.1 Wheat flour production and characterization

Commercially available wheat flour of Type 550 ground in a roller mill was used as the base flour in this thesis. Furthermore, the mechanical modification of wheat flour was received through treatment in an industrial ball mill (ATR-31; Hosokawa Alpine, Augsburg, Germany) by varying the retention time and rotational speed. Flours produced this way were used in Hackenberg et al. (2017), Hackenberg et al. (2018a), Hackenberg et al. (2018b) and Hackenberg et al. (2019). Additionally, wheat flour was mechanically modified with a planetary ball mill (PM 100; Retsch, Haan, Germany) on a laboratory scale. Detailed information regarding the grinding process can be found in Hackenberg et al. (2019). The measurement of the water-holding capacity (WHC) of wheat flours was described in Hackenberg et al. (2017). The enzymatic activity (α- and β-amylase) of wheat flours was analyzed using the Ceralpha and Betamyl Assay Kits by Megazyme International Ireland (Wicklow, Ireland), respectively (applied in Hackenberg et al. (2017)). The MSM level of wheat flours was measured by the SDmatic (Chopin Technologies, Villeneuve la Garenne, France) according to AACC International Method 76-33.01 (amperometric method) (applied in Hackenberg et al. (2017), Hackenberg et al. (2018a) and Hackenberg et al. (2019)). The particle size of dry wheat flours and of flour-water suspensions produced from them was analyzed via laser diffraction technology using a Mastersizer 3000 (Malvern, Herrenberg, Germany). Sample preparation and measurement were described in Hackenberg et al. (2018a). The sugar spectrum (glucose, fructose, maltose, sucrose and maltotriose) of flour-water suspensions was analyzed using high-performance anion-exchange chromatography with electrochemical detection (HPAEC-ED) (Dionex ICS 5000 ion chromatograph, Software Chromeleon 6.0, Thermo Scientific, Hennigsdorf, Germany). Thereby, enzymatic starch hydrolysis and thus the formation of fermentable carbohydrates was inhibited by $AgNO_3$. Details regarding the enzymatic inactivation, sample preparation, and extraction and determination of saccharides in flour can be found in Hackenberg et al. (2018b). The solubility of proteins in wheat flour was measured using a modified Osborne fractionation. Furthermore, the protein concentrations of the fractions were quantified using reversed-phase high-performance liquid chromatography (RP-HPLC). Details about the analysis can be found in Hackenberg et al. (2019).

2.2 Wheat dough analysis

Wheat dough was prepared in a spiral-kneader-type SP 12 A-3 (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). Information regarding the recipe, kneading parameters, and dough variations (water additions) can be found in Hackenberg et al. (2017) and Hackenberg et al. (2018b). Furthermore, the dough was produced in a Z-kneader (DoughLab; Perten Instruments, Hägersten, Sweden) with optimum bulk water addition according to AACC International Method 54-21.02 as shown in Hackenberg et al. (2018a) and Hackenberg et al. (2019). The visual evaluation of the dough microstructure was performed via confocal laser scanning microscopy using an eclipse Ti-U inverted microscope with an e-C1 plus confocal system (Nikon GmbH, Düsseldorf, Germany). Information regarding the staining of dough samples and details concerning the CLSM settings (resolution, wavelength, and number of recorded images) can be found in Hackenberg et al. (2018a) and Hackenberg et al. (2019). Furthermore, the protein network structure was quantitatively evaluated using the protein network analysis (PNA) method developed by Bernklau et al. (2016). The settings of image analysis and a description of the protein network attributes are described in Hackenberg et al. (2018a) and Hackenberg et al. (2019). Thereby, the dough was analyzed at various kneading times. The dough development time ($Peak_{time}$) was determined according to AACC International Method 54-21.02. Dough fermentation properties, such as gas volume, gas-holding capacity (time of porosity (T_x) and gas retention coefficient), and the maximum dough height (Hm), were analyzed using a rheofermentometer F3 from Chopin (Chopin Technologies; Villeneuve la Garenne Cedex, France). Additional details concerning the measurements can be found in Hackenberg et al. (2017) and Hackenberg et al. (2018a). Dough elongation behavior (maximum resistance to extension (R_{max}) and maximum extensibility (Ext_{max})) was evaluated using a texture profile analyzer (TPA) with an SMS/Kieffer Extensibility Rig (Stable Micro Systems Ltd., Godalming, UK) directly after kneading. Texture profile analysis was selected because a high correlation is present between the bread volume and R_{max} of the dough (Dobraszczyk and Salmanowicz 2008). Details regarding the measurements can be found in

Hackenberg et al. (2018a) and Hackenberg et al. (2019). Furthermore, the maltose concentration was analyzed in dough based on the MSM level and the kneading and proofing times by using an HPAEC-ED. The formation of maltose via enzymatic starch hydrolysis was inhibited using $AgNO_3$. Details regarding enzymatic inactivation, sample preparation, and the extraction and determination of maltose in dough can be found in Hackenberg et al. (2018b). Furthermore, dynamic mechanical thermal analysis (DMTA) with oscillatory measurements was performed to investigate the starch gelatinization in dough during baking. For this purpose, an AR-G2 rheometer (TA instruments, New Castle, USA; software Rheology Advantage 5.7.2.0) with a Smart Swap Peltier plate temperature system with a 40 mm plate geometry and a gap of 2,000 mm was used. After placing the dough between the plates, the dough was pre-heated to 30 °C. The oscillation measurement was performed using a strain amplitude of 0.1% and a frequency of 1 Hz (linear viscoelastic region). Prior to starting the test, a relaxation phase of 2 min, based on the time determined by sweep tests in pre-trials, was adjusted. The initial temperature was held for 1 min before the temperature increased by 4.25 °C/min until reaching 100 °C. In preliminary tests, the heating rate of 4.25 °C/min is approximately equivalent to the temperature increase in dough during baking and thus simulates the baking process (Jekle et al. 2016). At the end of the measurement, the temperature was held at 100 °C for 3 min. During the measurement, the complex shear modulus (G*) was determined.

2.3 Wheat bread analysis

The baking process of wheat bread is described in Hackenberg et al. (2017). The specific volume is an important quality issue for wheat bread. Thus, the specific volume of the wheat breads was measured using a laser-based volumeter (BVM-L370, Perten Instruments, Hägersten, Sweden).

2.4 Statistical analysis

To show significant differences, the results were analyzed with Graph Pad Prism (GraphPad Software, Inc., La Jolla, USA) using a one-way analysis of variance (ANOVA; 5% significance level) followed by Tukey’s Multiple Comparison Test. Linear relationships were evaluated using correlation tests, and the degree of linear correlation was determined using Pearson’s Coefficient of Variation (r). The results are visualized as the mean value, including the standard error of mean.

3 Results (Thesis Publications)

3.1 Summary of the results

The publications created within the scope of the dissertation are listed as original copies.

Part I Section 3.2 Pages 26–33	Mechanical wheat flour modification and its effect on protein network structure and dough rheology

Altered properties of starch and protein might influence dough development and therefore the rheological dough behavior, such as the maximum extensibility (Ext_{max}) and the maximum resistance to extension (R_{max}). The elongation properties of dough strongly affect the maximum dough height (Hm) during proofing and thus the final bread quality. To clarify the effect of mechanical flour treatment by a ball mill on dough microstructure formation, the dough was produced with five significantly different mechanical starch modification (MSM) levels, ranging from 4.78 to 8.15 g 100 g^{-1} flour and visualized at dough development time ($Peak_{time}$) according to AACC International Method 54-21.02 using a confocal laser scanning microscope (CLSM). The quantitative evaluation was conducted using protein network analysis (PNA). The dough produced from high-MSM flour was not fully developed at $Peak_{time}$. In contrast to the standard dough, which was characterized by a continuous cross-linked gluten structure with regularly allocated starch granules, the dough produced from high-MSM flour exhibited primarily gluten agglomerates with separated accumulations of starch granules at $Peak_{time}$. The protein network's reduced branching rate (-14%), increased protein end-point rate (+25%), and high values for lacunarity (+139%) indicate poor network connectivity with network interruptions. It is supposed that the viscosity up to $Peak_{time}$ in dough produced from high-MSM flour primarily increased through the cold swelling of mechanically modified starch and not through the development of a completely interconnected protein network structure. Furthermore, the protein network attributes were highly correlated with the dough rheological properties. The maximum extensibility (Ext_{max}) decreased by 28% in dough produced from high-MSM flour, whereas R_{max} decreased by 49%, resulting in a significantly decreased Hm during proofing (-54%). In conclusion, the poor rheological properties of dough produced from high-MSM flour were responsible for decreased Hm during fermentation and thus might be responsible for the lower baking volume of bread produced from high-MSM flour.

Contribution: The doctoral candidate co-designed and conducted the survey, performed the data management and analyses, and drafted, revised, and approved the final manuscript.

Part II Section 3.3 Pages 34–41	Impact of altered starch functionality on wheat dough microstructure and its elongation behavior

As discussed in Part I, dough produced from highly mechanically modified wheat flour was not fully developed at dough development time ($\text{Peak}_{\text{time}}$) according to AACC International Method 54-21.02. Part II clarifies the effect of the kneading time on protein network formation, dough rheology, and specific bread volume by applying highly mechanically modified wheat flours. The standard dough exhibited only a maximum peak ($\text{Peak}_{\text{time}}$) according to AACC International Method 54-21.02. The dough development curves produced from severely mechanically modified wheat flours with MSM levels between 6.92 and 8.15 g 100 g^{-1} flour displayed a second peak of less intensity after $\text{Peak}_{\text{time}}$. The increase in dough viscosity up to the second peak can be explained by the formation of a more highly branched and interconnected protein network structure (protein branching rate +14.8%, protein end-point rate -24.3%, mean lacunarity -64%). This improved network connectivity is achieved due to increased energy input during prolonged kneading. More energy in the form of kneading is required because large, swollen, mechanically modified starch granules appear to adhere more strongly to one another. Thus, a higher amount of kneading energy was needed to separate them, particularly during the initial kneading phase. The improved protein microstructure with an elongated kneading time increased the resistance to extension (R_{max}) of the dough by 52.5%, resulting in an increase of the specific bread volume by 24.4%. Nevertheless, the specific volume of the standard bread was larger than the specific volume of the bread produced from high-MSM flour despite the adapted kneading time. This might be explained by the lowered interfacial interactions between starch and protein and the larger voids within the protein network, both arising due to starch swelling. The cohesion of the protein network on the molecular level consequently appears to be weaker in dough produced from high-MSM flour. In summary, it can be ascertained that the specific volume and thus the quality of wheat bread produced from high-MSM flour could be significantly improved by the adaption of mechanical energy input in the form of kneading.

Contribution: The doctoral candidate co-designed and conducted the survey, performed the data management and analyses, and drafted, revised, and approved the final manuscript.

Part III Section 3.4 Pages 42–47	Maltose formation in wheat dough depending on mechanical starch modification and dough hydration

Evidence exists that mechanical wheat flour modification improves the accessibility of starch granules for amylolytic enzymes and thus influences the formation of fermentable carbohydrates during dough processing. Since the functional effect of mechanical flour, or rather starch modification, during proofing and baking was presented in Parts I and II, Part III aims to clarify the mechanisms regarding the saccharide release based on the MSM (4.78–8.37 g 100 g^{-1} flour) and hydration levels after grinding and during kneading and proofing (without yeast) or fermentation (with yeast) by means of a high-performance anion-exchange chromatography with electrochemical detection (HPAEC-ED). The maltose concentration significantly increased after grinding in a ball mill. This effect might be explained by the improved starch accessibility for β-amylases, which mainly hydrolyze β-maltose from starch polymers even at low water concentrations. The largest maltose increase was detected after kneading (up to 334%). During this process, the maltose formation was facilitated by high MSM and reduced dough hydration caused by an improved interaction between the amylolytic enzymes and mechanically modified starch. However, the increased maltose concentrations in dough issuing from high MSM were not utilized by yeast during 60 min of fermentation. This indicates that the reaction speed and substrate concentration were independent of each other. Therefore, it could be suggested that higher maltose concentrations due to mechanical flour or rather starch modification have no relevant effect on gas formation during proofing.

Contribution: The doctoral candidate co-designed and conducted the survey, performed the data management and analyses, and drafted, revised, and approved the final manuscript.

Part IV Section 3.5 Pages 48–57	Effect of mechanically modified wheat flour on dough fermentation properties and bread quality

The functionality of wheat dough during dough processing is primarily determined by the structural and functional properties of the individual wheat flour components (e.g., starch), which are influenced during mechanical flour treatment. Mechanically modified wheat flour had an increased water-holding capacity (WHC + ~30%), which influenced the dough viscosity and elasticity and thus its fermentation and baking properties. To evaluate the effect of mechanical starch modification (MSM) and the resulting alterations in dough hydration on dough functionality during proofing and baking, dough was produced from wheat flour with various MSM levels (4.78–8.37 g 100 g^{-1} flour) and water additions (58–108 g 100 g^{-1} flour). Compared with the standard, the maximum dough height (Hm) during proofing and the specific bread volume after baking decreased significantly using high-MSM flours despite various water additions. During proofing, the time of porosity (T_x) decreased in dough produced from high-MSM flour with adapted water addition compared with the standard. However, the gas retention coefficient was almost 100% in dough produced from high-MSM flour with optimum water addition at 60 min fermentation time. The study reveals that the β-amylase activities of the flours dropped from 30.65 U g^{-1} to 22.05 U g^{-1} with increased MSM caused by mechanical flour treatment in the ball mill. Therefore, it was assumed that gas production in dough produced from high-MSM flour might be reduced compared with the standard, which would negatively affect the dough height during proofing. However, the results demonstrate that decreased enzymatic activity with high MSM levels did not affect gas production. The gas production of dough produced from high MSM was not significantly different compared with the standard dough at 60 min fermentation time when using the optimum water addition for dough preparation according to AACC International Method 54-21.02. The substrate availability appeared to prevail the effect of reduced enzyme activity. The poor bread quality therefore could not be immediately associated with a reduced gas-holding capacity, or rather reduced gas production, during proofing.

Contribution: The doctoral candidate co-designed and conducted the survey, performed the data management and analyses, and drafted, revised, and approved the final manuscript.

3.2 Mechanical wheat flour modification and its effect on protein network structure and dough rheology

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Mechanical wheat flour modification and its effect on protein network structure and dough rheology

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A R T I C L E I N F O

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A B S T R A C T

Mechanical flour modification is frequently associated with a reduced bread volume due to changed structural and functional properties of protein and starch. To clarify the effect of mechanical flour treatment on the protein network formation at the optimum kneading time ($\text{Peak}_{\text{time}}$), dough was produced with various mechanical starch modification (MSM) levels and visualized by confocal laser scanning microscope before being characterized by protein network analysis (PNA). Dough produced with high MSM showed a reduced branching rate (−14%), a high end-point rate (+25%) and an increased lacunarity (+139%), indicating a poor network connectivity with network interruptions. Alterations of the protein microstructure were closely related to the rheological dough properties. In this regard, reduced extensibility and resistance to extension of dough produced with high MSM levels were responsible for decreased dough height (Hm) during fermentation and thus might be the cause for lower baking volume of bread produced with high MSM.

1. Introduction

Mechanical flour treatment has an influence on the biopolymers of flour and thus on its structural and functional properties. The level of mechanical flour modification is commonly characterized by the value of starch damage. Starch damage is a collective term, describing a variety of effects on different structural levels of the polysaccharide (Li, Dhital, & Hasjim, 2014). In the study at hand, the term "mechanical starch modification" (MSM) was used instead of starch damage according to Hackenberg, Verheyen, Jekle, and Becker (2017). In general, the evaluation of the flour modification level is only in consideration of starch but fails to recognize the protein component, which may be also affected by grinding. However, the MSM level is currently the most appropriate method for describing the flour modification degree. Many studies examined the effect of mechanical flour treatment on the structural and functional properties of starch and protein (Di Stasio, Vacca, Piciocchi, Meccariello, & Volpe, 2007; Ivanova, 2006; Li et al., 2014; Licon, 1995; Pojić, Spasojević, & Atlas, 2014; Yu et al., 2015). The investigation of mechanically modified wheat starch granules ground by a disk mill were done by using scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM). The native starch exhibited a smooth surface, whereas the severely modified starch granules showed a high degree of roughness and less uniformity (Barrera, Calderón-Domínguez, et al., 2013). The hydration properties of flour were crucially influenced by the MSM level. Thus, the water holding capacity (WHC) was significantly enhanced with high MSM (Hackenberg et al., 2017). Furthermore, mechanically modified starch had the ability to swell in cold water (Barrera, Bustos, et al., 2013). The cold swelling of mechanically modified starch (Barrera, Bustos, et al., 2013) might increase its relative phase volume in the dough and conceivably affect the dough development mainly based on the protein network development. In addition to the effect of starch, grinding also influenced the chemical and functional characteristics of the wheat protein (Ivanova, 2006; Licon, 1995). The solubility of albumin/globulin, gliadin and the HMG protein fraction was reduced after impact grinding (Licon, 1995). Alterations of the protein component could affect the protein network formation during kneading. In the course of our study, flour was mechanically modified by grinding in a ball mill. The application of wheat flour re-ground by a ball mill significantly reduced the maximum dough height (Hm) during proofing with increasing flour modification or rather MSM level (Hackenberg et al., 2017). The specific bread volume was negatively affected using flours with high MSM levels despite optimum bulk water addition (Barrera, Pérez, Ribotta, & León, 2007; Brütsch, Huggler, Kuster, & Windhab, 2017; Hackenberg et al., 2017). The reason for a lower quality of bread produced from high MSM flours has not yet been clarified in detail. It is assumed that the lower baking quality was caused by decreased gas retention capacity as a consequence of starch degradation during

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proofing (Barrera et al., 2007) or due to weakening of the protein network structure (Brütsch et al., 2017). Contrary, Hackenberg et al. (2017) demonstrated that the gas holding capacity of dough produced with high MSM using the optimum water addition was not reduced during a fermentation cycle of 60 min. However, the maximum dough height (Hm) and the specific bread volume was significantly lower. Thus, the reduced bread volume could not immediately be associated with a reduced gas retention capacity during proofing (Hackenberg et al., 2017). Another explantation for poor quality of bread produced of high MSM levels was the incomplete formation of the gluten structure caused by altered starch functionality (Barrera et al., 2007). There is some evidence that mechanical flour modification affects the dough microstructure formation and the dough rheological properties, such as the dough elongation behavior. Nevertheless, the consequence of altered structure-function relationship of starch and protein on the dough development, particularly on the protein network formation as well as on the dough rheology at the optimum kneading time ($\text{Peak}_{\text{time}}$) has not been examined yet.

The objective of this study was to clarify the effect of mechanical flour modification on the maximum dough height (Hm) during fermentation by analyzing the influence of the MSM level on the protein network development and the rheological dough properties after kneading ($\text{Peak}_{\text{time}}$; according to AACC method 54.21). To achieve significantly higher MSM levels, commercial wheat flour (ground by a roller mill) was re-ground by a ball mill. The protein microstructure of dough produced with various mechanically modified flours was visually evaluated by CLSM. The quantitative evaluation of the protein structure was carried out with the protein network analysis (PNA). To clarify the relation between Hm and the biaxial dough elongation, the maximum dough height was correlated with the dough extensibility and the resistance to extension.

2. Experimental

2.1. Materials

The following values of mechanical starch modification (MSM) measurements of the flour samples were analyzed amperometrically (SDmatic, Chopin Technologies, Villeneuve la Garenne, France) according to AACC 76-33 (starch damage) in the study of Hackenberg et al. (2017). The MSM level of the standard wheat flour of Type 550 (ground by a roller mill) was 4.78 g 100 g^{-1} flour. The standard flour was re-ground in a ball-mill (ATR-315, Alpine Hosokawa, Augsburg, Germany) by variation of the process parameters settings, such as rotational speed and retention time in order to cause significantly higher MSM levels of the flours. The obtained MSM levels were 6.56, 7.46, 7.85 g 100 g^{-1} flour. Additionally, the flour sample of MSM value 8.15 g 100 g^{-1} flour was produced with the same procedure and the same raw material. Further flour specifications can be obtained from Hackenberg et al. (2017).

2.2. Flour particle sizes

The flour particle sizes of the various mechanical modified flours as well as of the flour-water-suspensions produced from them, were analyzed with a Mastersizer 3000 (Malvern, Herrenberg, Germany). Laser diffraction within a measuring range from 0.01 to 3500 µm using a red He-Ne-laser (632.8 nm) was applied. For determining the particle sizes of the flours, the samples were transferred into an automated dry powder dispersion unit (Aero S), before analysis was performed. For evaluating the swelling properties of the flour particles, flour-water-suspensions with various MSM levels were prepared. For this purpose, 10 ml bi-distilled water was mixed with 0.5 mg flour sample for 20 s by using a vortex mixer (Vortex-Genie2, Scientific Industries Inc., Bohemia, New York). Furthermore, flour-water-suspensions were admitted into a manual wet dispersion unit (Hydro EV) containing distilled, deaerated water (room temperature), until achieving an obscuration value of 5%. Particle size values based on the number of particles as well as on the volume of particles are shown as *d90 (*90% of the particles are smaller than this value)*, d50* (mean particle size) and *d10* (10% of the particles are smaller than this value). Five measurements were taken from each sample. Analysis was performed in triplicate.

2.3. Dough preparation

Dough was produced with wheat flour (100 parts), compressed yeast (3 parts) of *Saccharomyces cerevisiae* obtained from Wieninger (Passau, Germany) and water. The dough produced for protein network analysis (PNA) and rheological dough characterization contained no yeast. According to the AACC method 54.21 a torque measuring Z-kneader system (DoughLab; Perten Instruments, Germany) was used for determining the optimum water addition of the flours, which was defined as achieving a FU line between 480 and 520. The optimum bulk water amount of the flour samples was analyzed in the study of Hackenberg et al. (2017). The optimum water addition per 100 g flour was 58 g, 63 g, 73 g, 78 g and 83 g with increasing MSM level. The dough development time ($\text{Peak}_{\text{time}}$) according to the AACC method 54.21 (DoughLab method) complied with the maximum dough consistency during kneading, which was reached before the consistency drops again.

2.4. Visual evaluation of dough microstructure

The protein in dough was stained with Rhodamine B (Merck, Darmstadt, Germany) by the application of the bulk water technique described by Lucas, Stauner, Jekle, and Becker (2017). To receive a homogenous distribution of the dye, a part of the bulk water was replaced by Rhodamine B solution (0.1 g 1 L^{-1} water). The dye-water-solution was added during kneading (Lucas et al., 2017). To visualize the starch granules, 10 µl of Nilblue solution (AppliChem GmbH, Darmstadt, Germany) was added onto the surface of the Rhodamine B stained dough samples. For CLSM measurement 2 g of the dough was placed on a microscope slide with a cylindrical score (Ø 18 mm, height 7 mm), before being sealed with a coverslip (24 × 50 mm). The samples were examined by an eclipse Ti-U inverted microscope with an e-C1 plus confocal system (Nikon GmbH, Düsseldorf, Germany) with a Plan Apo 20 ×/0.75 objective. For visualization, a laser with a wavelength of 534 nm (emission 590/50 nm) was used. All doughs were analyzed after optimum kneading ($\text{Peak}_{\text{time}}$).

2.5. Quantitative analysis of dough microstructure

Dough was produced in triplicate, whereby eight independent images with a resolution of 1024 × 1024 pixel and an image size of 686 × 686 µm were taken from each sample. The quantitative characterization of the protein network was followed using the PNA according to Bernklau, Lucas, Jekle, and Becker (2016) with AngioTool64 version 0.6a (National cancer Institute, National Institute of Health, Maryland, USA). The analysis of each image was performed with the following settings: protein diameter 2 and 3, low and high threshold 15–255, small particles were removed under 10 and the function "fill holes" was deactivated. Calibration was set on 1.49 pixel/µm. The protein network was evaluated using the attributes branching rate (number of junctions per protein area), end-point rate (number of endpoints per protein area), mean lacunarity (degree of gaps and irregularities), average protein length (average length of protein threads) and protein width (protein area per total length).

2.6. Empirical rheology

To evaluate biaxial elongation properties of dough produced with various MSM levels rheological dough properties were measured with a texture profile analyzer (TPA) by using a SMS/Kieffer Extensibility Rig (Stable Micro Systems Ltd., Godalming, United Kingdom). The dough samples were inserted into the Kieffer sample plate directly after kneading. Afterwards the samples were equilibrated in the clamping device for 20 min at 30 °C and 90% RH. The settings were initial speed $2\,\text{mm}\,\text{s}^{-1}$; test speed $3.3\,\text{mm}\,\text{s}^{-1}$, retaining speed $10\,\text{mm}\,\text{s}^{-1}$, distance 75 mm and triggering force 0.05 N. The output values were the extensibility (E_{max}), which is the distance in mm until the dough starts to disrupt and the resistance to extension (R_{max}) characterized by the force (N), which is necessary for dough rupture. Analyses were performed in triplicate.

2.7. *Maximum dough height during fermentation*

The maximum dough height (Hm) was measured with a Rheofermentometer F3 from Chopin (Chopin Technologies; Villeneuve la Garenne Cedex, France). The doughs were produced as described in the section 'dough preparation'. After optimum kneading, 315 g of the dough was proofed in a fermentation basket during a fermentation cycle of 180 min at 30 °C. Measurements were conducted as triplicates.

2.8. *Statistical analysis*

The statistical analysis was carried out with Graph Pad Prism (Version 5.03, GraphPad Software, Inc., La Jolla, USA). Significant differences between the samples were evaluated using a one-way analysis of variance (ANOVA; 5% significance level) followed by Tukey's Multiple Comparison Test. Correlation tests were applied in order to investigate linear relationships. The goodness of the fit was described as Pearsons Coefficient of Variation (r). Results are shown with mean value including the standard error of mean (SEM).

3. Results and discussion

3.1. *Maximum dough height during fermentation depending on mechanical flour modification and dough rheology*

The maximum dough height (Hm) during fermentation was closely related to the bread volume (Gandikota & MacRitchie, 2005) and therefore it is a good attribute for predicting the final product quality. Fig. 1a visualizes a high linear correlation between the MSM level and the maximum dough height (Hm) achieved during 180 min fermentation ($r = -0.97$, $p < .001$). The maximum dough height (Hm) was significantly reduced by 54% with heightened MSM level of the flour. Dough expansion capacity during fermentation and baking strongly depended on dough rheological characteristics (Dobraszczyk & Salmanowicz, 2008; Kenny, Wehrle, Dennehy, & Arendt, 1999; Kieffer, Wieser, Henderson, & Graveland, 1998). Biaxial dough expansion, as it occurs during fermentation, can be adjusted with rheological methods, such as texture profile analysis (TPA) with a SMS/Kieffer Extensibility Rig, describing extensional dough behavior. The resistance to extension (R_{max}) characterized by the force required for dough rupture, proved to be a suitable attribute to assess the bread volume (Dobraszczyk & Salmanowicz, 2008; Kenny et al., 1999). The results of our study clearly demonstrate that altered rheological dough behavior, such as decreased R_{max} and reduced extensibility (E_{max}) of doughs produced with high MSM levels were strongly correlated with a low Hm during 180 min of fermentation. The maximum extensibility (Ext_{max}) was 28% lower for dough produced with high MSM levels (Fig. 1b). The resistance to extension (R_{max}) of dough produced with highly mechanically modified flour was significantly reduced by 49% in comparison to the standard dough (Fig. 1c). The rheological properties of suspensions and dough depended on the structure and functionality of flour biopolymers (Barrera, Bustos, et al., 2013; Licon, 1995). The dough rheology was also influenced by protein-starch interactions (Petrofsky & Hoseney, 1995). It seems that starch is not only acting as a filler particle in the protein phase but its surface is also interacting with the surrounding gluten and is influencing the dough rheological behavior (Edwards, Dexter, & Scanlon, 2002). Mechanical treatment in a mill changed the starch granule surface (Barrera, Calderón-Domínguez, et al., 2013) and thus might influence starch-protein and starch-starch interactions, resulting in alterations of the dough rheological behavior. Petrofsky and Hoseney (1995) presumed a relation between the dough extensibility and gluten-starch interactions. Soft wheat gluten doughs had a lower extensibility (high values of elastic and loss moduli) in comparison to doughs produced from hard wheat, which is possibly caused by enhanced starch-gluten interactions (Petrofsky & Hoseney, 1995). It can further be assumed that the chemical and functional characteristics of protein and starch in combination with its interaction affect the formation of the visco-elastic dough matrix during kneading. The protein microstructure influenced the dough rheological behavior (Bernklau et al., 2017; Jekle & Becker, 2011). Dough with a low protein network connectivity, as a consequence of a reduced protein branching rate, was characterized by a decreased R_{max}. Furthermore, a low protein branching rate, a reduced protein length and a high protein width can be related to a low E_{max} of the dough (Bernklau et al., 2017). In our study, the resistance to extension (R_{max}) and E_{max} of doughs produced with high MSM levels were significantly reduced in comparison to the standard dough, indicating a poor protein network connectivity. Brütsch et al. (2017) also observed a reduced R_{max} and E_{max} of doughs produced with highly mechanically treated wheat flours. They suppose that the gluten network structure has been weakened (Brütsch et al., 2017). To clarify the effect of mechanical wheat flour modification on the dough matrix after optimum kneading ($\text{Peak}_{\text{time}}$), the protein microstructure and the starch granule distribution in doughs produced with various MSM levels were analyzed by CLSM using the optimum water addition according to the doughLab method.

3.2. *Protein network analysis of dough depending on mechanical flour modification*

Investigations on hydrated unmixed flours showed a continuous but irregular gluten structure, which was characterized by a low network coherence and a large number of open areas. The starch granules were only partially enclosed by the protein (Unbehend, Lindhauer, & Meuser, 2004). Thermal or mechanical energy input combined with water addition transferred the protein strands into a continuous, hyperbranched gluten structure stabilized by various bonds, such as disulfide crosslinks (Faridi & Faubion, 1990; Létang, Piau, & Verdier, 1999; Van der Mijnsbrugge, Auger, Frederix, & Morel, 2016). During this process, the starch granules were embedded into the filamentous protein structure (Amend & Belitz, 1991; Létang et al., 1999). The hydration of flour components in conjunction with the formation of gluten strands caused an increase of the dough viscosity up to a maximum (Auger, Morel, Lefebvre, Dewilde, & Redl, 2008). The maximum dough consistency is commonly associated with the optimum kneading time ($\text{Peak}_{\text{time}}$) in the doughLab and a good bread quality (Faridi & Faubion, 1990). Van der Mijnsbrugge et al. (2016) visualized widespread allocated protein filaments throughout the dough as the dough consistency elevated to $\text{Peak}_{\text{time}}$, whereas only few residual gluten agglomerates can be detected. An optimal gluten network is developed when the gluten is fully in a filamentous structure and completely infused the dough (Auger et al., 2008). The microstructure of dough can be visualized by CLSM. The CLSM image of the standard dough (58 g water $100\,\text{g}^{-1}$ flour; 500 FU ± 4%) illustrates a homogenous gluten matrix characterized by a hyperbranched, regular structure with relatively uniform and separately allocated starch granules (Fig. 2a). In contrast, the dough produced with increased MSM level had a heterogenous gluten structure with areas comprising mainly starch accumulations at $\text{Peak}_{\text{time}}$ (Fig. 2b and Fig. 2c). This phenomenon of separated gluten and starch was also visualized in the initial phase of kneading (Peighambardoust, van der

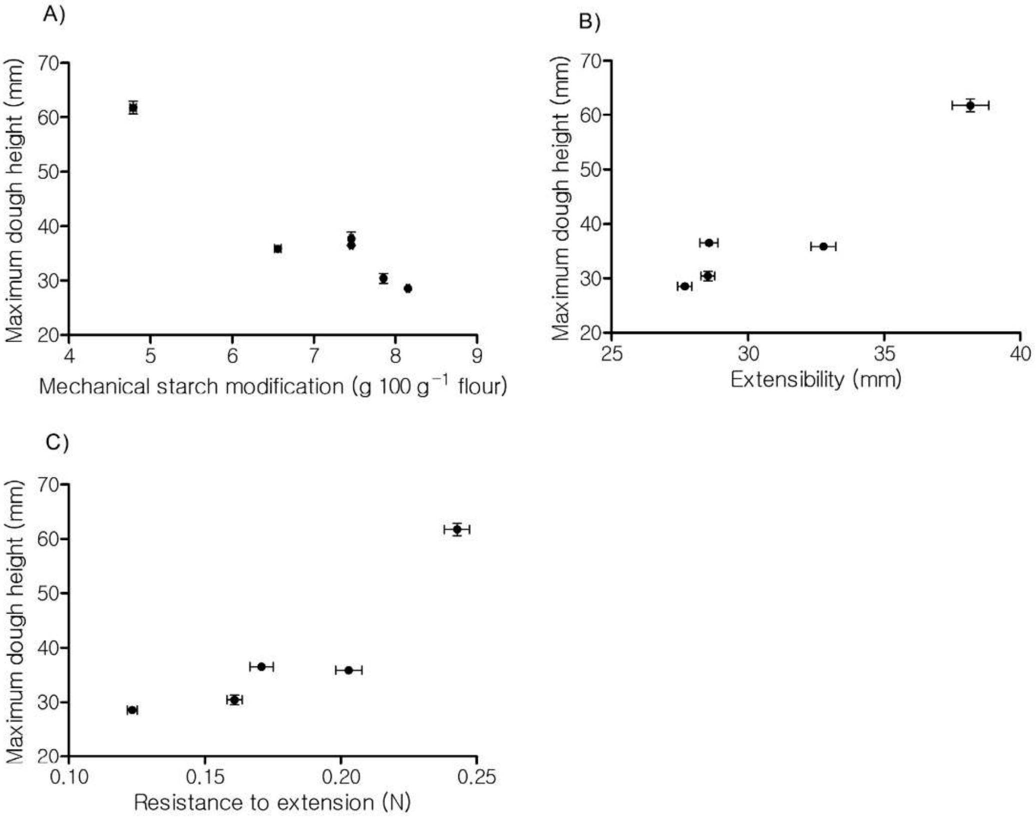

Fig. 1. (a) Maximum dough height (Hm) during 180 min of fermentation, depending on the MSM level of wheat flour as well as on the (b) extensibility and (c) the resistance to extension of the dough. All doughs were produced according to the doughLab method using the optimum water addition. To evaluate significant differences between the samples a one-way ANOVA (5% significance level) followed by Tukey's Multiple Comparison Test was applied. Means are shown with standard error of mean (n = 3).

Goot, van Vliet, Hamer, & Boom, 2006). In general, the irregular allocated protein domains should be transferred into a homogenous gluten structure with embedded starch granules at the optimum kneading time ($Peak_{time}$). The visual evaluation of the CLSM images of the doughs produced with high MSM levels indicate that $Peak_{time}$ seems not to be the optimum kneading time when using highly mechanically modified flours.

While the protein network was evaluated visually above, the protein microstructure of dough produced with various MSM levels was analyzed quantitatively at $Peak_{time}$ by image processing using PNA according to Bernklau et al. (2016). The branching rate of the protein network of dough made from highly mechanically modified flour was significantly reduced by 14% in comparison to the branching rate of dough prepared with the standard flour (Fig. 3a). This indicates that the protein network was less interconnected, resulting in a reduced viscoelastic behavior of the dough (Bernklau et al., 2016). The end-point rate of the gluten network of dough produced with high MSM levels was 25% higher than of dough produced with the standard flour (Fig. 3b). Furthermore, observing the mean lacunarity of the high MSM doughs, it can be shown that the structural irregular form significantly increased

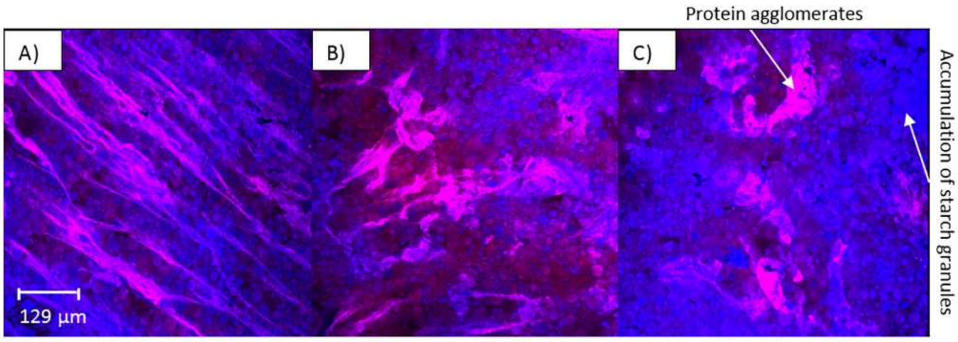

Fig. 2. Microstructure of dough produced with (a) standard wheat flour (MSM level of 4.78 g 100 g^{-1} flour), (b) MSM level of 7.46 g 100 g^{-1} flour and (c) MSM level of 8.15 g 100 g^{-1} flour was visualized by CLSM The starch granules were dyed with Nilblue (blue), whereas the protein was stained with Rhodamine B (red). Doughs were produced according to the doughLab method using the optimum water addition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

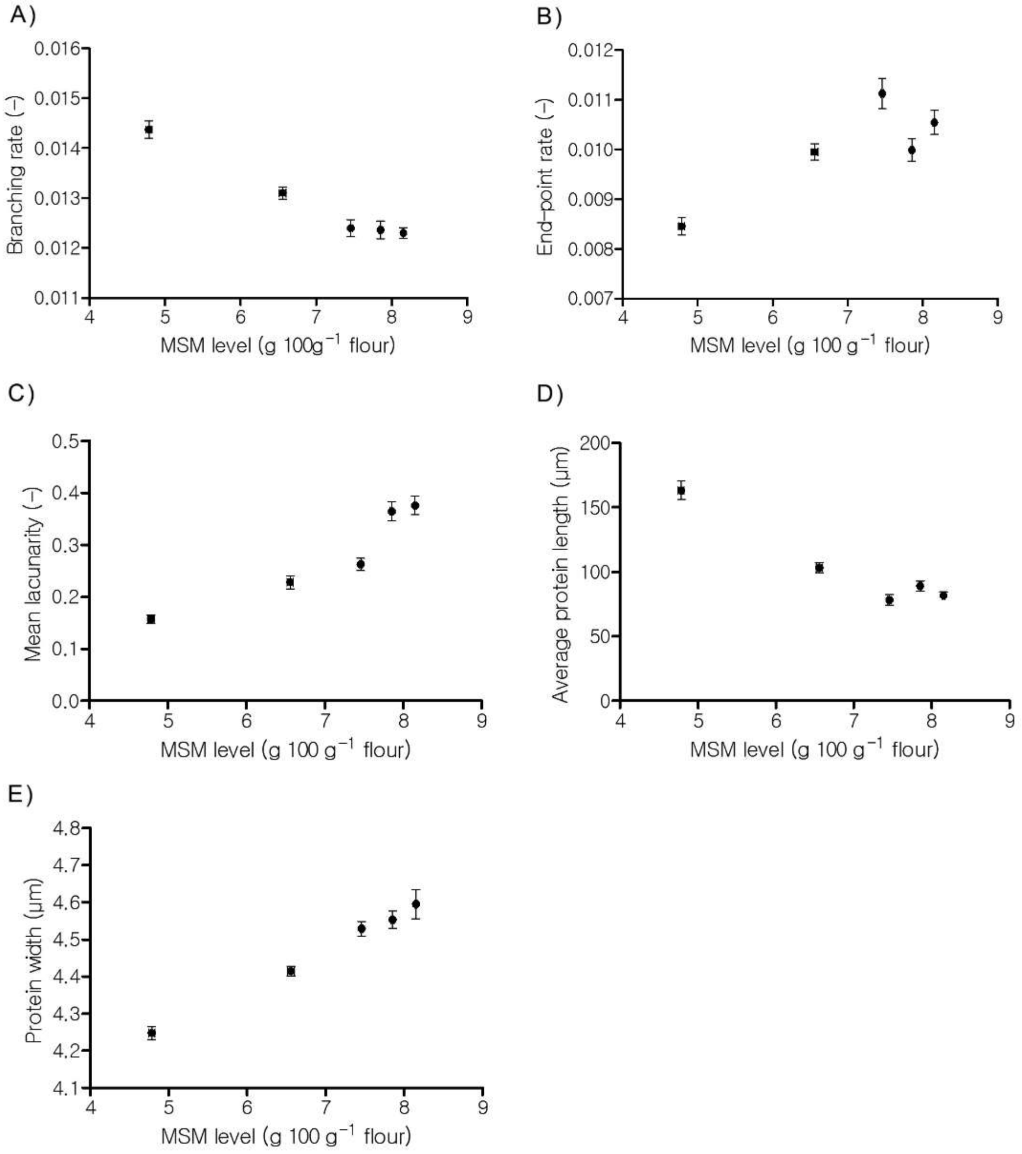

Fig. 3. Protein network characteristics of dough produced with mechanical starch modification (MSM) levels of 4.78–8.15 g 100 g^{-1} flour were investigated by CLSM. (a) The branching rate, (b) the end-point rate, (c) the mean lacunarity, (d) the average protein length and (e) the protein width were analyzed with PNA according to Bernklau et al. (2016). Doughs were produced according to the doughLab method using the optimum water addition. To evaluate significant differences between the samples a one-way ANOVA (5% significance level) followed by Tukey's Multiple Comparison Test was applied. Means are shown with standard error of mean (n = 3).

by 139% (Fig. 3c). A lower branching rate, higher end-point rate and higher values for lacunarity of dough produced with high MSM point out a poor network connectivity characterized by network interruptions. The average protein length in dough produced with highly mechanically modified flour was 50% lower than that for dough prepared with the standard flour (Fig. 3d), whereas the protein width was 8% higher (Fig. 3e). The protein threads were shorter and thicker indicating, in combination with an increased lacunarity, the formation of protein agglomerates. The results of the PNA were in agreement with the visual evaluation of the CLSM images. A linear correlation between the MSM level (degree of the mechanical flour modification) and the protein network attributes branching rate ($r = -0.99$, $p < .05$), mean lacunarity ($r = 0.93$, $p < .05$), average protein length ($r = -0.96$, $p < .05$) and protein width (0.99, $p < .05$) can be observed. The results of the PNA demonstrate that changes of the protein network structure at Peak$_{\text{time}}$ were directly connected to the MSM level. The results also reveal that the gluten network appears to be insufficiently developed in doughs produced with enhanced MSM level at Peak$_{\text{time}}$. In general, the optimum dough development (Peak$_{\text{time}}$), which is normally characterized by a fully hydration of flour components and a progeressively formed protein network structure can be well determined with the doughLab method when using commercial wheat flours with a moderate MSM level (~4–6 g 100 g^{-1} flour). In our study, the flour with high MSM levels required more bulk water to achieve a dough consistency between 480 and 520 FU in accordance with the doughLab method in comparison to the standard flour caused by increased hydration capacity of mechanically modified starch. Altered functionality of mechanical modified starch, such as cold swelling ability (Barrera, Bustos, et al., 2013) might enhance its relative phase volume in the dough and thus affected the dough development curve when using the doughLab method.

3.3. *The flour particle size, depending on grinding, and its effect on swelling properties*

The energy input during kneading in the doughLab was quite similar between the standard (~0.478 kJ/h) and the dough made from MSM level of 8.15 g 100 g^{-1} flour (~0.482 kJ/h) with adapted water addition at $Peak_{time}$. However, as shown in chapter 3.2, no homogenous protein network structure had been developed in dough produced with MSM level of 8.15 g 100 g^{-1} flour at $Peak_{time}$. This raises the question, what causes the enhance in dough strength during the initial phase of kneading. Several studies observed cold swelling properties of mechanical modified flour or rather starch (Barrera, Bustos, et al., 2013; Hackenberg et al., 2017), which might increase the dough viscosity and thus affect the dough development curve when using the doughLab method. Hence, in the following, the effect of mechanical flour modification on flour particle size and swelling properties will be observed.

In agreement with Oh, Seib, Ward, and Deyoe (1985) and Barak, Mudgil, and Khatkar (2014) mechanical flour treatment reduced the flour particle size (Table 1). Based on the particle number distribution, the d_{90} value was reduced from 10.5 to 6.3 µm (−40%) with increased MSM level. For the d_{50} and the d_{10} value a clear decrease by 36% and 29% can be detected. Based on the volume distribution the same tendency of predominance concerning the flour particle sizes can be observed. Thereby, the d_{90} value was reduced by 62% due to e.g. crushing of protein agglomerates and protein-starch aggregates during grinding. The d_{50} and d_{10} values were also lowered by 66% and 57%. Thus, the degree of fineness of mechanically modified flours rose during ball milling. A high fineness of flour heightened its mass-specific surface, influencing the hydration capacity of flour components (Ivanova, 2006). This changed hydration properties of flour might affect dough processing, such as the dough development during kneading. In the following, the swelling properties of the flour particles were investigated using flour-water-suspensions combined with laser diffraction (Table 2). For this purpose, 10 ml bi-distilled water was mixed with 0.5 mg flour for 20 s by using a vortex mixer (Vortex-Genie2, Scientific Industries Inc., Bohemia, New York). Based on the volume distribution, the particle sizes in the flour-water-suspensions were lower than in the flour samples. This could be explained by a partial separation of protein-starch aggregates as well as protein agglomerates during vortex mixing. Nevertheless, a strong rise in flour particle sizes based on particle number and particle volume distribution in the suspension-models can be perceived with heightened MSM level. Wheat flour contains of mainly two kinds of starch granules: the large A-granules (> 10 µm) and the small B-granules (< 10 µm) (Brocklehurst & Evers, 1977; Peng, Gao, Abdel-Aal, Hucl, & Chibbar, 1999). The particle sizes based on the number distribution mainly focused on the small B-granules, which account for up to 98% of granules in wheat flour depending on the wheat variety (Evers & Lindley, 1977). The particle sizes, based on the volume distribution describe protein agglomerates or rather protein-starch aggregates. The swelling behavior of the larger A-granules, comprising ~58–90% (Raeker, Gaines, Finney, & Donelson, 1998; Zhang et al., 2016) regarding to the volume percentage were also taken into account. Barrera, Bustos, et al. (2013) analyzed the swelling behavior of mechanically modified wheat starch in starch-water-suspensions at room temperature. They observed an increase of the d_{50} (+ ~15%) and d_{90} (+ ~36%) value with high MSM levels. The d_{10} value was not influenced. The particle diameter and the relative volume of the large particles increased with high MSM level. Barrera, Bustos, et al. (2013) presume that improved hydration and swelling properties of mechanical modified starch by alterations of its granular surface (peel-off) were the cause of this effect. In our study a growth of the d_{50} (+25%) and the d_{90} (+31%) value referred to the volume-based particle size can also be observed. The d_{10} value rose by only 6%. In the study at hand not starch but wheat flour was analyzed, which had a similar swelling behavior in water at room temperature as the wheat starch samples of Barrera, Bustos, et al. (2013). It can be assumed that heightened swelling of mechanically modified wheat flour was mainly caused by enhanced MSM. The cold swelling properties of mechanically modified starch effect its relative phase volume in the dough and thus influenced the initial dough strength or rather the dough development during kneading. Considering our results, there is some evidence that dough produced with high MSM level was not sufficiently kneaded at $Peak_{time}$ but rather swelling of mechanically modified starch increased the dough viscosity and thus affected the formation of a protein network. The poor network connectivity in dough produced with high MSM level at $Peak_{time}$, in turn, had a negative effect on the rheological dough properties, resulting in a lower Hm during proofing and thus might be the reason for lower baking quality of bread produced with high MSM levels. The results clearly demonstrate that mechanical flour modification had a strong influence on the protein network functionality at $Peak_{time}$. As starch is acting as a filler particle in the gluten network it can be assumed that its enhanced phase volume influenced the protein network formation, the protein microstructure and the rheological dough properties. Furthermore, mechanical treatment in a mill led to alterations of the starch granule surface (Barrera, Calderón-Domínguez, et al., 2013). It is recognized that its surface interacts with the surrounding gluten (Edwards et al., 2002). Altered starch-protein and starch-starch interactions could therefore affected the dough rheological behavior. Moreover, the mechanical flour treatment can influence the protein structure and functionality. In our study, the temperature of the flours was measured after grinding and kept below the protein denaturation temperature (around 40 °C). The applied steel balls still had a temperature of ~60 °C measured directly after grinding. Several studies have investigated the temperature increase of the balls during grinding in different mills depending on the ball diameter, the milling power, and the beaker size (Kwon, Gerasimov, & Yoon, 2002; Takacs & McHenry, 2006). Takacs and McHenry (2006) revealed that the temperature of the balls was above 100 °C after 10 min grinding in a planetary mill using a 7.5 cm bowl, 50 balls and 280 rpm. In general, a heat transfer between the balls and the ground material can occur by

Table 1
Particle size of the mechanically modified dry wheat flour based on the number and on the volume of particles is shown as d_{10} (10% of the flour particles are smaller than this value), d_{50} (mean value) and d_{90} (90% of the flour particles are smaller than this value). Means are shown with standard error of mean (n = 3).

MSM level (g 100 g^{-1} flour)	Flour Particle size based on number of particles (µm)			Flour particle size based on volume of particles (µm)		
	d_{10}	d_{50}	d_{90}	d_{10}	d_{50}	d_{90}
4.78	2.5 ± 0.2	3.6 ± 0.3	10.5 ± 1.7	21.1 ± 2.4	82.0 ± 2.7	189 ± 5.8
6.56	2.0 ± 0.3	2.9 ± 0.4	8.3 ± 1.7	15.2 ± 0.6	53.0 ± 1.5	146.0 ± 12.0
7.46	1.8 ± 0.0	2.8 ± 0.0	7.4 ± 0.0	11.7 ± 0.1	35.6 ± 0.7	99.6 ± 11.4
7.85	1.7 ± 0.1	2.7 ± 0.1	7.0 ± 0.3	10.7 ± 0.0	31.6 ± 0.0	82.3 ± 1.0
8.15	1.6 ± 0.1	2.5 ± 0.1	6.3 ± 0.2	9.1 ± 0.1	28.0 ± 0.4	71.3 ± 6.0

Table 2
Size of swollen flour particles with various MSM levels based on the number and on the volume of particles is shown as d_{10} (10% of the flour particles are smaller than this value), d_{50} (mean value) and d_{90} (90% of the flour particles are smaller than this value). Means are shown with standard error of mean (n = 3).

MSM level (g 100 g^{-1} flour)	Size of swollen flour particles based on number of particles (µm)			Size of swollen flour particles based on volume of particles (µm)		
	d_{10}	d_{50}	d_{90}	d_{10}	d_{50}	d_{90}
4.78	2.6 ± 0.0	3.7 ± 0.1	8.6 ± 0.6	8.1 ± 1.2	22.0 ± 1.0	39.5 ± 1.7
6.56	2.9 ± 0.0	4.2 ± 0.0	9.6 ± 0.2	7.9 ± 0.5	23.1 ± 0.6	43.2 ± 1.1
7.46	3.5 ± 0.0	5.1 ± 0.0	11.0 ± 0.1	8.4 ± 0.2	26.3 ± 0.5	50.1 ± 1.1
7.85	3.5 ± 0.0	5.1 ± 0.1	11.1 0.1	8.5 ± 0.2	27.0 ± 0.3	50.7 ± 0.5
8.15	3.7 ± 0.0	5.4 ± 0.0	11.3 ± 0.1	8.6 ± 0.2	27.5 ± 0.6	51.7 ± 0.7

means of convection. It can be assumed that the local temperature obtained during grinding in a ball mill has probably been much higher than the protein denaturation temperature caused by ball-flour particle friction and collision, which could influenced the protein. Temperatures above 70 °C affected the chemical and functional properties of the protein: the protein solubility and the maximum shear stress were strongly reduced (Mann, Schiedt, Baumann, Conde-Petit, & Vilgis, 2014). Licon (1995) demonstrated that the energy input during impact grinding caused a decrease of the protein solubility. A reduced protein solubility indicates the formation of protein agglomerates by oxidation of SH-groups to S—S-bonds (Licon, 1995; Mann et al., 2014). Additionally, not only the temperature but also the moisture content of the heat-treated flours were important factors influencing the protein aggregation during grinding. The protein molecules had a higher mobility with increasing moisture content of the flours and thus conformational alterations were facilitated (Mann et al., 2014). Due to the formation of S—S bonds during grinding, the proteins might be fixed in a more compact state and thus impeded to re-structure during kneading. Therefore, it can be assumed that more energy is required to re-structure S—S-bonds as in a standard wheat dough development. Hence, the network connectivity in doughs produced from high MSM levels could be improved by higher energy input during kneading or rather a longer kneading time.

4. Conclusions

This study revealed that doughs produced with enhanced MSM levels were not fully developed at $\text{Peak}_{\text{time}}$, which is normally defined as the optimum kneading time. The increase of the dough viscosity in the initial kneading phase can be primarily explained by cold swelling of mechanically modified starch but not by the formation of a completely interconnected protein network. In contrast to the standard dough, which had a continuous cross-linked gluten structure with embedded starch granules, the doughs produced with high MSM levels showed mainly gluten agglomerations with separated spots of starch granules at $\text{Peak}_{\text{time}}$ may be caused by changes of starch or rather protein structure and functionality as a consequence of mechanical flour treatment. The protein microstructure of doughs produced with high MSM levels was characterized by a low branching rate, a high end-point rate and high values for lacunarity, resulting in a poor network connectivity with network interruptions at $\text{Peak}_{\text{time}}$. Therefore, the doughs produced with high MSM levels had a low extensibility and a low resistance to extension. These rheological dough characteristics were highly correlated with the maximum dough height (Hm) during fermentation. A reduced dough extensibility as well as a decreased resistance to extension caused a low Hm during fermentation and thus might be responsible for lower baking volume of bread produced with high MSM levels. The study clearly demonstrate that grinding had an influence on the development of the dough matrix during kneading and hence affected the dough rheology and the final baking quality. The results indicate that gluten stretching and the protein network connectivity in doughs produced from high MSM levels might be improved by increasing energy input during kneading induced by re-structuring of S—S-bonds. Consequently, the final bread quality might be enhanced by the adaption of the process parameter settings during kneading.

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3.3 Impact of altered starch functionality on wheat dough microstructure and its elongation behavior

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Impact of altered starch functionality on wheat dough microstructure and its elongation behaviour

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ABSTRACT

The effect of kneading on dough microstructure development, dough elongation and bread volume was investigated using wheat flour with a high mechanical starch modification (MSM) level. The resistance to extension (R_{max}) of dough produced from wheat flour with a high MSM level increased by 52.5% because of higher protein network connectivity with prolonged kneading time. The improved network structure was caused by an increased protein branching rate (+14.8%), a decreased protein end-point rate (−24.3%) and a decreased mean lacunarity (−64%). R_{max} was highly correlated with specific bread volume ($r = 0.97$, $P < 0.05$) only if the dough was not over-kneaded. Kneading time adaptation of the dough produced from high MSM flour significantly increased specific bread volume by 24.4%. Differences compared with the standard can be attributed to weakened network connectivity because of weakened protein interfacial interactions and larger cavities within the gluten network, both caused by starch swelling.

1. Introduction

Mechanical wheat flour treatment such as grinding modifies starch structure and functionality (Li, Dhital, & Hasjim, 2014). Therefore, the degree of mechanical wheat flour modification is commonly measured as the mechanical starch modification (MSM) level, previously known as starch damage. The use of mechanically modified wheat flour is associated with improved storage properties of bread because of the enhanced water retention capacity of mechanically modified wheat starch. However, the bread volume is significantly decreased when wheat flour with a high MSM level is used (Barrera, Pérez, Ribotta, & León, 2007; Brütsch, Huggler, Kuster, & Windhab, 2017; Hackenberg, Verheyen, Jekle, & Becker, 2017). In general, wheat dough properties are strongly dependent on dough development, particularly its microstructure (Bernklau, Lucas, Jekle, & Becker, 2016; Hackenberg, Jekle, & Becker, 2018; Jekle & Becker, 2011). Dough development is influenced by the hydration of flour ingredients and kneading parameters (Auger, Morel, Lefebvre, Dewilde, & Redl, 2008; Kilborn & Tipples, 1972a,b; Letang, Piau, & Verdier, 1999; Peighambardoust, Van der Goot, Van Vliet, Hamer, & Boom, 2006; Van der Mijnsbrugge, Auger, Frederix, & Morel, 2016). The optimum water addition and kneading time of wheat dough is commonly determined with rheological methodologies, such as AACC International Method 54–21. During the analysis, dough is kneaded until a maximum consistency is achieved, centred on the 500 ± 4% farinograph unit (FU) line, which is in accordance with the dough development time ($Peak_{time}$). During this, widespread protein filaments streak through the dough (Van der Mijnsbrugge et al., 2016), such that starch granules are enclosed within the gluten matrix (Letang et al., 1999).

Mechanical flour treatment in a ball mill may influence dough development during kneading by altering the functionality of wheat flour ingredients such as starch and protein. Starch granules in dough produced from wheat flour with a moderate MSM level of $4.78\,g\ 100\,g^{-1}$ flour (ground using a roller mill) are relatively uniformly dispersed within the cross-linked protein structure at $Peak_{time}$. With regard to dough produced from highly mechanically modified wheat flours with MSM levels between 7.46 and $8.15\,g\ 100\,g^{-1}$ flour (re-ground using a ball mill) at $Peak_{time}$, it can be observed that the gluten network is not completely interconnected and starch granules form dense accumulations (Hackenberg et al., 2018). This effect occurred although approximately equivalent amount of energy was applied during kneading.

A linear correlation between the water-holding capacity of wheat flour and the MSM level was shown by Jakobi, Jekle, and Becker (2018). Furthermore, mechanically modified wheat starch has the ability to swell in water at room temperature (Barrera, Bustos, et al., 2013), resulting in an increased volume of starch if water is added to

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the flour during dough preparation. In the presence of a liquid, adhesion forces are induced by the formation of capillary bridges in the cavity between adjacent particles (Dörmann & Schmid, 2015). Dörmann and Schmid (2015) observed that the capillary force enhances with increasing particle size. Thus, swollen starch granules can interact more strongly with each other. In addition, mechanical flour treatment influences the starch granule surface (Barrera, Calderón-Domínguez, et al., 2013); therefore, interactions between the granule surface and the surrounding protein at the molecular level are probably altered during dough preparation.

Mechanical flour treatment affects not only starch but also protein in wheat flour. Licon (1995) revealed that wheat protein solubility decreases as a consequence of impact milling. A decreased protein solubility indicates that gluten is aggregated (Mann, Schiedt, Baumann, Conde-Petit, & Vilgis, 2014). Protein agglomeration is associated with oxidation reactions, such as the oxidation of thiol (SH) groups to disulphide bonds (Licon, 1995). Thus, the application of higher energy may be required to re-structure disulphide bonds during kneading. Moreover, this may be a critical factor in protein microstructure formation.

This study hypothesizes that the microstructural protein network development of dough produced from wheat flour with a high MSM level can be improved by kneading process adaption, which may positively influence dough elongation during proofing and specific bread volume after baking. However, to date, no quantitative analysis of the protein microstructure of dough produced from high MSM wheat flour has been performed with regard to kneading time. Furthermore, the effect of prolonged kneading and the associated alterations of the rheological dough behaviour and the influence on the final baking quality of bread have not yet been explored.

2. Experimental

2.1. Materials

The following values of wheat flours were analysed in the studies by Hackenberg et al. (2017) and Hackenberg et al. (2018). MSM levels of type 550 wheat flour obtained after roller milling and grinding in an industrial ball mill (ATR-31; Alpine Hosokawa, Augsburg, Germany) were 4.78 g 100 g^{-1} flour (standard flour 1) and 8.15 g 100 g^{-1} flour (MSM flour 1), respectively. In addition, two commercially available type 550 wheat flours ground using a roller mill (standard flours 2 and 3) were re-ground using a planetary ball mill (PM 100; Retsch, Haan, Germany) on a laboratory scale with a beaker size of 500 mL with four steel balls of 40 mm diameter. A rotational speed of 500 rpm was applied for 2 min to enhance the MSM levels of these flours, termed as MSM flours 2 and 3. MSM levels were measured amperometrically according to AACC International Method 76-33 (starch damage) using the SDmatic (Chopin Technologies, Villeneuve la Garenne, France). The MSM levels of the standard wheat flours 2 and 3 were 5.09 and 5.51 g 100 g^{-1} flour, respectively. The MSM levels of the flours produced from them using a planetary ball mill on a laboratory scale were 6.92 and 7.33 g 100 g^{-1} flour (MSM flour 2 and 3). The flour moisture content was determined according to AACC International Method 44-01. The moisture contents of standard flours 1, 2 and 3 were 14.1, 14.9 and 14.1 g 100 g^{-1} flour, respectively, whereas those of MSM flours 1, 2 and 3 were 12.8, 13.2 and 12.7 g 100 g^{-1} flour, respectively. The decreased moisture content of the MSM flours is caused by an increase in flour temperature during ball milling. The maximum flour temperature after grinding was below 40 °C.

2.2. Determination of protein solubility

The proteins in standard flour 1 and MSM flour 1 were separated into three different solubility fractions (albumins/globulins, gliadins and glutenins) using a modified Osborne fractionation procedure. The flours (100 mg) were extracted first with (a) salt solution (2 × 1.0 mL; 0.4 mol/L NaCl with 0.067 mol/L Na_2HPO_4/KH_2PO_4, pH 7.6) for 10 min at 22 °C (albumins/globulins); followed by (b) ethanol/water (60:40, v/v; 3 × 0.5 mL) for 10 min at 22 °C (gliadins); and then (c) glutelin solution [2 × 1.0 mL; 2-propanol/water (50:50, v/v)/0.1 mol/L Tris HCl, pH 7.5, containing 2 mol/L (w/v) urea and 0.06 mol/L (w/v) dithiothreitol] for 30 min at 60 °C under nitrogen (glutenins). The suspensions were centrifuged (3750 × g, 20 min, 22 °C) and the respective supernatants were combined to a total of 2.0 mL with the extraction solvents and filtered (0.45 µm). The protein concentrations of the three fractions were quantified with reversed-phase high-performance liquid chromatography (RP-HPLC) using UV absorption at 210 nm and external calibration with Prolamin Working Group (PWG)-Gliadin (Van Eckert et al., 2006; Vogel, Scherf, & Koehler, 2018a; Wieser, Antes, & Seilmeier, 1998).

2.3. Dough preparation and baking trials

Dough was produced from wheat flour and water. The optimum water addition was determined according to AACC International Method 54.21 using a doughLab. Accordingly, dough was kneaded to a consistency of 500 ± 4% FU. Thus, the maximum dough consistency represents $Peak_{time}$. The optimum water additions to standard flours 1, 2 and 3 were 58, 58 and 59 g water 100 g^{-1} flour, respectively. The optimum water additions to MSM flours 1, 2 and 3 were 83, 66 and 68 g water 100 g^{-1} flour, respectively. The values were approximated to two significant digits. The water additions to the high MSM wheat flours were increased compared with those to standard wheat flours because of the enhanced swelling capacity of the starch granules caused by the mechanical treatment during grinding. The fermentation and baking procedure was according to the method described in the study by Hackenberg et al. (2017).

2.4. Qualitative and quantitative analysis of dough microstructure using confocal laser scanning microscopy and protein network analysis

The protein microstructure of the dough produced from standard flour 1 and MSM flour 1 was analysed as a function of kneading time (2.4–30 min) using confocal laser scanning microscopy (CLSM). The protein microstructure of the dough produced from standard flour 1 was shown at 2.4 min ($Peak_{time}$), and that of the corresponding MSM flour was analysed after 2.9 min ($Peak_{time}$), 4 min, 8 min, 16 min (second peak), 21 min, and 30 min. The dough was over-kneaded from 21 min of kneading time.

Protein was dyed with Rhodamine B (Merck, Darmstadt, Germany) using the bulk water technique, which was developed by Lucas, Stauner, Jekle, and Becker (2018). The dough samples were produced in triplicate, such that seven different images with a resolution of 1024 × 1024 pixels and an image size of 686 × 686 µm were required from each sample. The protein microstructure was quantitatively evaluated using the protein network analysis (PNA) method described by Bernklau et al. (2016) with AngioTool64 version 0.6a (National Cancer Institute, National Institute of Health, Bethesda, MD, USA). The protein network was characterised using the following attributes: protein branching rate (number of junctions per protein area), protein end-point rate (number of end-points per protein area), mean lacunarity (degree of gaps), average protein length (average length of protein strands) and protein width (protein area per total length). These protein network attributes of dough produced from standard wheat flour 1 and the corresponding MSM flour 1 were visualized at $Peak_{time}$ in the study by Hackenberg et al. (2018).

2.5. Large-scale rheology using texture profile analysis

The elongation behaviour of the dough produced from standard flour 1 and MSM flour 1 was evaluated using the value of maximum resistance to extension (R_{max}) and the value of maximum extensibility (Ext_{max}) as a function of kneading time. Elongation properties of the dough produced from standard flour 1 were analysed at 2.4 min ($Peak_{time}$). Furthermore, the elongation behaviour of the corresponding MSM flour was analysed after 2.9 min ($Peak_{time}$), 4 min, 8 min, 16 min (second peak) and 21 min (over-kneaded dough). R_{max} and Ext_{max} were determined using a texture profile analyser with an SMS/Kieffer Extensibility Rig (Stable Micro Systems Ltd., Godalming, UK) directly after kneading. The measurement was conducted as described by Hackenberg et al. (2018).

2.6. Statistical analysis

The results were assessed statistically using of GraphPad Prism (Version 5.03; GraphPad Software, Inc., La Jolla, CA, USA) using a one-way analysis of variance (5% significance level) followed by Tukey's multiple comparison test. Linear relationships were investigated using correlation tests. The goodness of fit was shown using Pearson's coefficient of variation (*r*). Results are reported as the mean value ± standard error of mean (SEM).

3. Results

3.1. Dough development curves of mechanically modified wheat flours

Wheat dough resistance is mainly influenced by the hydration of flour components and gluten structure formation during kneading. In the initial phase of kneading, the protein aggregates. Short-term mixing leads to the formation of protein rich areas and spots with mainly starch granules in the dough (Peighambardoust et al., 2006). In the presence of a liquid, capillary forces are formed due to surface tensions at the contact points between particles (Borho, Polke, Wintermantel, Schubert, & Sommer, 1991). The formation of capillary bridges between particles results in an particle-particle adhesion (Dörmann & Schmid, 2015). Because of kneading, accumulated starch granules are separated. Concurrently, the protein network is developed, in which starch granules are quite homogenously embedded (Hackenberg et al., 2018; Peighambardoust et al., 2006). The dough achieves maximum consistency, which is generally in accordance with $Peak_{time}$. Presumably, structural and functional alterations of starch and protein because of mechanical flour treatment in a ball mill affect the dough development during kneading, dough rheology and baking quality of wheat bread.

Fig. 1 shows the development curves of dough produced from three different standard wheat flours ground using a roller mill (Fig. 1a) and the corresponding MSM flours re-ground using an industrial or a laboratory ball mill (Fig. 1b) depending on the kneading time. In contrast to the dough development curves of the standard wheat dough (Fig. 1a), those of dough produced from the flours treated using an industry or a ball mill on a laboratory scale showed an additional peak with a lower intensity between 13 and 18 min of kneading time (Fig. 1b).

The progression of the development curves of the dough produced from high MSM flours (Fig. 1b) can be explained by the following. The increase in dough viscosity in the initial kneading phase up to $Peak_{time}$ is because of an increase in volume of wheat starch granules caused by the cold swelling ability of mechanically modified starch. In this case, the increase in dough viscosity in the initial kneading phase is not caused by the commonly known formation of an evenly distributed and highly branched protein network structure, as expected for an optimally kneaded dough (Hackenberg et al., 2018). The slight decrease in dough consistency after $Peak_{time}$ could be associated with enhanced enzymatic hydrolysis of swollen starch granules and its effect on water release from the granules. The protein presumably began to develop a network structure with higher interconnections simultaneously. The formation of a highly branched gluten network caused a renewed increase in dough viscosity up to a second peak. This protein network structure was developed because of further application of mechanical energy during kneading. The prolonged kneading time of dough produced from high MSM flour might be explained by stronger interactions between swollen starch particles. The theoretical adhesion force proportionally increases with the increasing diameter of a spherical particle (Borho et al., 1991). With regard to microscopic particles, an approximately linear increase in capillary forces can be observed. For particles > 1 µm, the volume of capillary bridges increases linearly with the particle size (Dörmann & Schmid, 2015). The force effect of capillary bridges is maintained until they break (Borho et al., 1991) by, for example, application of mechanical energy.

Thus, a higher amount of energy in the form of kneading was required to separate large, swollen starch particles from each other, imitating a torque level comparable to a standard protein network development. This means, the required torque was overlapped by this separation energy between starch granules and less by the development of the protein network. Thus, more mechanical energy was necessary for the protein network development itself, as shown in the second peak. The dough development curves of MSM flours 1, 2 and 3 (Fig. 1b) indicate that this effect was stronger with increasing MSM levels. Therefore, the second peak of the development curve of the dough produced from high MSM wheat flour was visible later than that of the dough produced from low MSM wheat flour.

3.2. Protein microstructure development depending on the kneading time

3.2.1. Qualitative evaluation of the protein microstructure

Dough development and protein network structure are strongly dependent on mixing parameters such as kneading time. The dough microstructure is a relevant factor, which is associated with rheological dough behaviour (Bernklau et al., 2016; Hackenberg et al., 2018; Jekle & Becker, 2011). To investigate the effect of kneading on the protein microstructure formation in dough produced from high MSM flour, the protein network was first qualitatively assessed as a function of kneading time using a CLSM. Fig. 2a illustrates dough produced from

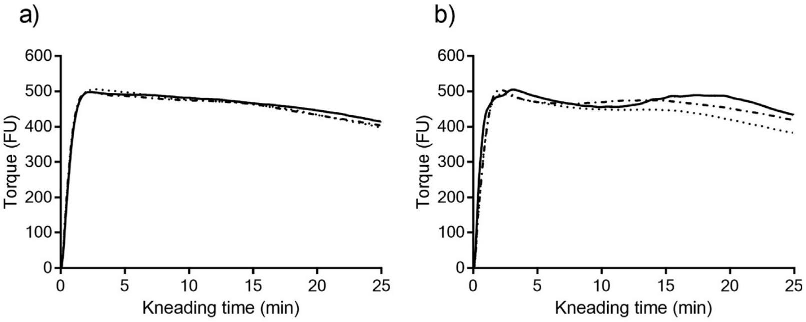

Fig. 1. Dough development curves of three different commercial wheat flours were analysed using a doughLab. (a) The dough development curves of standard wheat flours 1, 2 and 3 (ground using a roller-mill). (b) The dough development curves of the wheat flours re-ground using an industrial ball mill (MSM flour 1) and a ball mill on a laboratory scale (MSM flour 2 and 3). Wheat flours 1, 2 and 3 and their corresponding MSM flours are shown as (−), (····) and (-–-–), respectively. The curves represent the mean value (n = 3).

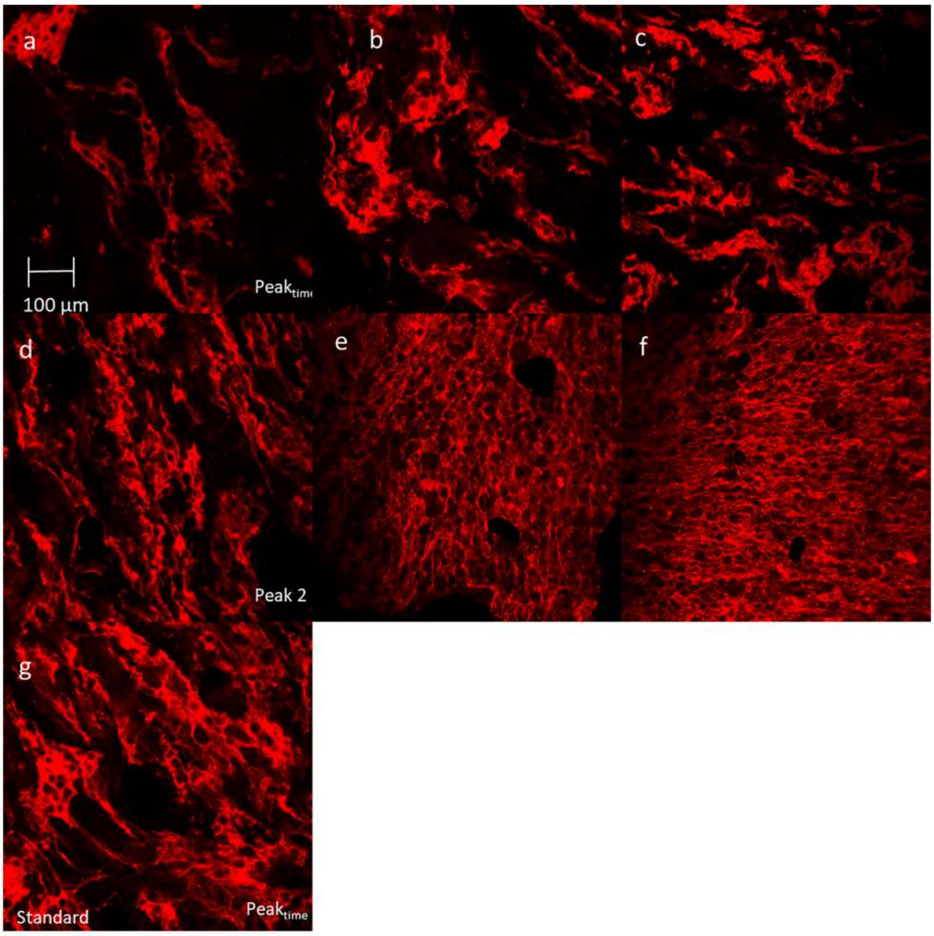

Fig. 2. The protein microstructure of dough produced from MSM flour 1 with kneading times of (a) 2.9 min, (b) 4 min, (c) 8 min, (d) 16 min, (e) 21 min and (f) 30 min was stained with Rhodamine B and visualised using a confocal laser scanning microscope. The protein network of the standard dough with a kneading time of 2.4 min is illustrated in image (g). All dough was produced with optimum water addition using a doughLab.

MSM flour 1 at $\text{Peak}_{\text{time}}$. High MSM wheat flours show mainly protein agglomerates and separated starch granules at $\text{Peak}_{\text{time}}$ (Hackenberg et al., 2018). The CLSM images of dough produced with kneading times of 4 and 8 min indicate that kneading beyond $\text{Peak}_{\text{time}}$ enhanced the protein network structure formation, resembling an ideal wheat dough protein network, and appeared to enhance the branching of protein threads (Fig. 2b and c). The protein network at 16 min of kneading time (Fig. 2d) was considerably similar compared with the protein microstructure of the standard dough and thus to an ideal protein network (Fig. 2g). After 16 min of kneading time, the dough consistency decreased again (Fig. 1b). Prolonged kneading (> 16 min) resulted in a close-meshed and fine protein network structure, in which the protein threads were densely accumulated. The protein matrix appears to be relatively homogenous and highly branched (Fig. 2e and f). Such protein network structures were particularly observed in over-kneaded dough. Peighambardoust et al. (2006) revealed a fine and homogenously distributed gluten network on a scale larger than 100 µm in dough produced with prolonged mixing times. Another study visualised thin gluten strands in dough, which was kneaded over a long period. Thus, the gluten threads appear to be ordered into bundles in a parallel orientation (Van der Mijnsbrugge et al., 2016). At the molecular level, strongly prolonged kneading can lead to a partially depolymerisation of the glutenin proteins caused by the cleavage of disulphide bonds (Letang et al., 1999). Because of over-kneading, the dough becomes weaker, which is termed breakdown (Gomez, Ferrero, Calvelo, Añón, & Puppo, 2011; Letang et al., 1999). Skerritt, Hac, and Bekes (1999) believe that the loss of high molecular weight glutenin subunits is the cause of breakdown during mixing.

3.2.2. *Quantitative evaluation of the protein microstructure*

For a more detailed evaluation of the protein microstructure of dough produced with various kneading times, dough was quantitatively analysed by image processing using the PNA method described by Bernklau et al. (2016). The protein branching rate significantly increased by 64.1% (Fig. 3a), whereas the protein end-point rate and the mean lacunarity strongly decreased by 57.7 (Fig. 3b) and 88.6% (Fig. 3c), respectively, in the dough produced from high MSM flour when the kneading time was prolonged from 2.9 min to 30 min. This indicates that the protein network had only few network interruptions and perforations after a longer kneading period. Thus, stronger connectivity was achieved because of the increased cross-linking of protein strands. Protein filaments in the dough produced from high MSM flour were significantly longer (+781.4%) and finer (−13.3%) when kneading time was prolonged by approximately 27 min (Fig. 3d and e). Interestingly, no significant differences between the protein branching rate, protein end-point rate, mean lacunarity, average protein length

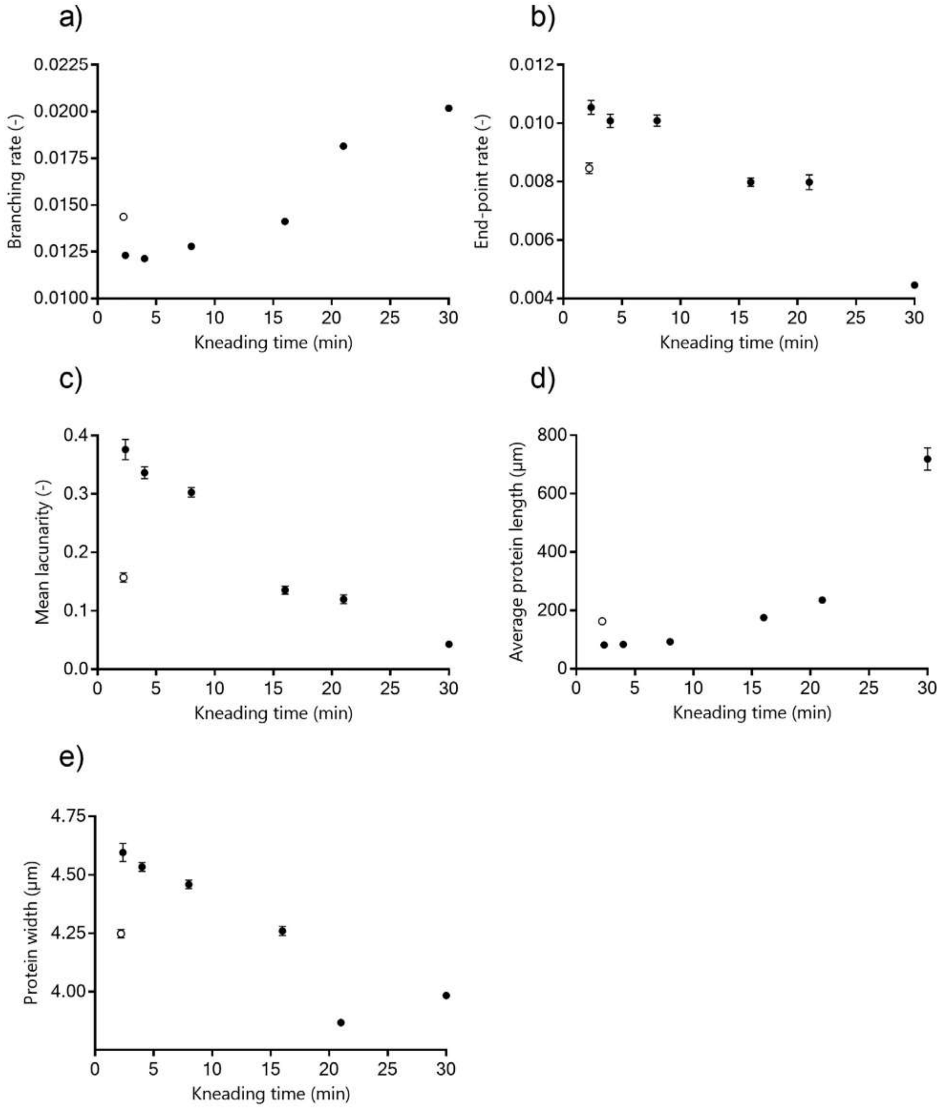

Fig. 3. The protein network characteristics of the dough produced from standard flour 1 and MSM flour 1 depending on kneading time were evaluated using a CLSM. (a) The protein branching rate, (b) the protein end-point rate, (c) the mean lacunarity, (d) the average protein length and (e) the protein width were quantified using protein network analysis. The protein microstructure attributes of the standard dough are shown as (○). All dough was produced with optimum water addition using a doughLab. Means are shown with the standard error of mean (n = 3).

and protein width in the dough produced from MSM flour 1 at 16 min of kneading time and the standard dough produced at Peak$_{time}$ were observed. The second peak in the dough development curve is exactly at this time after 16 min as illustrated in Fig. 1b. The results confirm the hypothesis of Hackenberg et al. (2018) that Peak$_{time}$ does not correspond to the optimum dough development when dough produced from high MSM wheat flour is used.

3.3. Quantitative analysis of wheat proteins using RP-HPLC

The viscoelastic behaviour is characteristic of wheat dough and strongly determines the baking quality of wheat bread. The viscoelastic properties of dough are mainly dependent on the ratio of gliadins to glutenins. These two main fractions have been comprehensively reviewed by Wieser (2007), whereas their importance for the gluten network formation was reviewed in detail by Kontogiorgos (2011). Several studies have shown that mechanical treatment of wheat flour can alter the protein structure and functionality (Licon, 1995; Mann et al., 2014; Vogel, Scherf, & Koehler, 2018a,b) and thus have an effect on protein microstructure formation during kneading. To clarify the effect of mechanical flour treatment in a ball mill on wheat protein functionality, the solubility of the main protein fractions of MSM flour 1 was analysed compared with that of the standard wheat flour using RP-HPLC.

The gliadin fraction mainly contains monomeric proteins. These proteins are insoluble in water and salt solutions, but soluble in aqueous alcohols (e.g. 60% ethanol or 50% propanol). Glutenins are polymerised by interchain disulphide bonds. These proteins are insoluble in water, salt solutions and aqueous alcohols under non-reducing conditions (Scherf, Koehler, & Wieser, 2016).

Table 1
Absolute concentrations of protein in standard flour 1 ground using a roller mill and MSM flour 1 re-ground using an industry ball mill. Means are shown with the standard error of mean (n = 3).

	Standard flour 1	MSM flour 1
Σ Albumins/Globulins (mg/g)	14.1 ± 0.2	12.1 ± 0.1
Σ Gliadins (mg/g)	50.0 ± 0.9	48.0 ± 0.1
Σ Glutenins (mg/g)	27.3 ± 1.0	27.1 ± 0.2
Σ Gluten (GLIA + GLUT) (mg/g)	77.3 ± 1.3	75.1 ± 0.2
Σ Osborne fractions (mg/g)	91.4 ± 1.2	87.3 ± 0.5
GLIA/GLUT ratio	1.83	1.77

The solubility of albumins/globulins decreased by 14.2%, whereas the solubility of gliadins was decreased by only 4% in the high MSM wheat flour (Table 1). Furthermore, mechanical wheat flour treatment using a ball mill had no significant effect on glutenin solubility (Table 1). The analysis results show that mechanical flour treatment with a ball mill had no relevant effect on the solubility of the main gluten proteins in the flour. If this was the case, a significant decrease in gliadins, particularly in α- and γ-gliadins, associated with an increase in glutenins would have occurred in MSM flour 1 compared with that in the standard flour. Because no significant differences were observed, it appeared that the treatment did not cause extensive thiol/disulphide exchange and no changes in molecular size distribution were expected. Thus, the delayed development of the protein network in dough produced from high MSM wheat flour may not be caused by functional alterations of the protein component.

3.4. Evaluation of dough rheology and baking quality of wheat bread

As discussed in Section 3.2, the protein network structure of the standard dough was considerably similar to the protein network in the dough produced from high MSM wheat flour on kneading process adaption.

To clarify whether these two protein networks cause similar rheological properties, the elongation behaviour of the dough produced from MSM flour 1 was analysed using texture profile analysis with an SMS/Kieffer Extensibility Rig after various kneading times compared with that of the dough produced from standard flour 1. In general, R_{max} and Ext_{max} are important properties associated with bread making. During the fermentation and baking stages, dough should be sufficiently extensible to expand in response to gas pressure and sufficiently elastic and strong to avoid breakage of gas cells for producing bread with an elastic inner structure and a large specific bread volume.

Fig. 4a shows that Ext_{max} of the dough produced from high MSM dough fluctuated only slightly. This could be explained by enhanced enzymatic hydrolysis of swollen starch granules and its effect on water release from the granules and changes of the water mobility in the dough. R_{max} significantly increased with prolonged kneading (Fig. 4b). Shear forces during kneading facilitate hydrated protein to form fibrils that align in the dough matrix resulting in an increase in R_{max} (Calderon-Dominguez, Vera-Dominguez, Farrera-Rebollo, Arana-Errasquin, & Mora-Escobedo, 2004). Fig. 3a–c visualize that the branching rate increased by 14.8%; however, the protein end-point rate and mean lacunarity, and thus the cavities within the network, decreased by 24.3% and 64%, respectively, with kneading time adaption. The protein network achieved a more hyperbranched structure with only few network interruptions resulting in stronger network connectivity. Consequently, more energy was required to break such a strong network. From the second peak, R_{max} significantly decreased by 29.3% (Fig. 4b) because of the cleavage of disulphide bonds by overkneading. The R_{max} of the dough produced from high MSM wheat flour was 22.1% lower than that of the dough produced from the standard wheat flour despite kneading time adaption (Fig. 4b). Furthermore,

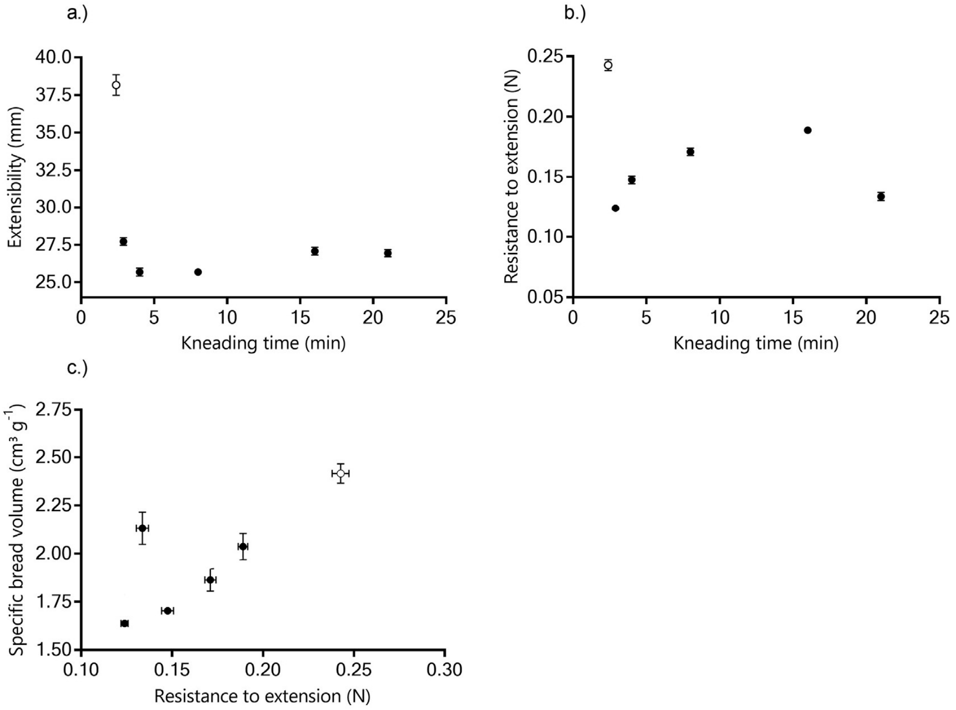

Fig. 4. (a) The extensibility (Ext_{max}) and (b) resistance to extension (R_{max}) of the dough produced from MSM flour 1 depending on kneading time (2.9–21 min) and (c) the specific bread volume as a function of R_{max} were investigated using a texture profile analyser. Values of the standard dough are shown as (○). All dough was produced with optimum water addition using a doughLab. Means are shown with the standard error of mean (n = 3).

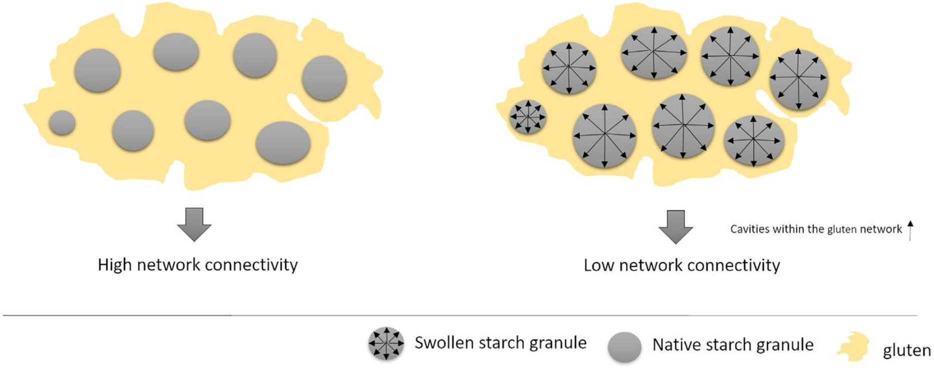

Fig. 5. Effect of starch granule size on gluten network strength in a dough system.

Ext_{max} of the dough produced from high MSM wheat flour was significantly decreased by 29.1% compared with that of the standard dough (Fig. 4a). The standard dough and the dough produced from high MSM wheat flour had a similar protein network structure following the kneading process adaptation; however, they differed with regard to their rheology.

The question raises why R_{max} and Ext_{max} were significantly lower than those of the standard dough. The decreased R_{max} may be because of weakened protein–filler interfacial interactions in the dough produced from high MSM wheat flour because of a lower specific surface area of swollen starch. Therefore, the dough had a reduced reinforcement. The dependency between reinforcement, particle size and the interaction of polymers and fillers was shown with poly(dimethylsiloxane)–silica composites and fumed silica particles using various sizes. Fumed silica particles with a diameter of 7 nm had the highest values of energy of rupture, E_r (area under the stress–strain curve), and therefore provided the greatest reinforcement. In contrast, series of fumed silica particles with a diameter of 40 nm had a low E_r. The larger surface area is considered to improve the interactions between polymers and fillers, which highly influence the strength (Yuan & Mark, 1999). Thus, the reinforcement decreases for networks with larger filler particles.

Furthermore, the significantly lower R_{max} and Ext_{max} may be because there are larger cavities within the gluten network caused by an increased volume of swollen starch granules. A simplified illustration is shown in Fig. 5. Therefore, the network connectivity appears to be weakened compared with that of the standard dough. Poor interactions between starch and protein and large voids within the gluten network, both caused by starch swelling, induce that less energy was required for dough rupture, characterised by a decreased R_{max} in the dough produced from high MSM wheat flour. In addition, dough breaks earlier during extension.

Kenny, Wehrle, Dennehy, and Arendt (1999) and Dobraszczyk and Salmanowicz (2008) observed high correlations of $r = 0.86$, $P < 0.01$ and $r = 0.78$, $P < 0.001$ between R_{max} and baking volume, respectively.

In the present study, R_{max} was highly correlated with specific bread volume ($r = 0.97$, $P < 0.05$; Fig. 4c) only if the dough was not over-kneaded. Because there is a strong demand for the gluten network of over-kneaded dough, there is no association between R_{max} and specific bread volume. The dependency between Ext_{max} and bread volume was not significant. The specific bread volume with regard to MSM flour 1 increased by 24.4% with a kneading time of 16 min (second peak in the dough development curve). However, the specific bread volume of $2.4\,\text{cm}^3\,\text{g}^{-1}$ for the standard dough could not be achieved with MSM flour 1 despite the kneading time adaptation (Fig. 4c).

The reason for this could be due to poor rheological dough properties during proofing. Another factor that could have influenced bread volume may be the increased water addition for dough preparation using high MSM wheat flour, which affects the gelatinisation temperature during baking. Nevertheless, the specific bread volume of dough produced from high MSM wheat flour can be significantly improved by kneading time adaptation.

4. Conclusions

The dough microstructure, rheology and specific bread volume of dough produced from high MSM wheat flour can be significantly improved by kneading process adaption by prolonging kneading time. In contrast to the dough development curve of the standard flour, which has only one peak ($\text{Peak}_{\text{time}}$), that of high MSM wheat flour displayed a second peak with less intensity following the first peak. The increase in the dough viscosity up to the second peak is caused by the formation of a more hyperbranched and interconnected gluten network with prolonged kneading. Because of enhanced protein network coherence, the rheological dough behaviour changed with prolonged kneading time, resulting in an increased R_{max}. However, the cohesion of the protein network appears to be weakened in dough produced from high MSM wheat flour probably because of weakened protein–filler interfacial interactions and larger voids within the protein network. Both were caused by the volume increase of mechanically modified wheat starch because of enhanced water absorption capacity. This study clearly demonstrates that the final quality of bread produced from high MSM wheat flour can be significantly improved by prolonging the kneading time. Therefore, wheat bread quality can be controlled and improved by kneading process adaptation.

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Declaration of interest

None.

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3.4 Maltose formation in wheat dough depending on mechanical starch modification and dough hydration

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Maltose formation in wheat dough depending on mechanical starch modification and dough hydration

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ABSTRACT

This study investigates the maltose formation during grinding of flour and dough processing (kneading, fermentation) attributed to mechanical flour or rather mechanical starch modification (MSM) and dough hydration. A strong rise of maltose formation up to 334% was observed during kneading due to improved accessibility of mechanical modified starch to amylolytic enzymes, whereas during proofing only a minor rise was detectable. The maltose release was more pronounced with high MSM and low dough hydration. Low dough hydration levels of 158 g water 100 g^{-1} flour instead of 178 g water 100 g^{-1} flour also intensified the maltose utilization by yeast (+37% with MSM of 4.78 g 100 g^{-1} flour; +27% with MSM of 7.46 g 100 g^{-1} flour), whereas increased maltose concentrations were not utilized by yeast during a fermentation cycle of 60 min. Consequently, high maltose concentrations based on MSM should have no relevant effect on the gas production during 60 min fermentation. However, the gas production by yeast might be prolonged due to enhanced substrate availability and thus could be relevant for elongated proofing methods.

1. Introduction

Mechanical wheat flour modification by grinding ensures an encompassing modification of the ground product. Impact grinding, for instance, caused an agglomeration of proteins due to oxidation of SH-groups to S—S-bonds (Licon, 1995). However, this study primarily focuses on starch. Depending on the wheat variety and the process parameter settings of the grinder, a varying amount of starch granules was separated from the protein matrix during mechanical flour processing (Ivanova, 2006). The mechanical treatment of wheat flour, such as grinding was strongly related to mechanical starch modification (MSM), formerly known as starch damage (Banafa, 2004; Dhital, Shrestha, & Gidley, 2010; Hackenberg, Verheyen, Jekle, & Becker, 2017; Yu et al., 2015). This modification was caused by changes of the starch structure on different length scales (Li, Dhital, & Hasjim, 2014). Due to alterations of the granule structure, the polymer chains of starch become more susceptible to amylolytic enzymes (Dhital et al., 2010), which might affect the release of fermentable saccharides (monosaccharides up to dextrins). In particular, the maltose concentration was highly correlated with the MSM level of wheat flour (Banafa, 2004). It is generally known that the type as well as the quantity of mono- and disaccharides influence the bread quality. In addition to the effect on browning and flavor attributes, mono- and disaccharides had a positive influence on the bread volume (Martins, Jongen, & van Boekel, 2000; Spieleder, 2006; Voica & Codină, 2009). A sucrose addition of 2%–2.4% resulted in a higher fermentation capacity by yeast and increased the bread volume (Voica et al., 2009).

As mechanical flour modification improves the susceptibility of starch to amylolytic enzymes (Li et al., 2014), it must be assumed that the amount of fermentable carbohydrates increases during dough production and thus the substrate availability for yeast is also improved. This might have a positive effect on yeast utilization and therefore on the gas production during fermentation and on the final bread volume. However, there are no detailed investigations regarding the generation of fermentable mono, di- and trisaccharides during grinding in a ball mill and the following dough processing (kneading and proofing), depending on mechanical flour modification and altered starch granule properties. It shall be taken into account that mechanical flour modification affected the dough hydration properties caused by elevated water holding capacity (Hackenberg et al., 2017) as well as cold swelling ability of the starch granules (Barrera et al., 2013). In the study of Hackenberg et al. (2017) was shown that more bulk water was required using flours with high MSM levels for reaching the optimum dough consistency according to the AACC method 54.21 (doughLab method). In general, the saccharide release is related to the enzymatic activity, which was strongly affected by the hydration level (Rezaei,

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Jenab, & Temelli, 2007). Dumont, Barth, Corbier, Branlant, and Perrut (1992) investigated the influence of various water additions on the reaction rate of lipase, which is an enzyme of the hydrolase class. They showed in their study that the reaction rate of the enzyme strongly depended on the water concentration and the composition of the reaction medium (Dumont et al., 1992). Dependencies between (dough) hydration, the value of MSM measurement and the saccharide production were not examined in closer detail. The utilization of saccharides by yeast during fermentation as a function of dough hydration and the MSM level were also not investigated.

The aim of the present study is to determine the saccharide profile, with a strong focus on maltose depending on (dough) hydration, MSM level and yeast fermentation during dough processing by using a HPAEC-ED. Therefore, commercial wheat flour (ground by a roller mill) was re-ground by a ball mill until it achieved four significantly higher MSM levels. Various hydration levels were obtained by the application of water concentrations between 1500 g water 100 g^{-1} flour (suspension) and 58 g water 100 g^{-1} flour (dough). Changes of maltose concentration in dough depending on the yeast utilization were evaluated during fermentation.

2. Experimental

2.1. Wheat flour characterization and mechanical modification

The following flour values were analyzed in the study of Hackenberg et al. (2017). The obtained values of MSM measurement (MSM levels) of the five wheat flours used in this study were 4.78 g 100 g^{-1} flour (standard flour, ground by a roller mill) and 6.56, 7.46, 7.85, 8.37 g 100 g^{-1} flour (mechanically modified by a ball mill). The MSM level was analyzed amperometrically with the SDmatic by Chopin (Villeneuve la Garenne, France) according to AACC 76-33 (starch damage). It was determined by the inclusion of iodine in the amylose helix of starch. Thereby, it was influenced by several factors: the accessibility of starch granules in the flour matrix and the starch granular structure. The α-amylase activity was measured using the Ceralpha Assay Kit by Megazyme International Ireland (Wicklow, Ireland). In all samples no α-amylase could be detected (detection limit 0.05 U mL^{-1}). The β-amylase activity of the samples based on Betamyl Assay Kit by Megazyme International Ireland (Wicklow, Ireland) dropped from 30.7 U g^{-1} ± 0.5 (standard flour) to 29.4 U g^{-1} ± 0.3, 28.2 U g^{-1} ± 1.0, 25.2 U g^{-1} ± 0.4 and 22.1 U g^{-1} ± 0.7 with increasing MSM level. Further specifications of the flour samples can be taken from the study of Hackenberg et al. (2017).

2.2. Sample preparation

The saccharide profile, composed of glucose, fructose, maltose, sucrose and maltotriose of flour-water-suspensions with five MSM levels (4.78, 6.56, 7.46, 7.85 and 8.37 g 100g^{-1} flour) was evaluated as a function of the resting time (0, 5, 10, 20, 60, 120, 180 min). For this purpose, 3 ml bi-distilled water was mixed with 0.2 g flour sample for 20 s by using a vortex mixer (Vortex-Genie2, Scientific Industries Inc., Bohemia, New York). The samples were rested in a proofing chamber at 30 °C.

In contrast to the suspensions, which contained excess water, the doughs have limited water contents depending on the MSM level. The maltose release was analyzed in non-yeasted and yeasted dough-models with two water additions during kneading and proofing. For this purpose, dough was produced with wheat flour (100 parts), NaCl (1.75 parts) (esco, Hannover, Germany), compressed yeast (3 parts) of *Saccharomyces cerevisiae* obtained from Wieninger (Passau, Germany) (yeasted dough) and water. The optimum water addition is commonly determined with a torque measuring Z-kneader system (DoughLab; Perten Instruments, Germany) according to AACC 54.21 (doughLab method). The optimum water addition of flour was defined as achieving a FU line of 500 ± 4%. In general, this water addition is associated with a good dough development and a good bread quality (Faridi & Faubion, 1990). The main aim of our study was to apply two comparable water levels. The optimum water addition of MSM level of 4.78 g 100 g^{-1} flour (standard flour) was 58 g 100 g^{-1} flour according to the doughLab method, whereas a water addition of 78 g 100 g^{-1} flour increased the dough hydration level and as a consequence reduced the dough viscosity. Hackenberg et al. (2017) have shown that the specific bread volume produced from the standard dough was not significantly different using water additions of 58 g 100 g^{-1} flour or higher. But, the specific bread volume of dough produced with high MSM level (7.46 g 100 g^{-1} flour) was significantly lower with a water addition of 58 g 100 g^{-1} flour. In their study, the highest specific bread volume was achieved using a water addition of 78 g 100 g^{-1} flour (Hackenberg et al., 2017). The bread volume is an important quality criterion for bread. Hence, the water addition of 78 g 100 g^{-1} flour was defined as the optimum water amount for dough preparation from flour of MSM level of 7.46 g 100 g^{-1} flour in our study. A water addition of 58 g 100 g^{-1} flour reduced the dough hydration level. For dough processing all ingredients were mixed for 1 min at 100 rpm and additionally kneaded for 6 min at 200 rpm in a spiral-kneader type SP 12 A-3 (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). Afterwards, the dough samples were rested in a proofing chamber at 30 °C with 90% RH. The saccharide concentration was analyzed at various processing steps (0 min- after grinding; 6 min- after kneading; 20 − 60 min- corresponds with the proofing time (non-yeasted dough) or rather fermentation time (yeasted dough).

2.3. Extraction and determination of saccharides in flour and dough

Enzymatic reactions can be inhibited by using $AgNO_3$ (Rittenauer, Gastl, & Becker, 2017). In the study at hand, the amylolytic enzymes in the flour-water-suspensions (0.2 g flour 3 ml^{-1} water) were inhibited by using 1 ml $AgNO_3$ solution (1.5 ppm; 5 ml $AgNO_3$ solution g^{-1} flour) during resting. The enzymatic reaction in the doughs was stopped with 150 ml $AgNO_3$ solution (30 ppm; 5 ml $AgNO_3$ solution g^{-1} flour). Due to a possible decrease of diffusion processes in the dough samples a factor of 20 was used to ensure the inhibition of amylolytic enzymes. To assure the same flour to water ratio (1:20) as applied for the suspensions, the dough-$AgNO_3$-slurries were mixed with 300 ml bi-distilled water at 5200 rpm by using an ultra-turrax (Ultra-Turrax T50 basic, IKA-Werke GmbH & Co KG, Staufen, Germany). Afterwards, the flour and the dough samples were centrifuged at 4000 rpm for 10 min at 20 °C, before the supernatant was filtered through a 0.45 μm membrane. The filtrate contained the fermentable saccharides, which were verified by using a high performance anion exchange chromatography with electrochemical detection (HPAEC-ED) (Dionex ICS 5000 ion chromatograph, Software Chromeleon 6.0, Thermo Scientific, Hennigsdorf, Germany) according to the method described by Mellado-Mojica & López (2015) with a CarboPac PA-10 guard column (4 mm × 50 mm) and a CarboPac-PA10 analytical column (4 mm × 250 mm). From each sample 25 μl were injected into the HPAEC-ED. The saccharides were separated by using a gradient of 0.25 M NaOH in deionized water at a flow of 0.25 ml/min and a column temperature of 25 °C. The potentials implemented for detection by the amperometric pulse were E1 (400 ms), E2 (20 ms), E3 (20 ms), and E4 (60 ms) of + 0.1, − 2.0, + 0.6, and − 0.1 V.

2.4. Statistical analysis

The statistical analysis was performed with Graph Pad Prism (version 6.01, GraphPad Software Inc., La Jolla, USA). To show significant differences between the samples a one-way analysis of variance (ANOVA; 5% significance level) was applied following Tukey's Multiple Comparison Test. Results are shown with mean value including the standard error of mean (SEM). All measurements were carried out in

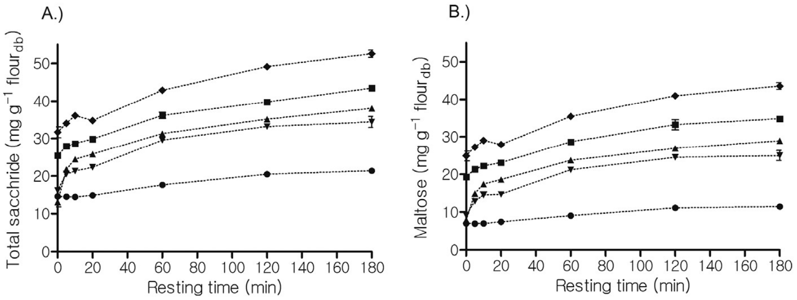

Fig. 1. Total saccharide concentration, composed of glucose, fructose, sucrose, maltose and maltotriose (A) and pure maltose concentration (B) depending on MSM level and resting time in flour-water-suspensions was analyzed by HPAEC-ED. The enzymatic hydrolysis was inhibited by using $AgNO_3$ solution (1.5 ppm) at various times of resting. Saccharide concentration, based on dry mass of flour (flour$_{db}$), is shown with MSM level 4.78 g 100 g^{-1} flour (●), 6.56 g 100 g^{-1} flour (▼), 7.46 g 100 g^{-1} flour (▲), 7.85 g 100 g^{-1} flour (■) and 8.37 g 100 g^{-1} flour (◆). Means are shown with standard error of mean (n = 3).

triplicate.

3. Results

3.1. *Influence of the MSM level on the saccharide formation in non-yeasted suspensions during resting*

The type as well as the quantity of mono- and disaccharides are not only affecting the gas formation during proofing but also the starch gelatinization during heating (Perry & Donald, 2002; Li, Li, & Gao, 2015; Kohyama & Nishinari, 1991). For instance, an addition of 10% (w/w) maltose to corn-starch-slurries resulted in a shift of gelatinization to higher temperatures (Li et al., 2015). In addition, the amount of saccharides also influences the sensory of baking goods and therefore determine, among other factors the final product quality. Hence, in this section, the total saccharide concentration of wheat flour, which is composed of glucose, fructose, maltose, sucrose and maltotriose was initially characterized depending on the value of MSM measurement (4.78–8.37 g 100 g^{-1} flour) after grinding, and during resting in flour-water-suspensions with excess water (1500 g water 100 g^{-1} flour) and without yeast by using a HPAEC-ED (Fig. 1A). The enzymatic reaction was inhibited by $AgNO_3$ solution (1.5 ppm) directly before analysis at various resting times.

At first, the effect of MSM on the saccharide composition during grinding was evaluated. Fig. 1A illustrates that the total saccharide concentration rose by 116% with enhanced MSM level after grinding (time 0). It showed that a major part of saccharides was already formed during the mechanical flour treatment. These heightened total saccharide concentrations are mainly caused by increased maltose concentration (Fig. 1B). Schlesinger (1964) and Banafa (2004) also reported an elevated concentration of maltose in flour ground in a ball mill. In the study at hand, the maltose concentration of the standard flour was 7.0 ± 0.1 mg g^{-1} flour$_{db}$. The maltose concentrations after grinding rose significantly with increasing MSM level of the flour up to 25.0 ± 1.3 mg maltose g^{-1} flour$_{db}$ (time 0). The results indicate that enzymatic reactions already occur during grinding despite low water contents of the mechanically modified flours, which ranged between 12.8% and 14.1% (Hackenberg et al., 2017). Enzymatic activity of a hydrolase without water addition was also observed by Dumont et al. (1992). However, the particles used in this study contained 10% water (Dumont et al., 1992), indicating that this negligible water concentration seems to be sufficient for this enzymatic reaction. The experimental flours mainly showed β-amylase activity. In general, β-amylases produce β-maltose by splitting from the non-reducing end of the α-1,4-linked poly- and oligoglucan chains until reaching an α-1,6-branching point of the substrate (Ziegler, 1999). The β-amylase activities of the samples in our study, based on Betamyl Assay Kit by Megazyme International Ireland, dropped from ~30.7 U g^{-1} (standard flour) to ~22.1 U g^{-1} with increasing MSM level (Hackenberg et al., 2017). It should be taken into account that altered substrate availability due to mechanical flour modification or rather MSM may influence the assay of enzyme activity. With the assumption that mechanical treatment led to a relative decrease of enzymatic activity, it can be concluded that substrate availability clearly predominated the effect of reduced enzyme activity.

In the next step, the saccharide composition depending on the MSM level was investigated during a resting period of 180 min. Due to excess water in the suspensions, viscosity effects can be excluded. The total saccharide concentration of the standard increased from 14.6 ± 0.0 mg g^{-1} flour$_{db}$ to 21.4 ± 0.2 mg g^{-1} flour$_{db}$ during resting, representing a growth of 46.3%. Again, the rise of total saccharides during resting was mainly caused by an increased maltose release (+4.3 mg g^{-1} flour$_{db}$). The glucose concentration elevated by 2 mg g^{-1} flour$_{db}$, whilst the sucrose concentration rose by only 0.8 mg g^{-1} flour$_{db}$. The fructose concentration did not increase significantly during the resting period and amounted around 0.8 mg g^{-1} flour$_{db}$, whereas the maltotriose already dropped by 0.3 mg g^{-1} flour$_{db}$ resulting from further enzymatic hydrolysis. In quantity terms, the maltose had the largest proportion (53.5%) of total fermentable carbohydrates, followed by sucrose (19.6%), glucose (15.5%), maltotriose (7.4%) and fructose (4.0%). In contrast to the standard flour, the total saccharide concentration of flour of MSM level 8.37 g 100 g^{-1} flour heightened from 31.6 ± 1.5 mg g^{-1} flour$_{db}$ to 52.6 0.1 ± mg g^{-1} flour$_{db}$ during 180 min resting time, which was an increase of 146.6%. Thereby, the maltose concentration rose by 281%, whereas no significant changes of the maltotriose (~1.7 mg g^{-1} flour$_{db}$), sucrose (~3.1 mg g^{-1} flour$_{db}$) or fructose (~0.5 mg g^{-1} flour$_{db}$) concentration could be detected. The glucose concentration heightened from 1.3 mg ± 0.1 g^{-1} flour$_{db}$ to 3.4 mg ± 0.0 g^{-1} flour$_{db}$. Considering our results, the strongly rise of the total saccharide concentration with high MSM level after grinding and during resting were primarily caused by increased maltose concentration.

3.2. *Influence of hydration and MSM level on maltose concentration after kneading and during proofing in non-yeasted doughs*

In the previous chapter, five significantly different MSM levels were analyzed and interpreted in suspension-models (excess water). The maltose concentration rose as a function of the value of the MSM measurement and the resting time. As next step the dependencies between MSM, the hydration level and the maltose production in the real matrix (dough) were examined during dough processing. For this purpose, non-yeasted dough was produced with two hydration levels by the application of bulk water concentrations of 58 g water 100 g^{-1} flour and 78 g water 100 g^{-1} flour with two relevant MSM levels of 4.78 g^{-1} 100 g flour (standard) and 7.46 g^{-1} 100 g flour. These two MSM levels were used because they can also occur during the common grinding process. The dough samples were proofed 60 min at 90% RH in accordance with the straight-dough method. The enzymatic hydrolysis in dough after kneading and as a function of proofing time was inhibited by using $AgNO_3$ solution (30 ppm), before maltose was determined by HPAEC-ED.

As shown in chapter 3.1 the maltose concentration of the flour with MSM level of 4.78 g 100 g^{-1} flour was slightly lower (7.0 ± 0.1 mg

Table 1
Maltose concentration at various steps of dough processing (0 min- maltose concentration after grinding; 6 min- maltose concentration after kneading; 60 min- maltose concentration after proofing at 30 °C, 90% RH) in dough produced with MSM level of 4.78 g 100 g^{-1} flour (standard) and MSM level of 7.46 g 100 g^{-1} flour with two water additions (58 g water 100 g^{-1} flour and 78 g water 100 g^{-1} flour).

MSM (g 100 g^{-1} flour)	Water addition (g 100 g^{-1} flour)	Time (min), 0 after grinding, 6 after kneading, 60 after proofing	Maltose increase (%) in dough after kneading and during proofing
4.78	58[a]	0	(−)[d]
		6	190.0
		60	20.2
4.78	78[b]	0	(−)[d]
		6	128.6
		60	32.5
7.46	58[c]	0	(−)[d]
		6	334.2
		60	18.1
7.46	78[a]	0	(−)[d]
		6	230.4
		60	52.5

[a] Optimum dough hydration.
[b] Increased dough hydration.
[c] Reduced dough hydration.
[d] After grinding.

maltose g^{-1} flour$_{db}$) than with MSM level of 7.46 g 100 g^{-1} flour (7.9 ± 0.5 mg maltose g^{-1} flour$_{db}$) after grinding. The data in Table 1 demonstrates that the majority of maltose was formed by amylolytic enzymes during kneading. A strong increase of maltose concentration up to 334.2% was detected during kneading, whereas during proofing only a minor rise was observed. Potus, Poiffait, and Drapon (1994) also investigated the influence of mixing on the formation of mono- and disaccharides. They revealed that the maltose concentration was dependend on the mixing time: the maltose concentration increased by 59 times after 15 min of mixing. In the process higher MSM levels and growing amylolytic activity led to higher maltose concentrations (Potus et al., 1994). The study at hand shows that a reduced dough hydration intensified the maltose release during kneading. Thus, the probability of interaction between amylolytic enzyme and its substrate (MSM) was heightened with lower dough hydration level. It seems that the positive effect of high substrate availability has predominated the negative influence of limited diffusion due to higher viscosity.

Fig. 2A visualized the maltose formation of dough produced with MSM level of 4.78 g 100 g^{-1} flour after grinding (0 min), kneading (6 min) and during proofing (6 min − 60 min) depending on dough hydration.

The maltose concentrations significantly increased during a kneading period of 6 min with both dough hydration levels. An elongation of incubation from 20 to 40 min did not show an effect with low dough hydration level, whereas the maltose concentration increased further with high dough hydration. With a proofing time of 40 min to 60 min the maltose concentration did not increase any further for both hydration levels. The doughs produced with flour of MSM level 4.78 g 100 g^{-1} flour with a reduced dough hydration level had significantly enhanced maltose concentrations at the same time of proofing.

Further the influence of water addition on maltose release in dough produced with a higher MSM level (7.46 g 100 g^{-1} flour) was investigated (Fig. 2B). The changed hydration properties of highly mechanically modified starch caused a change of the optimum water addition of non-yeasted dough produced with MSM level of 7.46 g 100 g^{-1} flour to a value of 78 g water 100 g^{-1} flour (Hackenberg et al., 2017). To imitate the effect of low hydration, dough was also produced with a water addition of 58 g 100 g^{-1} flour. Fig. 2B visualizes the same curve progression as shown in Fig. 2A. At a proofing time of 20 min a low difference in maltose formation between dough produced with high and low hydration levels can be observed. The high substrate availability compensated the effect of the dough hydration level on the saccharide formation during proofing with high MSM levels. In general, the results indicate that the maltose concentration was elevated with high MSM level during the whole dough production process caused by a higher substrate availability (MSM). Additionally, it could be demonstrated that most of the maltose was released during the kneading process.

3.3. Influence of hydration and MSM level on maltose concentration after kneading and during fermentation in yeasted doughs

To clarify the effect of the MSM level and dough hydration on the yeast maltose utilization, changes of maltose concentration in dough during fermentation were evaluated. Fig. 3A visualizes the maltose concentration of yeasted dough produced with MSM level of 4.78 g 100 g^{-1} flour (standard) with two water additions. The optimum water addition was 58 g 100 g^{-1} flour. Thus, a water addition of 78 g 100 g^{-1} flour resulted in an increased dough hydration level. The maltose concentration in the dough was significantly reduced by using an increased hydration level after kneading and during 40 min fermentation time. At 60 min fermentation time, no significant differences between the dough produced with varied water additions could be detected. The continuously decreasing maltose concentration from 20 min fermentation time indicates that the maltose utilization by yeast exceeds the maltose formation from starch hydrolysis by amylolytic enzymes resulting from reduced substrate availability in low mechanically modified flour.

The maltose concentrations of yeasted dough prepared with a MSM level of 7.46 g 100 g^{-1} flour produced with the optimum water additions of 78 g 100 g^{-1} flour and 58 g 100 g^{-1} flour (reduced hydration level) are plotted in Fig. 3B. In contrast to Fig. 3A, starting from 20 min fermentation time, no significant differences regarding the maltose concentrations between doughs produced with the two water additions could be revealed. Furthermore, no increase of maltose can be determined with both dough hydration levels between 20 min to 60 min fermentation time. Contrary to the experiments with low MSM level, the maltose utilization by yeast seems to exceed the maltose formation from starch hydrolysis by amylolytic enzymes at a later point of time

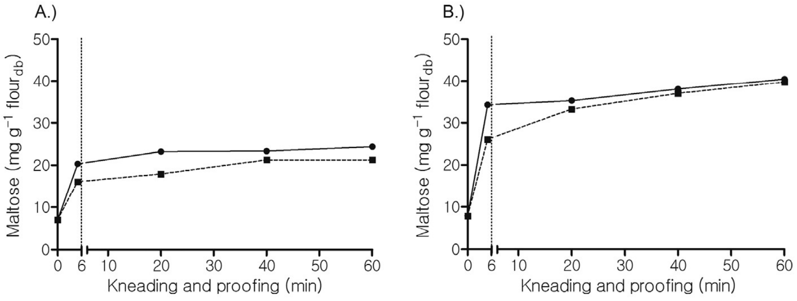

Fig. 2. Maltose concentration of non-yeasted dough produced with MSM level of 4.78 g 100 g^{-1} flour (A) and MSM level of 7.46 g 100 g^{-1} flour (B) with two water additions (58 g water 100 g^{-1} flour (●); 78 g water 100 g^{-1} flour (■)) as a function of processing (0 min- maltose concentration after grinding; 6 min- maltose concentration after kneading; 20 − 60 min- maltose concentration during fermentation at 30 °C, 90% RH). Means are shown with standard error of mean (n = 3).

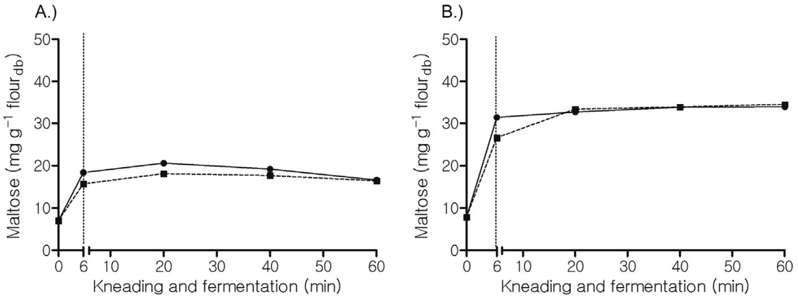

Fig. 3. Maltose concentration of yeasted dough produced with MSM level of 4.78 g 100 g^{-1} flour (A) and MSM level of 7.46 g 100 g^{-1} flour (B) with two water additions (58 g water 100 g^{-1} flour (●); 78 g water 100 g^{-1} flour (■)) as a function of processing (0 min- maltose concentration after grinding; 6 min- maltose concentration after kneading; 20 – 60 min- maltose concentration during fermentation at 30 °C, 90% RH). Means are shown with standard error of mean (n = 3).

Table 2
Comparison of maltose concentration of non-yeasted and yeasted wheat dough produced with MSM level of 4.78 g 100 g^{-1} flour (standard) and MSM level of 7.46 g 100 g^{-1} flour with two water additions (58 g water 100 g^{-1} flour and 78 g water 100 g^{-1} flour) after 60 min proofing (non-yeasted dough) or rather fermentation time (yeasted dough). Means are shown with standard error of mean (n = 3).

MSM (g 100 g^{-1} flour)	Water addition (g 100 g^{-1} flour)	Maltose concentration (mg g^{-1} flour$_{db}$) of non-yeasted dough after 60 min of proofing	Maltose concentration (mg g^{-1} flour$_{db}$) of yeasted dough after 60 min of fermentation	Maltose utilization by yeast during 60 min (mg g^{-1} flour$_{db}$)
4.78	58[a]	24.4 ± 0.0	16.6 ± 0.4	7.8 ± 0.4
4.78	78[b]	21.2 ± 0.3	16.4 ± 0.2	4.8 ± 0.1
7.46	58[c]	40.5 ± 0.6	33.9 ± 0.3	7.0 ± 0.9
7.46	78[a]	39.8 ± 0.2	34.5 ± 0.2	5.3 ± 0.0

[a] Optimum dough hydration.
[b] Increased dough hydration.
[c] Reduced dough hydration.

caused by a higher substrate availability for enzymatic hydrolysis with high mechanical flour modification.

3.4. *Influence of dough hydration and MSM level on yeast maltose utilization*

For evaluating the maltose utilization depending on MSM level and dough hydration during fermentation, the maltose concentrations of non-yeasted and yeasted dough prepared with two different MSM levels (4.78 and 7.46 g 100 g^{-1} flour) and two water additions (58 and 78 g 100 g^{-1} flour) have been compared after proofing (non-yeasted dough) or fermentation (yeasted dough), respectively. For this purpose, the maltose concentrations of the yeasted dough samples were subtracted from the maltose concentrations of the non-yeasted dough samples after 60 min fermentation or rather 60 min proofing. The results indicate that low dough hydration improved maltose utilization by yeast with both MSM levels during 60 min of fermentation (Table 2). It is assumed that the reaction rate was high at low dough hydration caused by high concentrations of reactants, which have prevailed the negative effect of higher viscosity. Furthermore, the same dough hydration level with various MSM levels resulted in nearly the same maltose utilization by yeast despite higher substrate was available with enhanced MSM level during a fermentation cycle of 60 min. The results demonstrate that elevated maltose concentrations were not utilized by yeast. Consequently, it can be assumed that high maltose concentrations in dough should have no perceptible influence on the gas production in a fermentation cycle of 60 min. Nevertheless, the gas formation by yeast might be prolonged during fermentation due to enhanced substrate availability with elevated MSM level. This may become relevant for elongated proofing methods. The high maltose concentrations with heightened MSM level can also affect the sensory of the baking goods and thus have an influence on the product quality.

4. Conclusions

The functional importance of MSM during dough processing was already shown in several studies. The publication at hand partially clarified the mechanisms regarding the saccharide formation depending on the value of MSM measurement and the hydration level. Mono- and disaccharides, especially maltose, were formed during grinding of wheat flour and during the processing steps of dough depending on MSM level and dough hydration. Considering that mainly the maltose concentration increased after dry grinding in a ball mill, it is supposed that this effect is caused by an improved starch digestibility by amylolytic enzymes, in particular β-amylases, which are responsible for β-maltose release from starch polymers. The majority of maltose was produced during dough kneading. In general, kneading with high MSM levels as well as a reduced dough hydration intensified the maltose formation. It seems that the interaction between amylolytic enzymes and their substrates (starch) was improved with low dough hydration. It is assumed that the positive effect of high substrate availability has prevailed the negative effect of limited diffusion due to a enhanced viscosity. Lower dough hydration also intensified the maltose utilization by yeast. However, heightened maltose concentrations in dough due to increased MSM levels were not utilized by yeast during 60 min fermentation, which indicates that the reaction speed was independed of the substrate concentration. In general, the results of our study have a relevant importance in the field of fermentation technology, such as prolonged fermentation methods.

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3.5 Effect of mechanically modified wheat flour on dough fermentation properties and bread quality

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ORIGINAL PAPER

Effect of mechanically modified wheat flour on dough fermentation properties and bread quality

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Abstract The swelling properties and thus baking performance of starch strongly depend on mechanical starch modification (MSM), which can be influenced by grinding. To analyze the effect of starch influencing factors on dough fermentation properties and specific bread volume, different MSM levels were obtained using a ball mill. This treatment led to an increase in the water-holding capacity and a decrease in the β-amylase activity of the flour. Baking tests were conducted with varied starch modification levels and water additions to analyze the effect of mechanical flour modification and the resulting changes in hydration and gas formation properties of the dough on bread quality. The specific bread volume was lower with high-MSM flour than with low-MSM flour, regardless of the adapted water addition. Therefore, the effect of MSM on dough fermentation properties was examined with respect to different water additions. Despite increasing the water addition, the time of porosity (Tx) of high-MSM dough was significantly lower than that of low-MSM dough. However, the amount of gas leakage after 60 min was quite low for high-MSM dough (gas retention coefficient ~99.5 %) and thus not considered significant. By adding 58 and 83 g water 100 g^{-1} flour, the gas retention coefficient after 60 min was 100 %. The results also show that lower enzymatic activity with high MSM has no significant effect on the produced gas volume during fermentation. However, increasing MSM leads to a reduced dough height (Hm).

Keywords Damaged starch · Fermentation · Water absorption · Bread quality · Rheofermentometer · Gas retention

Introduction

Structural changes during processing of protein–starch matrices are induced especially by the enzyme activity in flour and the swelling properties of starch, both depending strongly on the level of starch damage. Due to chemical, enzymatic or physical treatment, starch granules lose their native properties and are designated as damaged [1]. In this regard, flour can be processed mechanically by grinding in a mill. Thereby, the extent of starch damage depends on grinding parameters such as shear forces, which lead to different changes in the starch granular and molecular structure. The extent of starch damage increases with the mechanical force and grinding time [2–4]. Since starch damage is a collective term for effects on the starch structure, with either a negative or positive impact on the end product, the term 'mechanical starch modification' (MSM) is more suitable than 'starch damage' and will thus be used in this work. Several studies have been conducted to determine the positive influence of MSM on bread quality, staling and crust browning [5]. However, a high level of MSM might destabilize the integrity of the dough matrix and reduce the bread volume. Barrera et al. [6] evaluated the effect of MSM on bread quality. First, the kernels were ground with a roller laboratory mill, before the flours were re-ground by a disk mill, to obtain different MSM levels. With higher MSM, the bread volume

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decreased and the water-binding capacity increased. In this case, the water binding, as evaluated with the Farinograph method, approached the 500 FU line for an optimal dough network. However, it is still unclear why the bread volume suffered from higher MSM despite the adapted water content. The authors assumed that the decreasing dough consistency and gas retention capacity of the dough after starch degradation during dough fermentation caused the reduced baking quality. In this context, there are no sufficient fundamental investigations clarifying the decreasing bread quality, especially in view of changes in the hydration properties of mechanically modified starch. It is presumed that mechanical flour modification has a negative effect on the gas-holding capacity of the dough. In addition, MSM facilitates starch hydrolysis by amylolytic enzymes, which affects the quality of dough and bread, because it affects CO_2 formation by yeast as well as starch gelatinization properties [7, 8]. Moreover, enzymatic hydrolysis and molecular mobility might be limited with less available water, due to the increased hydration of the modified starch granules. The grinding process itself might also affect the enzymatic activity. In this regard, Charm et al. [9] examined the effect of shearing on the enzymatic activity using a viscometer. In this context, a combination of shear intensity and exposure time determined the extent of enzyme inactivation. Thus, mechanical forces during grinding might influence the enzyme structure to such an extent that they could be inactivated. The effect of grinding on the starch structure has been extensively studied, but its influence on the enzymatic activity has not been considered.

The present study aims to clarify the effect of mechanical flour treatment on starch modification and its consequence on fermentation and baking performance. For this purpose, flour (ground by a roller mill) was re-ground by a ball mill with varying rotational speeds and retention times until it reached a significantly higher MSM level, which was identified through an amperometric method. The influence of increasing MSM on the water-holding capacity (WHC) of flour was determined, since dough hydration plays a major role in the development of the viscoelastic dough network and on the specific bread volume. Consequently, dough and baking tests were conducted with differently modified flours. The water addition was varied in a full factorial experimental design in order to evaluate the effect of MSM and hydration on dough fermentation properties (gas volume, gas-holding capacity, dough height) and baking performance. The direct influence of grinding on enzymatic hydrolysis was analyzed and assessed in relation to the gas formation during fermentation and thus to the end product quality. This study provides an improved knowledge of the impact of MSM on bread quality.

Experimental

Wheat flour characterization and modification

For mechanical modification, commercial wheat flour Type 550 (ground by a roller mill) obtained from Rosenmühle (Landshut, Germany) with an MSM level of 4.78 g 100 g^{-1} flour (standard flour) was re-ground by a ball mill (ATR-315, Alpine Hosokawa, Augsburg, Germany) for four more MSM levels. Grinding was performed by varying the rotational speed and retention time until a significantly higher MSM level was reached. The modification level was determined during grinding amperometrically using the SDmatic by Chopin (AACC 76-33). During grinding, the temperature was below the protein denaturation temperature (~40 °C). In addition, depending on the MSM level, the mechanically modified flours contained 14.1, 13.1, 13.3, 12.9 and 12.8 % moisture (AACC 44-01). This difference was caused by the different temperatures during grinding, resulting from the different parameters set for the process. All modified flours contained 67.4 % starch (AACC 76-13) and 12 % protein (AACC 40-16, N × 5.7). The activity of amylolytic enzymes (α- and β-amylase) was analyzed using the Ceralpha and Betamyl Assay Kits by Megazyme International Ireland (Wicklow, Ireland), respectively. All measurements were performed three times.

Dough water absorption and WHC

In compliance with the AACC method 54.21.02, a torque-measuring Z-kneader system (DoughLab; Perten Instruments, Germany) was used for determining the optimal water absorption of the modified flour. For this purpose, the optimal water addition was defined by a FU line reached between 480 and 520 for all mechanically modified flour samples. The WHC of the modified flour was analyzed and calculated according to Abebe et al.'s method [10]. For this analysis, 2.0 g flour sample was mixed with 20 mL distilled water. The suspension was stored for 24 h at room temperature before the supernatant was determined gravimetrically. WHC is defined as the absorbed water amount in the sample in grams per grams of the initial flour weight. All measurements were conducted three times.

Preparation of dough and bread samples

The dough was prepared with wheat flour (100 parts), water, NaCl (1.75 parts) (Esco, Hannover, Germany) and compressed yeast (three parts) of *Saccharomyces cerevisiae* kindly provided by Wieninger (Passau, Germany). This formulation was based on 14 % flour moisture. In order to evaluate the effect of hydration, the dough was prepared

Table 1 Water addition for dough preparation per 100 g flour for each MSM level

	MSM 4.78 g 100 g^{-1} flour	MSM 6.56 g 100 g^{-1} flour	MSM 7.46 g 100 g^{-1} flour	MSM 7.85 g 100 g^{-1} flour	MSM 8.37 g 100 g^{-1} flour
Water addition (g 100 g^{-1} flour)	58[a]	58	58	58	–
	63	63[a]	63	63	–
	68	68	68	68	68
	73	73	73[a]	73	73
	78	78	78	78[a]	78
	83	83	83	83	83[a]
	–	–	–	88	88
	–	–	–	93	93
	–	–	–	98	98
	–	–	–	–	103
	–	–	–	–	108

The fields marked with '–' were not processable

[a] Optimal water addition

with varying water additions (58–108 g 100 g^{-1} flour in steps of 5 g). Table 1 shows the water additions for each MSM level. Thereby, the added amount of water was limited by the processability of the dough. Due to the higher WHC of high-MSM flour, the dough was too solid and thus not processable with a water addition of 58 g 100 g^{-1} flour. Thus, high-MSM flour started with a water addition of 68 g water 100 g^{-1} flour. In turn, due to their lower water-binding properties for low-MSM flour, the highest possible water addition was 83 g 100 g^{-1} flour. With higher water addition, the samples became too liquid to form a dough.

All ingredients were mixed for 1 min at 100 rpm and for additional 6 min at 200 rpm in a spiral-kneader-type SP 12 A-3 (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). The dough was separated into 200 g pieces before it was rested in a proofing chamber at 30 °C with 90 % RH for 60 min. Afterward, the pieces were baked in a Matador 12.8 oven (Werner & Pfleiderer Lebensmitteltechnik GmbH, Dinkelsbühl, Germany) for 26 min at 220 °C upper heat and 230 °C bottom heat with 0.5 L initial steam. After cooling at room temperature for 60 min, the specific bread volume (cm^3 g^{-1}) was analyzed by a laser-based volumeter (BVM-L370, Perten Instruments, Hägersten, Sweden). All measurements were performed in triplicate.

Determination of fermentation characteristics of wheat dough

The gas formation, gas-holding capacity and the dough development were investigated with a Rheofermentometer F3 from Chopin (Chopin Technologies; Villeneuve la Garenne Cedex, France). For this purpose, the dough was prepared as described in 'Preparation of dough and bread samples' section. After kneading, 315 g of the dough sample was transferred into a fermentation basket. Subsequently, the proofing chamber was sealed off completely before the system started the measurement at 30 °C for 180 min. The total gas production (mL) and the time of porosity (Tx), which is defined as the time when gas leaks from the dough into the surrounding area, were examined. Accordingly, the retention coefficient, which represents a comparison between the retarded gas volume and the produced gas volume of the dough, was evaluated after 60 min and 180 min. All measurements were performed three times.

Statistical analysis

The statistical analysis was performed with GraphPad Prism (Version 5.03, GraphPad Software, Inc). To expose significant differences between the samples, a one-way analysis of variance (ANOVA) was applied following Tukey's test.

Results and discussion

Mechanical flour modification and its effect on WHC and water absorption

For mechanical modification, commercial wheat flour (ground by a roller mill) with an MSM level of 4.78 g 100 g^{-1} flour (standard flour) was re-ground by a ball mill for four more MSM levels. Thus, as shown in Fig. 1, five MSM levels were realized. The increased iodine absorption with increased mechanical flour treatment indicates a significantly higher MSM level.

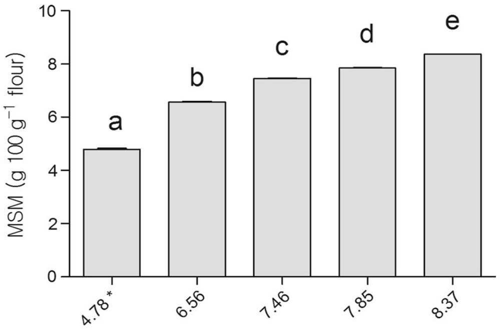

Fig. 1 MSM of different wheat flours. In all cases, a high significant difference between the flour samples could be detected (ANOVA) $p < 0.0001$. Means with different letters are significantly different. MSM refers to 100 parts flour, and the means are shown along with the standard error ($n = 3$). *Standard flour

WHC refers to the capacity of starch and other flour components to retain water molecules. To assess the effect of mechanical flour modification, the WHC of the different flours was examined according to Abebe et al.'s method [10]. A clear increase in WHC as a function of mechanical flour modification (Fig. 2a) is observed, which can be described by a linear fitting ($R^2 = 0.82$). In particular, starch, as well as proteins and pentosans, can absorb large amounts of water during dough processing. Mechanically modified starch can absorb more water than native starch. This is due to the improved water access along the cracked surface of MSM starch as well as the increasing hydration of the internal areas of the starch granules [6, 11]. Barrera et al. [12] pointed out that fast hydration and swelling properties of MSM starch result from the modification of the granular surface (flake off). Li et al. [13] concluded that, due to the mechanical forces produced during grinding, starch granules break into smaller particulates, increasing their surface area and thus the hydration rate. Furthermore, the mechanical treatment of starch granules can lead to reduced stability of their amorphous growth rings, whereby hydroxyl groups may form hydrogen bonds with water molecules. Tester et al. [14] revealed that the swelling properties of starch are mainly caused by the leaching of polysaccharides. Their findings indicate that swelling is a property of amylopectin, whereas amylose and lipids inhibit swelling. Due to the increased water absorption capacity of mechanically modified starch, the dough is more solid, which in turn might influence its elasticity, and with changing viscosity, the formation of gas bubbles during fermentation. The water absorption of the flours was measured according to the AACC method 54.21.02, in order to obtain the optimal water absorption for the baking tests. Contrary to WHC, in this method, water absorption occurs during dough formation and thus depends on dough viscosity. Figure 2b illustrates the optimal water amount determined through different methodologies for the modified flours. There is a high positive linear relation between WHC and the optimal water amount of mechanically modified flours. However, the dough structure system is completely different from the flour system. In the dough, there is a gluten network with immersed starch granules. Thus, the water interaction is different in the two systems.

Specific bread volume affected by MSM and water addition

After determining the optimal water absorption of the mechanically modified flours, the resulting values were used for analyzing the baking performance. The specific bread volume was evaluated for different MSM levels with the optimal water amount. Figure 3a illustrates a negative relation between an increased MSM level and the analyzed specific bread volume. This may be caused by modified hydration during dough processing. As described in 'Mechanical flour modification and its effect on WHC

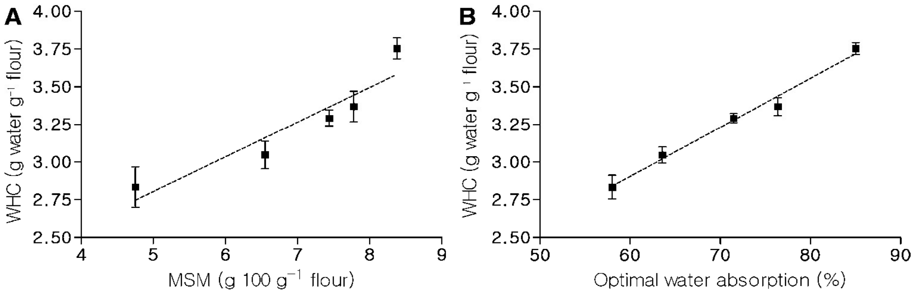

Fig. 2 **a** Effect of MSM on WHC and the optimal water absorption of the flours for dough formation in relation to **b** WHC of the flours. WHC was measured according to Abebe et al.'s method [10]. For WHC, a linear increase is indicated ($R^2 = 0.82$, $p < 0.0001$). The optimal water amount in each sample was determined according to the AACC method 54.21.02. A linear increase ($R^2 = 0.93$), $p < 0.0001$) in relation to WHC is observed. The means are shown with the standard errors ($n = 3$)

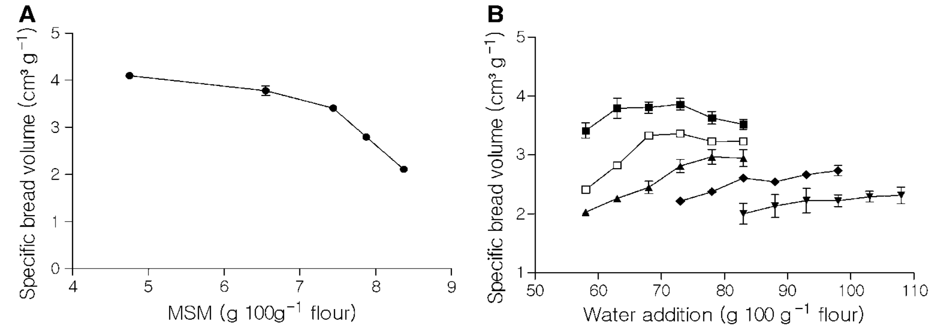

Fig. 3 Relation between MSM and specific bread volume with **a** the optimal water amount determined by the DoughLab method and **b** varying water addition. **a** Specific bread volume decreases significantly with increasing MSM level (ANOVA) $p < 0.05$. Means are shown with standard error ($n = 3$). **b** Specific bread volume based on the dough with MSM of 4.78 g water 100 g^{-1} flour *filled square*, 6.56 g water 100 g^{-1} flour *open square*, 7.46 g water 100 g^{-1} flour *pointed up filled triangle*, 7.85 g water 100 g^{-1} flour *filled diamond* and 8.37 g water 100 g^{-1} flour *pointed down filled triangle* was analyzed by a volumeter. The means are shown with the standard error ($n = 3$)

and water absorption' section, flours with a high-MSM level have higher WHCs, which results in a stronger dough structure due to a lower plasticizing effect of the mobile water amount [15]. Thus, the dough network might be less extensible and gas bubbles would not be able to expand accordingly. Indeed, starch with high levels of mechanical modification can be better hydrolyzed by amylolytic enzymes, contrary to native starch, which is resistant to enzymatic digestion [16]. It must therefore be assumed that the specific bread volume increases with higher MSM, due to increasing gas formation, albeit only if enough fermentable mono- and disaccharides are available for the yeast or when less fermentable carbohydrates are produced as compared to those in the higher MSM levels (substrate limitation). However, the higher the WHC (with increased MSM level), the lesser the mobile water is available in the dough system, which might have a diffusion-limiting effect on the interactions between the enzymes and the substrate (MSM). Moreover, less mobile water in the dough system might lead to incomplete starch gelatinization. This, in turn, leads to decreased enzymatic hydrolysis, which is necessary for the viscosity of the dough and to provide the yeast with fermentable mono- and disaccharides for CO_2 production. Furthermore, the grinding itself can influence the enzymatic activity, which in turn might have a negative effect on bread-making performance. Furthermore, only gelatinized starch can form structural–elastic interfaces with other flour components, e.g., lipids and proteins, which are essential for developing the crumb structure as well as for the gas-holding properties of the dough.

To clarify the effect of dough hydration on the specific bread volume, the dough was prepared with varied water additions in the range above and below the determined water optimum. An increase in water addition (Fig. 3b) results in an increase in the specific bread volume. Despite the adapted water addition, the specific bread volume prepared with high-MSM flour is lower than that produced with low-MSM flour. It can be assumed that the water amount in the dough system is sufficient for starch gelatinization. A diffusion limitation of the amylolytic enzymes due to the solid dough structure might also be excluded. Thus, the results indicate that there are other factors, apart from the hydration effects, that affect the specific bread volume. Therefore, the decreasing baking performance might also be a result of reduced enzymatic activity with increasing mechanical flour treatment. A lower enzymatic hydrolysis—resulting in lower starch degradation, which reduces substrate formation—causes lower yeast fermentation. Accordingly, CO_2 production could be decreased, resulting in a lower specific bread volume. To analyze the effect of water addition on the gas bubbles, the crumb structure was evaluated.

Figure 4 indicates that the size of the bread crumb pores increases with the water addition during dough preparation. However, despite the larger gas bubbles, the specific bread volume does not increase continuously. During dough preparation, an excess amount of water reduces the dough viscosity, whereas water deficiency produces a cohesive dough [17, 18]. This might favor coalescence and disproportionation during fermentation and baking, which might have a negative effect on the gas-holding properties of the dough, resulting in a lower specific bread volume. Furthermore, it seems that the enzymatic activity is influenced by grinding. This, in turn, influences the hydrolysis of fermentable carbohydrates, dough viscosity and dough rheology, which could also affect the resulting bread quality. For this reason, the influence of mechanical flour modification on enzymatic activity will be examined.

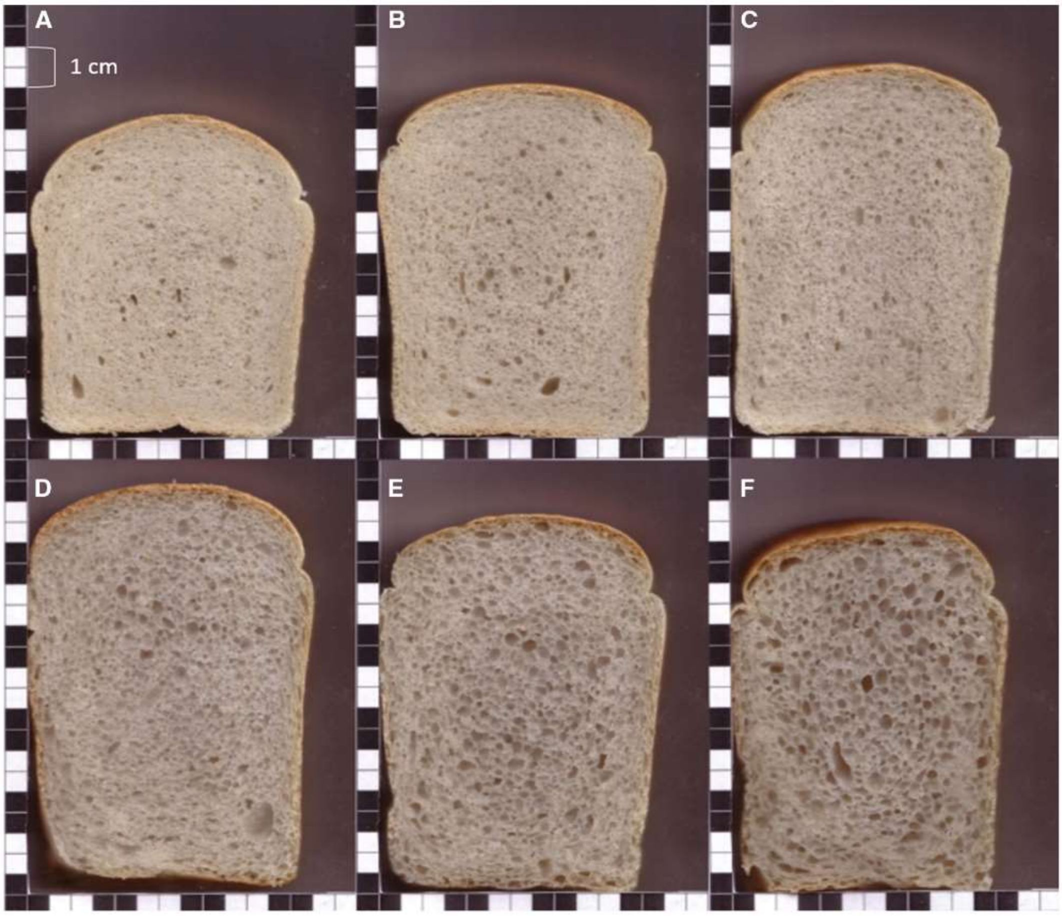

Fig. 4 Relation between water addition and crumb structure of wheat breads produced with flour based on MSM 6.56 g 100 g^{-1} flour. Breads are ranged in order of increasing water addition (**a**. 58 g water 100 g^{-1} flour, **b**. 63 g water 100 g^{-1} flour*, **c** 68 g water 100 g^{-1} flour, **d** 73 g water 100 g^{-1} flour, **e** 78 g water 100 g^{-1} flour, **f** 83 g water 100 g^{-1} flour). The size of the gas bubbles increases with water addition. The specific bread volume also increases with water addition; however, it starts decreasing from a water addition of 73 g 100 g^{-1} flour. *optimal water addition

Effect of mechanical flour modification on enzymatic activity

In order to evaluate the influence of grinding on the enzyme activity and its effect on the produced gas volume during fermentation and thus on the bread quality, the α- and β-amylase activity were determined for the different modified flours. Amylase activities can be assayed with the Ceralpha method [17] with a detection limit of 0.05 U mL^{-1}. α-Amylase was not detected in the samples. In general, α-amylase is formed during germination in the presence of gibberellic acid. For β-amylase, a significant decrease from 30.65 U to 22.05 U g^{-1} flour (Fig. 5) was detected with increasing mechanical flour modification. However, it should be taken into account that changes in substrate availability by MSM may affect the analysis of the enzymatic activity.

The influence of shearing on enzymatic activity has been also investigated by Charm et al. [9] using a capillary viscosimeter. They indicated that a combination of shear intensity and exposure time determines the quantity of inactivation. In accordance, van der Veen et al. [18] showed that the inactivation of thermally stable α-amylase can be described with a first-order process, wherein the inactivation energy strongly depends on shear stress. They assumed that inactivation is caused by the breaking of the tertiary structure when the enzyme is aligned to the shear field. Nevertheless, other studies have shown that shear stress leads to a decrease in enzymatic activity [19–21]. Thus, there is evidence that shear forces during grinding

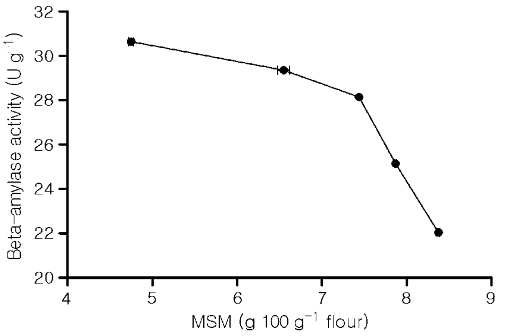

Fig. 5 Relation between MSM and β-amylase activity. Enzymatic hydrolysis was investigated with the Betamyl Assay Kit by Megazyme International Ireland. The activity is expressed as Betamyl-3® Units. The means are shown with the standard error ($n = 3$)

might influence the tertiary structure of proteins to such an extent that they can be inactivated. To evaluate the effect of decreasing enzyme activity on fermentation properties, the gas formation and the dough development were determined using the Rheofermentometer.

Influence of mechanical flour modification on gas volume and dough development

In the previous section, we focused on the influence of grinding on the enzyme activity, following investigations focused on the produced gas volume and dough development during fermentation of dough in relation to different MSM levels with optimal water addition. Figure 6a illustrates, for MSM levels of 4.78 g 100 g^{-1} flour and 6.56 g 100 g^{-1} flour, a sigmoidal curve of gas formation, which is typical for biological growth processes. The curve's characteristics mainly depend on the availability of the fermentable substrate. Due to grinding, starch granules lose their native properties, which can result in better hydrolysis by amylolytic enzymes and might increase the substrate concentration for yeast. For MSM 4.78 g 100 g^{-1} flour, the substrate concentration after approximately 150 min might be depleted, and thus, the gas formation does not increase any further. On the contrary, the gas formation for MSM 6.56 g 100 g^{-1} flour decreases later than for MSM 4.78 g 100 g^{-1} flour, perhaps due to higher substrate availability. The gas production for MSM 8.37 g 100 g^{-1} flour is almost linear within the 180-min interval, which might be due to a continual reproduction of the substrate by enzymatic hydrolysis. However, no significant differences in the gas volume of dough prepared with different MSM levels at 60 min fermentation time have been determined. Therefore, it can be clarified that reduced enzyme activity with increasing mechanical flour modification has no effect on gas formation during fermentation. Figure 6b illustrates the dough development curves during fermentation. It shows a significantly lower dough height (Hm) prepared with an MSM level of 8.37 g 100 g^{-1} flour at 60 min fermentation time. However, there are no significant differences between Hm of dough prepared with 4.78 and 6.56 g 100 g^{-1} flour. Furthermore, a continuous increase in Hm was observed for dough prepared with 4.78 g 100 g^{-1} flour and 6.56 g 100 g^{-1} flour until approximately 150 min fermentation time. The maximal dough height of high-MSM dough decreased slightly at approximately 60 min fermentation time after which it increased again moderately. Based on this, the results indicate that mechanical flour modification also has an effect on the dough stability during fermentation, despite adapted water addition. Subsequently, the gas retention properties and the time of porosity (Tx) of different MSM dough depending on water addition were investigated.

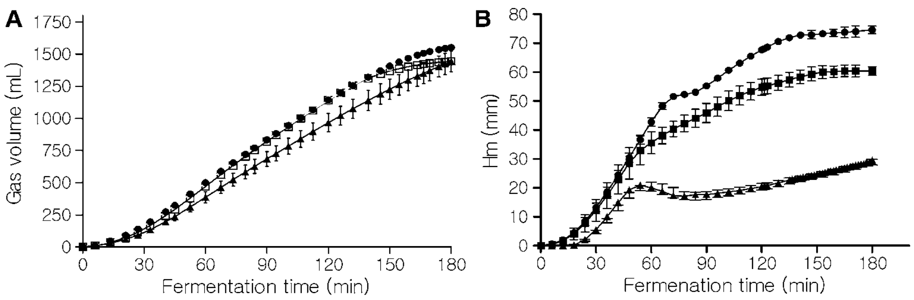

Fig. 6 **a** Gas volume and **b** dough development during fermentation of wheat dough of different MSM levels with optimal water additions. Gas volume (mL) and dough development (Hm in mm) based on flour exemplary shown with MSM levels of 4.78 g 100 g^{-1} flour *filled circle*, 6.56 g 100 g^{-1} flour *filled square* and 8.37 g 100 g^{-1} flour *pointed up filled triangle* were analyzed by a Rheofermentometer (n = 3)

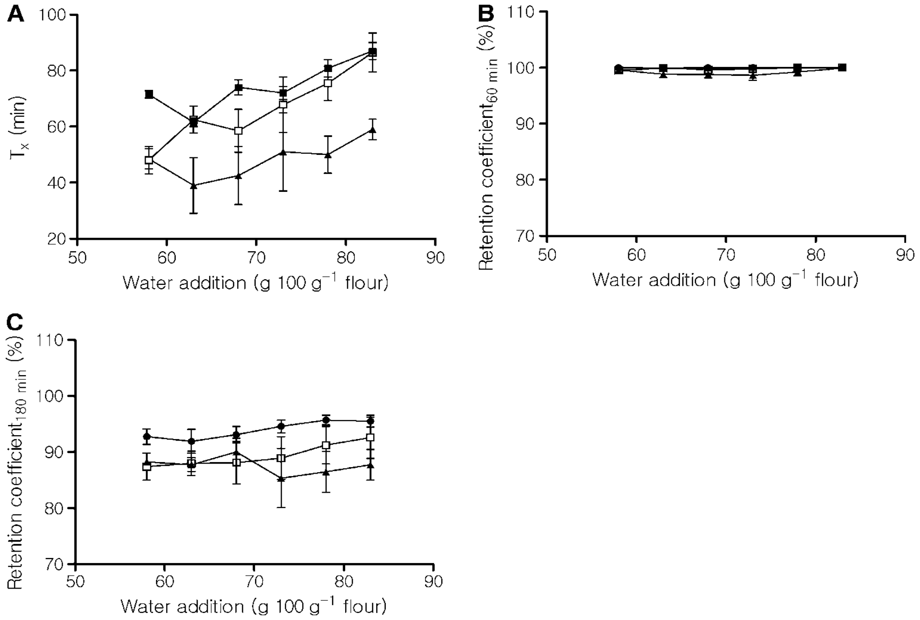

Fig. 7 Influence of water addition on the dough's **a** Tx and **b** gas retention coefficient after 60 min and **c** after 180 min with different MSM levels. Examples of the dough fermentation properties based on flours with MSM levels of 4.78 g 100 g^{-1} flour *filled circle*, 6.56 g 100 g^{-1} flour *filled square* and 8.37 g 100 g^{-1} *flour pointed up filled triangle* were analyzed by a Rheofermentometer. To expose significant differences between samples, ANOVA was applied following Tukey's test ($p < 0.05$). Means are shown with standard error ($n = 3$)

Time of porosity and gas retention coefficient of different MSM flours depending on water addition

To analyze the effect of dough hydration on gas retention properties of dough, Tx and the retention coefficient were evaluated using the Rheofermentometer. In this context, Tx of the dough prepared with different MSM levels, in relation to hydration, was evaluated as shown in Fig. 7a. The water addition had a significant effect on Tx in the dough prepared with flour of an MSM level of 4.78 g 100 g^{-1} flour. Thus, it can be shown that gas leakage is delayed with higher water addition. Dough prepared with flours of MSM level 6.56 g 100 g^{-1} flour showed a better gas-holding capacity only with a water addition of 63 g 100 g^{-1} flour, but not with a water addition of 58 g 100 g^{-1} flour. However, water addition had no significant effect on Tx in the dough prepared with the highest mechanically modified flour (8.37 g 100 g^{-1} flour). It seems that the water amount affects Tx only in the dough prepared with low mechanically modified flour. The water amount was adapted during kneading in each MSM flour sample because the dough hydration seems to change over time. Comparing different MSM levels with the same water volume indicates that there are significant differences in dough Tx. Time of porosity of the dough prepared with MSM 4.78 g 100 g^{-1} flour and water addition of 58 g 100 g^{-1} flour was significantly greater than that of the dough prepared with MSM 6.56 g 100 g^{-1} flour and 8.37 g 100 g^{-1} flour with the same water addition. However, no significant difference in Tx was observed between the different MSM levels for water additions of 63 g 100 g^{-1} flour, 68 g 100 g^{-1} flour and 73 g 100 g^{-1} flour. Nevertheless, Tx of the dough prepared with the highest water addition (83 g 100 g^{-1} flour) and an MSM level of 8.37 g 100 g^{-1} flour was significantly lower than that of the dough prepared with 4.78 g 100 g^{-1} flour and 6.56 g 100 g^{-1} flour. However, gas retention coefficient is crucial because it affects not only bread quality but also the amount of gas produced and lost from the dough matrix. Thus, the gas retention coefficient was analyzed from the dough prepared with varied water additions and flours with different MSM levels after 60 min (Fig. 7b) and 180 min (Fig. 7c). Figure 7b shows that neither the MSM level nor the water addition has any significant effect on the gas retention coefficient. Overall, gas leakage seems quite low at 60 min fermentation time, which is in accordance with the straight-dough process. To enable comparability with changes in gas retention over time, the gas retention capacity was also determined after 180 min, resulting in lower gas retention capacity than that observed after 60 min for all MSM levels (Fig. 7c). The gas retention capacity decreased with increasing fermentation time, but no significant differences were observed between the MSM levels. However, the water addition also has no significant effect on the retention coefficient at 180 min fermentation time. These results imply that mechanical flour modification does not affect the gas retention coefficient during the fermentation process. Initially, it was presumed that decreasing gas-holding properties during fermentation might responsible for a significantly lower Hm prepared with an MSM level of 8.37 g 100 g^{-1} flour at 60 min fermentation time. However, the study has shown that decreasing gas-holding properties were not the main reason for decreasing Hm with high-MSM dough. However, it has been demonstrated that Tx is earlier the higher the MSM level, which indicates that there are changes in dough structure which might strongly be influenced during thermal heating in the baking process and the following gas bubble expansion, which in turn might be responsible for low specific bread volume produced with dough with high MSM level. Another possible reason for the decreasing baking quality can be changes in dough viscosity during the baking process resulting from changes in enzymatic reaction due to higher substrate concentration with high MSM level. It is well known that mechanical flour modification affects gelatinization properties, which are associated with the enzymatic activity and digestibility of mechanically modified starch [13]. In this regard, changes in the amount of fermentable mono- and disaccharides might influence starch gelatinization properties during baking, which might also have an effect on the bread quality [22, 23].

Conclusions

The specific bread volume was negatively affected by the MSM level, regardless of the (optimal) water addition. The mechanical treatment (grinding) reduced the enzyme activity. However, it was shown that these effects had no influence on the gas formation at 60 min fermentation time. Furthermore, the results indicate that mechanical flour modification also has an effect on the dough stability during fermentation. In comparison to dough prepared with lower MSM flour and adapted water addition, a significantly lower dough height (Hm) was shown for dough prepared with high MSM at 60 min fermentation time. However, there were no significant differences in Hm between dough prepared with MSM 4.78 and MSM 6.56 g 100 g^{-1} flour at the same fermentation time. From the results it can be excluded that loss of gas during fermentation is the reason for low Hm with high MSM level. The results indicate that there are changes in dough structure, which additionally might be influenced during thermal heating in the baking process. These structural changes, in turn, might be responsible for low specific bread volume produced with dough with high MSM level.

The current study provides an improved understanding of the mechanically modified flour on dough processing and baking performance.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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4 Discussion

Starch has different functions throughout the various stages of bread making (kneading, proofing, baking), and thus, alterations of starch structure and functionality are critical factors that influence the final bread quality.

In this regard, the specific bread volume is negatively affected using flours with high-MSM levels despite optimum bulk water addition, according to the farinograph method (Barrera et al. 2007; Brütsch et al. 2017). The results indicate that the used water addition, or rather the applied energy, during kneading or its combination has not been optimal for this purpose. In this regard, the effect of various water additions on the specific bread volume was investigated. The results indicate that the specific bread volume of the standard could not be achieved with dough produced from high-MSM flour, even if water addition was optimized to the highest possible bread volume (see Fig. 3b of Hackenberg et al. (2017)). Thus, a simple adaption of the water amount did not result in a solution to the quality problem.

The present work provides a fundamental contribution to understanding the effect of mechanically modified starch on dough functionality during the non-heating steps of dough processing (kneading and proofing). Part of the first working hypothesis was that the altered starch functionality (swelling, increased water absorption capacity) caused by MSM negatively influences protein microstructure formation during kneading. It is highly probable that due to a poorly developed protein network structure, dough elongation behavior and therefore the gas-holding properties of the dough are negatively affected, resulting in a reduced dough height after proofing.

In this respect, this work initially clarifies in detail the effect of mechanically modified starch on protein network structure formation during kneading. The protein network was visually characterized by a CLSM and quantified using the PNA method developed by Bernklau et al. (2016) as a function of kneading time. The investigation of dough rheological properties, such as R_{max} and Ext_{max}, using texture profile analysis (TPA) enabled conclusions regarding the dough elongation behavior during proofing. In detail, the influence of increased swelling of mechanically modified wheat starch was set with respect to rheological alterations of dough produced from high-MSM flour compared with ideal wheat dough.

Mechanical energy input in the form of kneading induces gluten proteins to be bidirectionally extended. The energy input effects that the protein network receives a relatively homogenous distributed structure spread through the dough (Jekle and Becker 2015). According to Lucas et al. (2019), a standard protein network in dough might typically be categorized as a strengthened or spread network. In the literature, optimally kneaded wheat dough is described as an interconnected gluten structure covered with starch particles (Peighambardoust et al. 2010). Dough structure formation is associated with an increase in dough viscosity, commonly analyzed using the DoughLab (AACC International Method 54-21.02). The maximum dough consistency ($\text{Peak}_{\text{time}}$) is defined as the optimum kneading time. As discussed in Section 1.3.1, alterations of starch structure and functionality, particularly the increased swelling capacity of mechanically modified starch, might affect protein-starch or starch-starch interactions. The effect of the increased hydration capacity of mechanically modified wheat starch on dough microstructure formation during kneading has not yet been investigated and is thus explained below.

For this purpose, mechanically modified wheat flours used in this study are initially characterized regarding their particle size and swelling behavior in cold water. The particle size of mechanically modified dry wheat flour based on the number and volume distribution was analyzed with a Mastersizer 3000 using an automated dry powder dispersion unit. The results are presented in Table 1 of Hackenberg et al. (2018a). The wheat flour particle size based on number decreased with an increasing MSM level (d_{90} value -40%; d_{50} value -36%; d_{10} value -29%). Regarding the flour particle size based on the volume, the d_{90} value decreased by 62%, particularly caused by the separation of accumulated wheat flour particles. Moreover, the d_{50} and d_{10} values decreased by 66% and 57%, respectively (Hackenberg et al. 2018a). It is supposed that decreased flour particle sizes facilitate their hydration caused by increased relative surface area.

To evaluate the swelling behavior of the corresponding MSM flours, flour-water suspensions were prepared at room temperature and measured using a manual wet dispersion unit. The particle size based on the volume distribution increased with an increasing MSM level. Thus, the d_{50} and d_{90} values based on the volume of particles increased by 25% and 31%, respectively, whereas the d_{10} value increased by only 6%

(see Table 2 of Hackenberg et al. (2018a)). Barrera et al. (2013a) observed an increased swelling behavior of wheat starch granules with increasing MSM in water at room temperature. Thereby, the particle size of wheat starch based on the volume of particles increases from 20.2 ± 0.5 to 23.6 ± 0.9 and 33.9 ± 0.8 to 46.0 ± 1.2 for the d_{50} and d_{90} values, respectively (Barrera et al. 2013a). It is to be expected that the increased swelling behavior of wheat flour was primarily caused by the increased swelling capacity of mechanically modified wheat starch (Hackenberg et al. 2018a).

The possible effects of the increased water absorption and swelling capacity of mechanically modified starch on protein microstructure development are described in detail in Section 1.3.1. Thus, the increased water absorption of mechanically modified starch might cause starch and protein to compete for free water during dough processing. To prevent this, water addition for dough preparation needed to be adapted for all MSM flours according to AACC International Method 54-21.02. Furthermore, starch-starch interactions might affect the protein network development caused by increased swelling of the granules. This presumption could be supported by the investigation of the kneading curve of dough produced from high-MSM flour and its visualization at dough development time ($Peak_{time}$) by means of a CLSM.

A comparison of the dough development curves of the standard dough and the dough produced from high-MSM flour (see Fig. 1a and 1b of Hackenberg et al. (2019)) reveals that doughs produced from high-MSM flour displayed a second peak with lower intensity between approximately 13–18 min after $Peak_{time}$. The larger volume of mechanically modified wheat starch granules, caused by cold swelling, increased the dough viscosity up to $Peak_{time}$ (Hackenberg et al. 2019). At $Peak_{time}$, the torque level of dough produced from high-MSM flour was comparable to the torque level of the standard dough, which had a homogenously, hyperbranched gluten structure as expected for optimally kneaded dough (see Fig. 2g of Hackenberg et al. (2019)). By contrast, the dough produced from high-MSM flour primarily exhibited gluten agglomerations at $Peak_{time}$ but not an evenly distributed protein network structure (see Fig. 2a of Hackenberg et al. (2019)). This is the case despite the same amount of energy being applied during kneading (standard dough ~0.478 KJ/h; MSM dough ~0.482 KJ/h) (Hackenberg et al. 2018a). Thus, the swelling of starch granules imitated a torque level comparable to an optimally developed gluten network (Hackenberg et al. 2019). Two different assumptions explain why applied kneading energy might not be sufficient for optimal protein network development in dough produced from high-MSM flour at $\text{Peak}_{\text{time}}$.

First, this might be due to alterations of the protein fractions as a consequence of the mechanical treatment in the ball mill. Vogel (2019) discovered that the intensive ball milling of wheat flour leads to a thiol-disulphide exchange within the gluten proteins, resulting in a cross-linking of proteins (Vogel 2019). Thus, it can be presumed that additional energy might be needed to re-structure the disulphide bonds during kneading. However, the absolute concentration of the main gluten proteins did not substantially differ in the MSM flour compared with the standard flour. Based on the results, it can be concluded that the ball mill treatment caused no extensive thiol-disulphide exchange, and therefore, it is rather unlikely that altered protein functionality is the cause of a poorly developed protein network at $\text{Peak}_{\text{time}}$ (Hackenberg et al. 2019).

Second, the poorly developed protein network in dough produced from high-MSM flour at $\text{Peak}_{\text{time}}$ can be caused by a change in the starch functionality as a consequence of the ball mill treatment. As discussed in Section 1.3.1, starch-starch interactions might be influenced by increasing the diameter of starch granules due to swelling. Adhesion forces (capillary forces) between particles increase with increasing the diameter of a spherical particle (Borho et al. 1991), and therefore, starch swelling might enhance the strength of capillary bridges between starch granules. Thus, it is assumed that more energy in the form of kneading is required to separate swollen, accumulated starch granules from one another. The protein component therefore received less mechanical energy in the form of kneading, leading to a poorly developed protein network at $\text{Peak}_{\text{time}}$ (Hackenberg et al. 2019).

This assumption could be clearly confirmed by the qualitative and quantitative investigation of the protein microstructure development as a function of kneading time using a CLSM and the PNA method according to Bernklau et al. (2016). Kneading beyond $\text{Peak}_{\text{time}}$ increased the protein network structure formation, as presented in Fig. 2b–f of Hackenberg et al. (2019). The visual evaluation of the protein network structure of high-MSM dough at the second peak (see Fig. 2d of Hackenberg et al. (2019)) was quite similar to the protein network structure of the standard dough at $\text{Peak}_{\text{time}}$ (see Fig. 2g of Hackenberg et al. (2019)). Kneading beyond the second peak (see Fig. 2e–f of Hackenberg et al. (2019)) resulted in a homogenously, fine and close-meshed gluten network structure. Similar protein structures were observed in dough produced with prolonged mixing times, referred to as over-mixed dough (Peighambardoust et al. 2006; Van der Mijnsbrugge et al. 2016).

The visual evaluation of the protein microstructure confirmed by the quantitative analysis according to Bernklau et al. (2016) is illustrated in Fig. 3a–e of Hackenberg et al. (2019). Thus, the number of junctions per protein area (protein branching rate) increased by 64.1%, whereas the number of end-points per protein area (protein end-point rate) and the degree of gaps (lacunarity) decreased by 57.7% and 88.6%, respectively, when elongating the kneading time of high-MSM dough (MSM level 8.15 g 100 g^{-1} flour) produced with optimum water addition according to AACC International Method 54-21.02, from 2.9 min ($\text{Peak}_{\text{time}}$) to 30 min. Furthermore, the average protein length increased by 781.4%, whereas the protein width decreased by 13.3%; thus, the protein filaments became longer and finer with prolonged kneading. The protein network attributes branching rate, protein end-point rate, mean lacunarity, protein length, and protein width of dough produced from high-MSM flour observed at 16 min kneading time, which corresponds to the second peak in the dough development curve, did not significantly differ from the standard dough at $\text{Peak}_{\text{time}}$. Thus, high-MSM dough had a considerably similar protein network structure in terms of kneading process adaption (Hackenberg et al. 2019). In the further course, it is revealed whether these protein networks have equivalent rheological properties.

To clarify this, the rheological behavior of dough produced from high-MSM flour was compared with the standard dough and set with respect to the protein microstructure development. The maximum extensibility (Ext_{max}) of dough produced from high-MSM flour decreased by 28%, whereas R_{max} decreased by 49% with increasing MSM levels at $\text{Peak}_{\text{time}}$; as a consequence, the maximum height of the dough (Hm) decreased by 54%. The decreased Hm was attributable to poor extensional properties caused by poor protein network structure development at $\text{Peak}_{\text{time}}$ (Hackenberg et al. 2018a). By prolonging the kneading time up to 16 min (second peak), the specific volume of bread produced from high-MSM flour was significantly increased. This was pronounced by the improved rheological properties caused by coherent protein microstructure development. Thus, the maximum resistance to extension (R_{max}) significantly increased when high-MSM dough was kneaded up to the second peak. Due to stronger protein network connectivity more energy was required to break this network (Hackenberg et al. 2019). As a result, the dough should not break so rapidly during proofing.

The dough elongation properties, particularly the resistance to extension, are strongly positively correlated with the baking volume of bread (Dobraszczyk and Salmanowicz 2008; Kenny et al. 1999). Furthermore, Hackenberg et al. (2019) observed a high correlation of $r = 0.97$ and $p < 0.05$ between R_{max} and the specific bread volume. The specific bread volume significantly increased with increasing R_{max} by prolonging the kneading time of dough produced from high-MSM flour. Nevertheless, the R_{max} of the standard dough could not be achieved, despite similar network characteristics of dough being produced from high-MSM flour with kneading time adaption (Hackenberg et al. 2019). Therefore, the maximum resistance to extension (R_{max}) of dough produced from high-MSM flour decreased by 22.1% (see Fig. 4b of Hackenberg et al. (2019)). In addition, the maximum extensibility of dough (Ext_{max}) was significantly reduced compared with the standard presented in Fig. 4a of Hackenberg et al. (2019). To summarize, the rheological behavior of dough produced from high-MSM flour differs compared with the standard dough despite a similar protein network structure. The reason for this is discussed below.

The poor rheological properties and consequently the decreased bread volume compared with the standard were primarily caused by the increased swelling capacity of mechanically modified starch granules in dough produced from high-MSM flour. Two possible explanations exist for this effect. Due to the decreased relative surface area of mechanically modified starch granules as a consequence of its volume increase, the interactions between protein and starch might be reduced. As a result, the reinforcement of the dough might decrease, as characterized by a lowered R_{max} (Hackenberg et al. 2019). Moreover, a relationship between increased filler particle size and decreased reinforcement of an elastomer was observed by Yuan and Mark (1999) using a cathetometer. In addition, the larger volume of swollen starch granules might lead to larger cavities within the gluten network, resulting in poor network connectivity; therefore, the gluten network might be weaker compared with the standard, which was characterized by a significantly reduced Ext_{max} and R_{max} (Hackenberg et al. 2019). The influence of altered functionality of mechanically modified wheat starch in the form of increased swelling regarding dough microstructure formation is schematically summarized in Fig. 4.

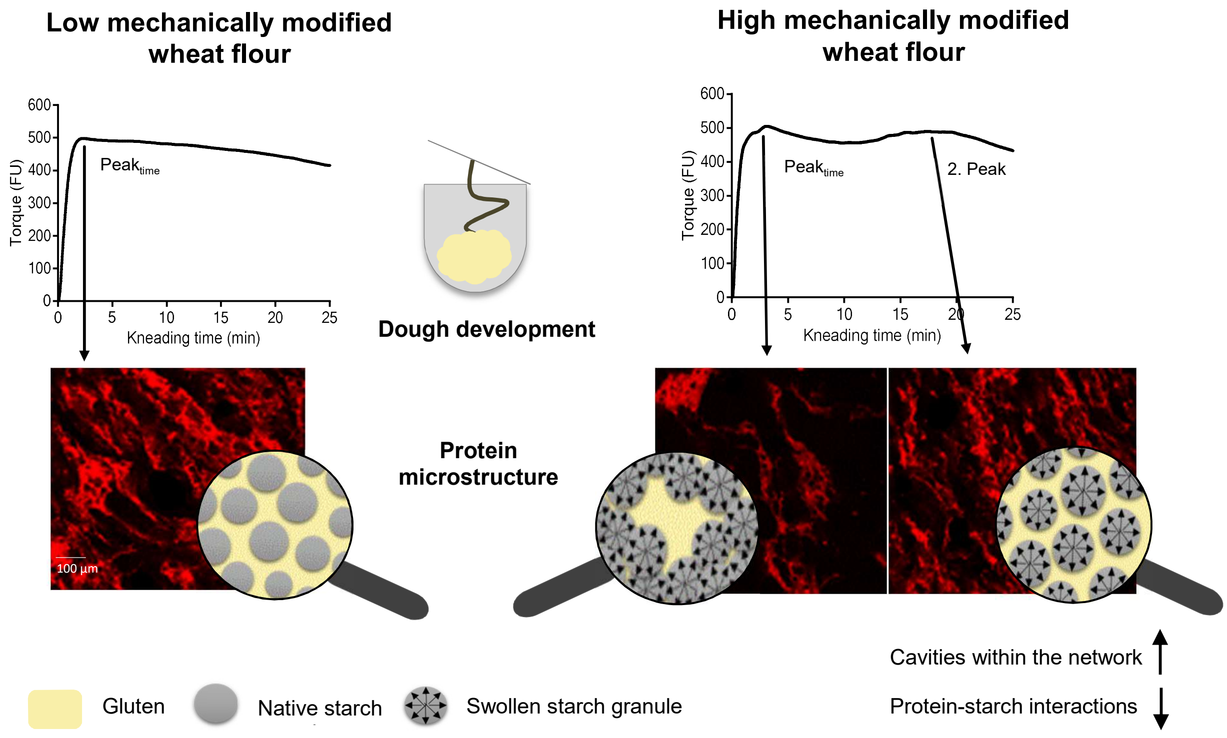

Fig. 4. Effect of altered functionality of mechanically modified wheat starch in the form of increased swelling on dough protein microstructure formation.

In this thesis, it is hypothesized that decreased dough elongation properties, such as decreased R_{max} and Ext_{max}, are the cause of poor gas-holding properties of the dough. In this regard, the gas-holding properties of dough produced from high-MSM flour were investigated. Therefore, the time when dough loses gas (time of porosity, T_x) and the gas retention coefficient were analyzed in dough produced from MSM levels between 4.78 and 8.37 g 100 g^{-1} flour with an optimum water addition according to AACC International Method 54-21.02 using a rheofermentometer (Hackenberg et al. 2017). As discussed above, the protein network in dough produced from high-MSM flour was not adequately developed at $\text{Peak}_{\text{time}}$ compared with the standard, revealing a highly interconnected and hyperbranched protein network. Furthermore, rheological properties (R_{max} and E_{max}) were poor compared with the standard (Hackenberg et al. 2019). Nevertheless, the gas-holding properties of high-MSM dough were comparable to those of the standard dough at a 60 min fermentation time and thus appear to not be relevant for the decreased Hm during proofing (Hackenberg et al. 2017). To summarize, the protein network formation and thus the elongation properties of high-MSM dough were negatively influenced by MSM. However, the gas-holding properties of high-MSM dough are not affected.

The maximum dough height (Hm) during proofing is not only influenced by the gas-holding properties of the dough but also by the gas amount produced by yeast. During mixing, gas cells are incorporated into the dough. Then, during proofing, these gas cells are filled with CO_2, which is released by yeast (Shehzad et al. 2010). As described in Section 1.3.2, yeast requires energy in the form of sugars for their metabolism. Fermentable carbohydrates in the form of sugars are becoming available for yeast due to enzymatic starch hydrolysis. Starch degradation depends on various factors, such as the accessibility of the substrate (starch) and the enzymatic activity. Due to the mechanical wheat flour treatment in a grinder, the accessibility of starch is improved; the possible reasons for this are summarized in Section 1.2, Table 1. Furthermore, the reaction rate of amylolytic enzymes depends on the water availability (Dumont et al. 1992) and water mobility in the reaction medium. These parameters might be influenced by the increased water absorption capacity of mechanically modified wheat starch.

In that respect, this study hypothesizes that altered dough hydration might affect the interactions between mechanically modified starch and amylolytic enzymes and thus could influence the release of fermentable saccharides. In addition, alterations of the substrate itself (MSM) might influence enzymatic hydrolysis and therefore affect substrate availability for yeast. These factors might influence gas production by yeast and consequently Hm during fermentation. To clarify the dependencies between MSM, (dough) hydration, and saccharide release during grinding in a ball mill and in the subsequent non-heating steps of dough processing, the produced fermentable carbohydrates were analyzed using significantly different MSM levels between 4.78 and 8.37 g 100 g^{-1} flour and hydration levels between 58 g H_2O 100 g^{-1} flour (dough) and 1,500 g H_2O 100 g^{-1} flour (suspension). The analysis was performed during kneading and proofing (without yeast) and fermentation (with yeast). The saccharides, such as glucose, fructose, maltose, sucrose, and maltotriose, were analyzed using high-performance anion-exchange chromatography with electrochemical detection (HPAEC-ED) (Hackenberg et al. 2018b).

The concentration of total saccharides already increased by 116% with a heightened MSM level of the wheat flour after grinding in a ball mill, as presented in Hackenberg et al. (2018b). The moisture content of the experimental wheat flours ranged between 12.8% and 14.1% (Hackenberg et al. 2017). It appears that these water concentrations were already sufficient for enzymatic hydrolysis during grinding (Hackenberg et al. 2018b). This could also be confirmed by Dumont et al. (1992), who observed enzymatic reactions as particles contained a water concentration of 10% (Dumont et al. 1992). In addition, the trend of increased total saccharides with high MSM could be observed in the suspension model during the 180 min resting time (see Fig. 1a of Hackenberg et al. (2018b)). These increases of total saccharides after grinding and during resting were primarily caused by enhanced maltose release (see Fig. 1b of Hackenberg et al. (2018b)). In general, maltose is hydrolyzed by exo-hydrolyses, such as β-amylase. In the experimental wheat flours, mainly β-amylase activity could be detected using the Betamyl Assay Kit by Megazyme International (Hackenberg et al. 2017). This aligns with the expectation, since *α*-amylase production is activated during germination by gibberellin (Jacobsen et al. 1995; Kaneko et al. 2002), whereas *β*-amylases were already contained in the resting cereal grain previously (Sopanen and Laurière 1989). The β-amylase activity of the wheat flours significantly decreased by ~28% with stronger mechanical treatment in the ball mill, as illustrated in Fig. 5 of Hackenberg et al. (2017). This result might be explained as follows: Changed substrate availability might affect the assay of enzyme activity. Thus, β-amylase in high-MSM flours might hydrolyze a greater amount of mechanically modified starch; therefore, a lower amount of labeled substrate of the assay is hydrolyzed within the specified time frame. Therefore, the β-amylase activity in the wheat flour might be much higher than the β-amylase activity value obtained from the assay. This assumption could be confirmed by the increased maltose release in high-MSM flours.

The maltose concentration in dough increased by approximately 334% during kneading, whereas during proofing, only a slight growth could be detected. Therefore, most of the fermentable saccharides had been released already during the kneading step. Thereby, maltose formation was facilitated by the decreased dough hydration and increased MSM level (Hackenberg et al. 2018b). This behavior aligns with the initial working hypothesis that increased dough hydration and thus reduced viscosity appears to decrease the interaction between mechanically modified starch and amylolytic enzymes. Nevertheless, the maltose concentration was significantly higher compared with the standard (Hackenberg et al. 2018b). Thus, high substrate availability appears to be the predominant factor regarding the sugar release by amylolytic enzymes.

To draw conclusions on yeast metabolism and thus indirectly on gas production, maltose concentrations of non-yeasted dough were compared with those of yeasted dough after fermentation as a function of MSM (MSM level 4.78 g100 g^{-1} flour and 7.46 g100 g^{-1} flour) and dough hydration (58 g water 100 g^{-1} flour, low dough hydration and 78 g 100 g^{-1} flour, high dough hydration) (see Hackenberg et al. (2018b)). Maltose utilization by yeast was favored by low dough hydration (+37% with MSM of 4.78 g 100 g^{-1} flour; +27% with MSM of 7.46 g 100 g^{-1} flour) (see Table 2 of Hackenberg et al. (2018b)). However, the increased maltose concentrations resulting from high MSM were not metabolized by yeast during a 60 min fermentation time, and therefore, the reaction speed was independent of the substrate concentration (Hackenberg et al. 2018b). The findings indicate that increased MSM should have no relevant effect on CO_2 production by yeast during proofing. To investigate this effect in greater detail, the gas formation of doughs produced from significantly different MSM levels between 4.78 (standard) and 8.37 g 100 g^{-1} flour with optimum water additions according to AACC International Method 54-21.02 was investigated during a fermentation cycle of 180 min using a rheofermentometer, as illustrated in Fig. 6a of Hackenberg et al. (2017). The gas volume of dough produced from high-MSM flour was not significantly different compared with the standard at a 60 min fermentation time (Hackenberg et al. 2017). In summary, the MSM level and dough hydration level had no relevant effect on gas formation during proofing, and thus altered gas formation with high MSM and changed dough hydration were not the cause of the reduced dough height during proofing or rather the lowered bread volume after baking.

Following dough processing and proofing, the baking step is performed. From the rheological point of view, the viscoelastic dough is converted into elastic bread (Schirmer 2014). Baking leads to several product alterations, such as a volume increase, formation of a porous structure, and crust formation and browning (Sablani et al. 1998). These attributes depend on protein denaturation and starch gelatinization. The effects of MSM on starch gelatinization properties are summarized in Section 1.2, Table 1. However, most experiments were performed in excess water, and thus the results do not directly apply to a system with limited water content, such as wheat dough. The effect of MSM and its altered functionalities on wheat starch gelatinization behavior in dough during the baking step has not yet been examined. In general, starch gelatinization strongly depends on the starch-to-water ratio. Thus, low water concentrations limit the gelatinization process displayed with CLSM micrographs of real baking and model systems using a heating plate (Schirmer 2014).

As mentioned, the bulk water addition for dough preparation must be adapted based on the MSM level of wheat flours caused by the higher water absorption capacity of mechanically modified starch. The relationship between MSM, water addition, and onset gelatinization temperature is presented in Fig. 5 adapted from Friess (2016). Thereby, structural alterations in dough during gelatinization were investigated by analyzing the complex shear modulus (G*), which describes the stiffness of dough (Schirmer et al. 2015). In the following G* was analyzed with an oscillation test by means of a rheometer.

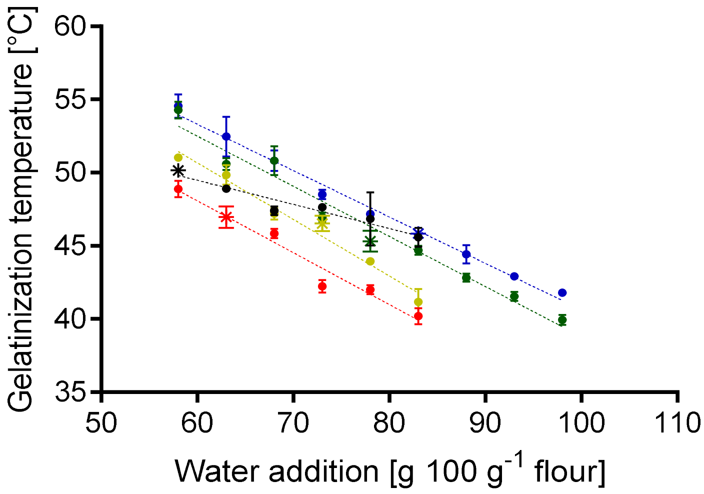

Fig. 5. Dynamic oscillatory measurement of wheat dough during heating. The increase of the complex shear modulus (G*) reflected the onset of gelatinization. Gelatinization temperatures were visualized as a function of various MSM levels and water additions. ● ≙ 4.78 g 100 g^{-1} flour; ● ≙ 6.65 g 100 g^{-1} flour; ● ≙ 7.46 g 100 g^{-1} flour; ● ≙ 7.85 g 100 g^{-1} flour; ● ≙ 8.15 g 100 g^{-1} flour. The optimum water addition according to AACC International Method 54-21.02 is shown with a diamond. Means are displayed with the standard error of mean (n = 3); ANOVA; $p < 0.05$.

The results demonstrate that the onset gelatinization temperature decreased with increasing bulk water addition, which aligns with the common literature. Furthermore, the results in Fig. 5 indicate that the gelatinization in dough produced with increased MSM levels, and the optimum water addition according to AACC International Method 54-21.02 was initiated at a lower temperature compared with the standard. However, the onset of gelatinization increased with increasing MSM when comparing dough with the same water addition. This could be due to the increased saccharide concentration in dough produced from high-MSM flour. The relationship between sugar addition and delayed gelatinization has already been observed in many studies (Li et al. 2015; Perry and Donald 2002; Spies and Hoseney 1982). Spies and Hoseney (1982) suppose that saccharides function in two independent ways. First, they reduce the water activity (A_w-value) of the solution. Second, the amorphous regions of starch are stabilized due to the interaction of saccharides with the starch chains. Both of these result in a delay of gelatinization temperature. They also discovered that the longer the chain length of the saccharides, the later the gelatinization (Spies and Hoseney 1982). Thus, the gelatinization is more inhibited by trisaccharides and tetrasaccharides than by disaccharides, whereas disaccharides have a more inhibitory effect than monosaccharides (Li et al. 2015). Therefore, it could be argued that maltose inhibits gelatinization in mechanically modified starch to a higher degree than glucose does. The influence of early starch gelatinization in dough produced from high-MSM flour with the optimum water addition during baking and its possible effects on the quality attributes of wheat bread, such as crumb and crust formation, has not yet been examined. Additionally, the effect of increased sugar concentration in dough produced from high-MSM flour on quality attributes of wheat bread, such as the taste, crust coloration, and flavor, has not yet been sufficiently evaluated.

This thesis clarified in detail the cause of poor-quality bread produced from wheat flour that was mechanically modified. As a basis, the fundamental understanding of the impact of mechanically modified starch in dough during processing was used. Thereby, this thesis was particularly focused on the non-heating steps of dough processing, such as kneading and proofing. Through the adaption of the kneading process, the protein microstructure could be significantly improved, resulting in a significantly increased baking volume of wheat bread. Gas-holding properties, or rather gas formation, during proofing had no relevant effect on differences in dough height between the standard and the dough produced from high-MSM flour. Thus, the dough height limiting factor was clearly the kneading step. The knowledge of the cause-effect relationship enables now the control and improvement of the quality of bread produced from wheat flour with a high MSM level.

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6 Appendix

6.1 Reviewed paper

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6.2 Non-reviewed paper

Hackenberg, S., Müller, E., Chen, X., Hussein, M., Begemann, J., Sciurba, E., Jekle, M., Köhler, P., Becker, T.: The baking quality of wheat– is it really predictable? Baking + Biscuit 6 (2016) 48–50.

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6.3 Oral presentations

Hackenberg, S., Jekle, M., Becker, T.: Mechanical starch modification and its effect on saccharide formation and dough functionality, AACC International Annual Meeting 2016, Savannah, USA, 2016-10-25.

Hackenberg, S., Jekle, M., Becker, T.: Steuerung der technologischen Funktionalität von mechanisch modifizierten Mehlen, Mühlensymposium, Würzburg, Germany, 2015-11-12.

Hackenberg, S., Jekle, M., Becker, T.: Einfluss der Stärkebeschädigung und der Enzymumsetzbarkeit auf die Gebäckstruktur hinsichtlich verschiedener Gärkonditionen, 1. D-A-CH-Tagung für angewandte Getreidewissenschaften, Wien, Austria, 2015-10-01.

Hackenberg, S., Verheyen, C., Bernklau, I., Jekle, M., Becker, T.: Innovationen und Zukunftsmärkte der Backbranche. Seminar: Einfluss der Vermahlungstechnologie auf Backwaren, Bakery Innovation Center, Uzwil, Switzerland, 2015-07-24.

Hackenberg, S., Jekle, M., Becker, T.: Einfluss von Stärkemodifikationen auf die Gebäckstruktur hinsichtlich verschiedener Gärkonditionen, 4. WIG Frühjahrstagung, Freising, Germany, 2015-04-22.

Hackenberg, S., Jekle, M., Becker, T.: Mehlqualitäten im 21. Jahrhundert: Welche Aussage bietet die Fallzahl? 3. WIG Frühjahrstagung, Freising, Germany, 2014-04-03.

Hackenberg, S., Jekle, M., Becker, T.: The influence of starch constitution and enzymes on raw material characteristics of wheat flour, 12th European Young Cereal Scientists and Technologists Workshop, Nottingham, England, 2013-04-12.

6.4 Poster presentation

Hackenberg, S., Jekle, M., Becker, T.: Mechanical flour modification impacts the kneading of wheat dough, AACC International Annual Meeting 2018, London, UK.