TECHNISCHE UNIVERSITÄT MÜNCHEN

Department Ingenieurwissenschaften für Lebensmittel und biogene Rohstoffe

Lehrstuhl für Brau- und Getränketechnologie

Prof. Dr.-Ing. Thomas Becker

System analytical approach for water macromolecule interactions of physically modified biopolymers in cereal based foams

Sabina Paulik

Vollständiger Abdruck der von der Fakultät TUM School of Life Sciences der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzende:	apl. Prof. Dr.-Ing. habil Petra Först
Prüfer der Dissertation:	1. Prof. Dr.-Ing. Thomas Becker
	2. Prof. Dr. Katharina Scherf

Die Dissertation wurde am 26.02.2020 bei der Technischen Universität München eingereicht und durch die Fakultät TUM School of Life Sciences am 15.10.2020 angenommen.

One never notices what has been done; one can only see what remains to be done.

Maria Skłodowska-Curie (1867 – 1934)

Acknowledgement

My deepest appreciations go to all those people who made this thesis an unforgettable and successful experience for me. First, I want to thank my supervisor Prof. Dr. Thomas Becker, who offered his continuous advice, who encouraged me to question the results, methodology and approaches throughout the course of this thesis and who challenged our scientific practice, so that we could become better researcher.

Further, I would like to offer my special thanks to Dr. Mario Jekle. Within the last years, he demanded and promoted not only a good scientific practice and a successful completion of the thesis, but also, he was an important driver for my personal development. I am deeply grateful for his support throughout the years.

I would like to thank Prof. Dr. Katharina Scherf for agreeing to evaluate this thesis and Prof. Dr. Petra Först for accepting the assignment as chairperson.

I owe a very important debt to all members of the Institute of Brewing and Beverage Technology, especially to the Research Group Cereal Process Engineering for the support and at all time enjoyable working atmosphere.

I would like to thank my good friends in Stuttgart, who remain good friends throughout the whole time, in spite the distance. Further, I would like to offer my special thanks to my parents, who believed in me since I was a little child and still do believe in me.

By far and from the bottom of my heart, I want to thank to my great love Daniel for his love, for his continuous trust in me, for his overall support over shorter and longer distances and for our wonderful little daughter.

Preface

The results and publications of this thesis were developed at the Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering from 2015 to 2019. The results of this thesis were published in five peer-reviewed journals.

Peer-Reviewed Publications

The following six peer reviewed publications (shown in chronological order) were generated in the period of this work:

1. Jakobi, S., Jekle, M., & Becker, T. (2018a). Direct link between specific structural levels of starch and hydration properties. Carbohydrate Polymers, 181(1), 159–166. https://doi.org/10.1016/J.CARBPOL.2017.10.062
2. Jakobi, S., Jekle, M., & Becker, T. (2018b). High-Pressure Treatment of Non-Hydrated Flour Affects Structural Characteristics and Hydration. Foods, 7(5), 78. https://doi.org/10.3390/foods7050078
3. Paulik, S., & Jekle, M. (2019). Novel approach to investigate the mechanical properties of crumb matrix during storage – Re-engineering of gas-free crumb pellets. Food Chemistry, 288, 333–340. https://doi.org/10.1016/J.FOODCHEM.2019.03.014
4. Paulik, S., Jekle, M., & Becker, T. (2019a). A review: Reverse approach to analyze the impact of starch modification on the inflation and gas holding properties of wheat-based matrices. Trends in Food Science & Technology, 91, 231–239. https://doi.org/10.1016/J.TIFS.2019.07.031
5. Paulik, S., Jekle, M., & Becker, T. (2019b). Mechanically and Thermally Induced Degradation and Modification of Cereal Biopolymers during Grinding. Polymers, 11(3), 448. https://doi.org/10.3390/polym11030448
6. Paulik, S., Wen Yu, W., Flanagan, B., Gilbert, R. G., Jekle, M., & Becker, T. (2019). Characterizing the impact of starch and gluten-induced alterations on gelatinization behavior of physically modified model dough. Food Chemistry, 125276. https://doi.org/10.1016/J.FOODCHEM.2019.125276

List of content

Abbreviation

AACC	American Association of Cereal Chemists
AM	Amylose
AP	Amylopectin
°C	Degree Celsius
CPMG	Carr-Purcell-Meiboom-Gill sequence
CG	Cryogenic grinder
CLSM	Confocal laser scanning microscopy
dm	Dry matter
DSC	Differential Scanning Calorimetry
FFF	Field-flow-fractionation
FU	Farinogramm Units
g	Gramm
HPAEC	High performance anion exchange chromatography
HPLC	High performance liquid chromatography
HPT	High pressure treatment
ICC	International Association of Cereal Science and Technology
IG	Impact grinder
J	Joule
K	Parameters of Mark-Houwink equation
M	Molecular weight
mG	Modified Gluten
min	Minute
mm	Millimeter
MPa	Megapascal
mS	Modified starch
MS	Mechanical stress
N	Newton
nm	Nanometer
NMR	Nuclear magnetic resonance
Pa	Pascal
rG	Reference gluten
rpm	Rounds per minute

rS	Reference starch
RVA	Rapid visco analyzer
s	Second
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SMD	Starch modification degree
SRC	Solvent retention capacity
TA	Dough yield
tan δ	Loss tangent
T_c	Conclusion temperature of gelatinization
TEM	Transmission electron microscopy
TMA	Thermomechanical analysis
TMS	Thermo-mechanical stress
T_o	Onset temperature of gelatinization
T_p	Peak temperature of gelatinization
TPA	Texture Profile Analyzer
TS	Thermal stress
UCG	Ultra-centrifugal grinder
WRC	Water retention capacity
X-ray	X-radiation (a form of electromagnetic radiation)
α	Parameters of Mark-Houwink equation
ΔH	Enthalpy of gelatinization
[η]	Intrinsic viscosity
µm	Micrometer

Summary

The formation of wheat based food foams depends on the non-covalent binding of water by the native polymers starch and gluten. Grinding, however, causes a multitude of structural alterations of starch polymers on molecular and microscopic scale, which affect the hydration properties of wheat flours. Previous research findings could not explain mechanistic interrelationships between starch structures and the interaction with water, since further wheat polymers are modified during grinding, which competitively interact with starch polymers and water. The aim of this thesis was to determine mostly isolated the effects of molecular and microscopic alterations of starch polymers on the hydration properties of wheat flours. For this purpose, functional changes of gluten were investigated and the influence of altered starch gluten interactions on the hydration characteristic of wheat flours was analyzed.

A methodology was developed at first, which enables to identify systematically causal relations between the polymer structure of starch and the foaming properties of physically modified, wheat based systems. Thus, a negative impact of an early gelatinization onset and a reduced viscosity during heating on the gas volume fraction of solid foams of wheat based dough was proven, which was evoked by an enhanced swelling of physically modified flour particles. A subsequent study demonstrated that the enhanced swelling and water binding of ground flours is caused by altered surface properties of starch-gluten agglomerates. Hence, the further focus of the work rested on strategies to determine the impact of different grinding types and the influence of gluten polymers on the hydration properties of physically modified wheat flours.

Targeted modifications of wheat flour and a selective modification of wheat starch and gluten by means of ultra centrifugal mill and cryogenic mill, thermal processes as well as high-pressure treatment revealed an increased water binding of wheat flour exclusively by mechanical forces during the grinding processes. Additionally, modified gelatinization properties of dry-thermally treated wheat flours were noticed, which were not observed on thermally treated wheat starch. The results indicate altered starch water interactions, which could be evoked by a physical modification of gluten polymers.

The hypothesis was confirmed by a nuclear magnetic resonance (NMR) analysis. Investigations on model dough, consisting of reference or ground starch, as well as reference or ground gluten demonstrated a reduced gelatinization enthalpy in modified starch gluten systems, inter alia, caused by the grinding of gluten. This was traced back to a tighter water binding of modified gluten leading to an intensified competition for water in starch gluten systems with limited water content. It can be concluded from this, that a reduced gelatinization enthalpy of physically modified starch gluten systems is caused by a destruction of crystalline parts of modified starch and furthermore by a tighter water binding of modified gluten.

The understanding of the impact of different mechanical and thermal modification methods allows an improved control of the hydration properties of wheat flours. This forms the basis to produce physical modified wheat flours and starches with defined hydration properties.

Zusammenfassung

Zur Ausbildung weizenbasierter Lebensmittelschäume ist die nicht-kovalente Bindung von Wasser durch die nativen Polymere Stärke und Gluten von entscheidender Bedeutung. Durch die Vermahlung von Getreide werden jedoch eine Vielzahl an strukturellen Veränderungen der Stärkepolymere auf molekularer und mikroskopischer Ebene hervorgerufen, die die Stärke-Wasser-Interaktionen beeinflussen. Bisherige Forschungsergebnisse konnten keine mechanistischen Zusammenhänge zwischen der Stärkestruktur und der Interaktion mit Wasser aufzeigen, da weitere Polymere des Weizens bei der Vermahlung modifiziert werden, die konkurrierend in Wechselwirkung mit den Stärkepolymeren und Wasser stehen. Das Ziel dieser Arbeit war es die Auswirkungen molekularer und mikroskopischer Veränderungen von Stärkepolymeren auf die Hydratationseigenschaften von Weizenmehlen möglichst isoliert aufzuklären. Zu diesem Zweck wurden funktionelle Veränderungen von Gluten systematisch untersucht, sowie der Einfluss von Stärke-Gluten-Interaktionen auf die Hydratation von Weizenmehlen ermittelt.

Zu Beginn der Thesis wurde eine Methodik entwickelt, die systematisch die Identifizierung des Wirkungsgeflechts zwischen der Polymerstruktur von Stärke und den Schaumeigenschaften in physikalisch modifizierten Weizensystemen ermöglicht. Damit konnte ein negativer Einfluss auf den Gasvolumenanteil von festen Schäumen durch einen früheren Verkleisterungsbeginn und eine geringere Viskosität bei der Erhitzung von weizenbasierten Teigen nachgewiesen werden. Diese konnte auf eine erhöhte Quellung von physikalisch modifizierten Mehlpartikeln zurückgeführt werden. In einer darauf aufbauenden Untersuchung wurde festgestellt, dass die erhöhte Quellung und Wasserbindung von physikalisch modifizierten Weizenmehlen auf die veränderten Oberflächeneigenschaften der Stärke-Gluten Agglomerate zurückgeführt werden kann. Der weitere Fokus der Arbeit lag daher auf Strategien zur Bestimmung der Auswirkungen verschiedener Vermahlungsarten, sowie des Einflusses von Gluten auf die Hydratationseigenschaften von physikalisch modifizierten Weizenmehlen.

Durch gezielte physikalische Modifikationen von Weizenmehl und die selektive Modifikation von Weizenstärke und Gluten mittels Ultrazentrifugalmühle und kryogener Vermahlung, thermischer Verfahren, sowie Hochdruckbehandlung konnte festgestellt

werden, dass die erhöhte Wasserbindung von Weizenmehlen ausschließlich auf eine mechanische Modifikation des Weizenmehls während des Mahlvorgangs zurückgeführt werden kann. Die trockene thermische Behandlung von Weizenmehl führte hingegen zu veränderten Verkleisterungseigenschaften, die jedoch nicht an modifizierter Weizenstärke festgestellt wurden. Dieses Ergebnis weist auf veränderte Stärke-Wasser-Interaktionen hin, welche durch eine physikalische Modifikation des Glutens hervorgerufen sein könnte.

Diese Hypothese wurde mittels einer Nuklearmagnetresonanz (NMR) Untersuchung bestätigt. Anhand von Modellteigen, bestehend aus Referenz-, sowie vermahlener Stärke bzw. vermahlenem Gluten, konnte gezeigt werden, dass durch die Vermahlung des Glutens eine reduzierte Verkleisterungsenthalpie von modifizierten Stärke-Gluten Systemen erzielt wurde. Dies wurde auf eine stärkere Wasserbindung des modifizierten Glutens zurückgeführt, die zu einer erhöhten Konkurrenz um Wasser in Stärke-Gluten Systemen mit limitiertem Wassergehalt führte. Daraus kann abgeleitet werden, dass eine reduzierte Verkleisterungsenthalpie von physikalisch modifizierten Stärke-Gluten Systemen sowohl auf die Zerstörung kristalliner Bereiche der modifizierten Stärke als auch auf eine engere Wasserbindung von modifiziertem Gluten zurückgeführt werden kann.

Durch das Wissen über die Auswirkungen von unterschiedlichen mechanischen und thermischen Modifikationsmethoden kann eine verbesserte Steuerung der Hydratationseigenschaften von Weizenmehlen erfolgen. Damit wird die Grundlage zur Herstellung physikalisch modifizierter Weizenmehle und -stärken mit definierten Hydratationseigenschaften gelegt.

1 Introduction

The texture formation of cereal food requires a complex interplay of biopolymers among each other and with water. Already minor raw material fluctuations of wheat flour components or the modification of flour components during grinding, however, result in an altered hydration of biopolymers, complicating a standardization of the texture of cereal products. In this thesis, water polymer interactions of hydrocolloids will be discussed and the unique role of wheat starch as a hydrocolloid in cereal/starch based food will be considered. In the following, structural and functional alterations of the main wheat components starch and gluten during physical modification processes will be identified, which contribute to variations of the flour hydration. Finally, the thesis outline will be presented.

1.1 Water macromolecule interactions in food systems

Hydrocolloids are used in a wide range of food products to improve their textural properties and the storage stability by the formation of water macromolecule interactions. Although, hydrocolloids are especially associated with products like sauces, soups, toppings and jams, they contribute to the textural formation of many more food products, like baked goods and dairy products (Altemimi, 2018; Phillips & Williams, 2009). Starch plays beside gelatin, the most important role as hydrocolloid in the food market (Seisun, Phillips, & Seisun, 2002). Specially to prepare baked goods, a high water binding ability of hydrocolloids (mainly starch) is a prerequisite for the transformation of a liquid foam into a solid sponge and consequently the formation of a crumb-like structure (Ferrero, 2017; Kohajdová & Karovičová, 2009; Mills, Wilde, Salt, & Skeggs, 2003).

The diversified group of hydrocolloids consists of long chain polymers of polysaccharides, as agar, cellulose or starch, as well as proteins, as gelatin. Despite structural differences, all hydrocolloids have in common that they can either form microscopically dispersed, insoluble particles in water, resulting in a significant rise in viscosity of the dispersion and/or they can form a gel (Milani & Maleki, 2012). This special function is primary traced back to the large number of hydroxyl groups of the hydrocolloids, which strongly interact with water molecules, giving colloids a strong hydrophilic character (Saha & Bhattacharya, 2010). Due to the thickening or gelling

ability of hydrocolloids, the stability of the mediate or final products can be improved and/ or additionally the texture of the product can be set (Laaman, 2011; Saha & Bhattacharya, 2010). In case of low-density (highly inflated) baked goods, the rise in viscosity during processing facilitates the stabilization of the gas phase during dough preparation and baking. Additional, gelling properties of hydrocolloids contribute to an appropriate texture impression in the final product (Figure 1.1).

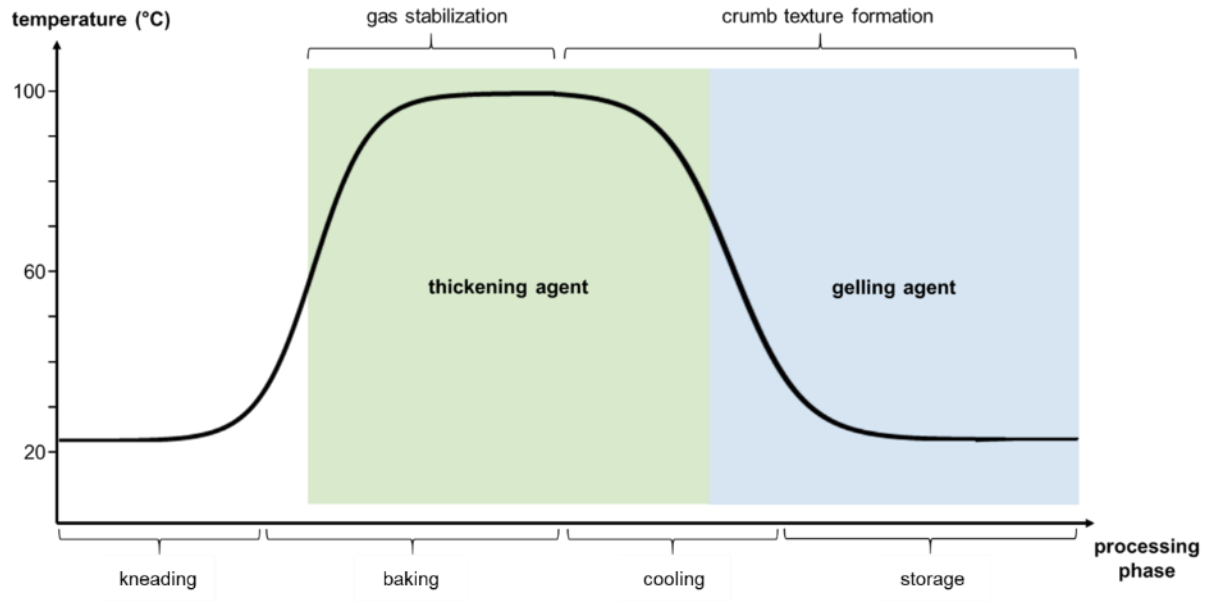

Figure 1.1: Functionality of the hydrocolloid starch in the production of baked goods.

Due to the presence of hydroxyl groups, all hydrocolloids are in a defined range soluble in water and strongly interact with water molecules. Thus, the addition of hydrocolloids to water based solutions results in a rise in viscosity (Marcotte, Taherian Hoshahili, & Ramaswamy, 2001). The quantity of restricted water by hydrocolloids depends on the composition, concentration, structure and accessibility of macromolecules (Laaman, 2011; Marcotte et al., 2001): while non-heated wheat starch, for instance, retain 0.6 g water compared to its dry mass, heating of starch suspensions leads to a 40 times higher water retention ability due to the changes in structural conformation of starch (Tegge, 2004). Thus, heat induced transformation of hydrocolloids is often used to increase the viscosity and stability of food systems, which contain a high amount of water. The type, in which the movement of water is restricted by hydrocolloids, is divided into a thickening and a gelling function of the hydrocolloids (Saha & Bhattacharya, 2010).

1.1.1 Thickening agents

Thickening agents, as xanthan, starch and cellulose derivates, are food compounds, which evoke a high rise in viscosity of a fluid, even when added in a low amount (Brunnschweiler et al., 2005; Williams, Phillips, & de Vries, 2004). The rise in viscosity in water based fluids, as most food systems are, is traced back to the reduction of non-bound, freely movable water. This is achieved by the swelling of the hydrocolloid and, additionally at higher concentrations, by the formation of loose entanglements in the food matrix (compare Figure 1.2).

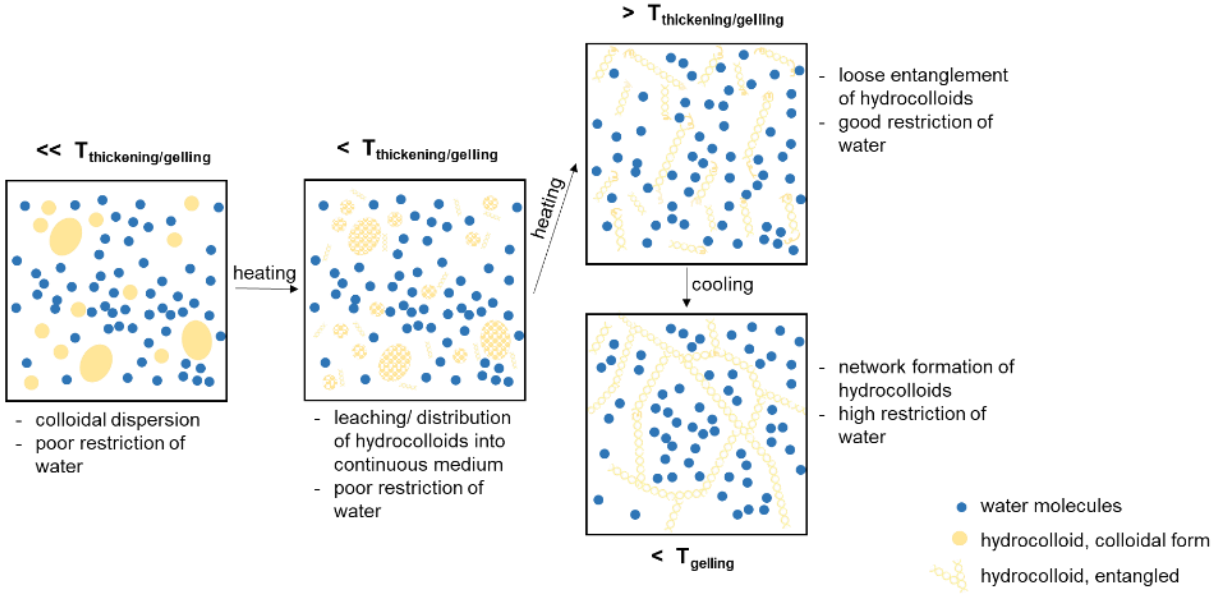

Figure 1.2: Thickening and gelling functionality of starch in aqueous matrices

The first mechanism causes only a slight increase in viscosity, whereby latter mechanism results in an over proportional increase in viscosity due to molecular interactions (hydrogen bonds, hydrophobic or ionic interactions) of hydrocolloids. Therefore, a minimum quantity of macromolecules have to be present in the dispersion, so that macromolecules could come into contact, restricting the mobility of single polymer chains (Milani & Maleki, 2012).

The extent of thickening depends predominantly on the structure and molecular weight of the hydrocolloid (polymer), as shown by the intrinsic viscosity ([η]) of a dispersion using Mark-Houwink equation (equation 1).

$$[\eta] = K \cdot M^{\alpha} \qquad \textbf{equation 1}$$

Thereby, K and α are empirically determined parameters of Mark-Houwink equation (Kasaai, 2007). M is the molecular weight of the hydrocolloid. Consequently, the molecular weight of a hydrocolloid is one of the main key factors, determining the intrinsic viscosity of a dispersion. Since the molecular weight of polymers can be reduced by physical treatments, for example by grinding procedures (Dhital, Shrestha, Flanagan, Hasjim, & Gidley, 2011), effects of grinding on polymer functionality must not be neglected, when analyzing processed cereals.

1.1.2 Gelling agents

All hydrocolloids can be used to thicken suspensions, whereby only few hydrocolloids show the ability to form a gel (Saha & Bhattacharya, 2010), as gelatin, pectin or agar (Williams et al., 2004). The gelation of water based matrix describes the process of the formation of a gel, whereat colloidal macromolecules develop a three-dimensional, highly viscous network (gel). In food industry, this transformation of a sol into a gel takes place especially after the heat treatment/ boiling of gelling agents in aqueous dispersions. During heat treatments, hydrocolloid particles disintegrate, and the polymers get distributed within the continuous medium of the food matrix. Thus, a heat treatment is a prerequisite for most gelling agents. In the second step, on condition that a minimal concentration of the gelling agents is exceeded, a gel can be formed during the cooling phase. Thereby, entanglements and/or junction zones are build, leading to an intense rise in viscosity (Bao & Bergman, 2004; Mandala, 2012; Wüstenberg, 2014), as illustrated in Figure 1.2.

The moment, when the hydrocolloids are connected on macroscopic scale (structural description), or, when the matrix is no longer flowable or deformable (functional description), is known as the gel point. Due to the immobilization (restriction) of a huge amount of water into a three-dimensional network structure through cross-linking (with mediate cation or by covalent bonds) or strong non-covalent interaction of the polymers (hydrogen bonds, hydrophobic attraction) (Banerjee & Bhattacharya, 2012; Shyichuk, 1997), gels are often seen as an intermediate form between a solid and liquid. Thereby, they do not show changes in their shape over a long period of time (Razavi, 2019). In contrast to a thickened dispersion, gels show a pronounced viscoelastic character by the formation of an extensive network (Banerjee & Bhattacharya, 2012; Bayarri, Izquierdo, Durán, & Costell, 2006; Mandala, 2012). In general, hydrocolloids can easily

be assigned to the category of thickening agents or gelling agents analyzing the storage modulus, loss modulus or loss tangent. In case of the hydrocolloid starch, however, the allocation is more difficult: starch polymers demonstrate characteristics of thickening and gelling agents during different processing steps, which can be referred to the unique structure of starch on molecular and microscopic scale (BeMiller, 2011; dos santos & da Silva, 2002; Fiszman, Costell, & Durán, 1986).

1.2 Starch – an important thickening and gelling agent in food industry

Starch constitution

Starch is a ubiquitously occurring biopolymer, consisting of the two macromolecules amylose and amylopectin, which function in plant cells as storage polymers. Although amylose and amylopectin are composed by the molecular unit 'D-glucose' (Bertoft, 2017), the arrangement of starch polymer chains to a crystalline and subsequent highly organized granular form creates specific functional properties of starch (see Figure 1.3).

Amylose is a linear polymer containing exclusively α-1,4 glycosidical bonds, resulting in a linear constitution. Its molar mass ranges between 10^5 and 10^6 g/mol. Which corresponds to a quantity of repeated D-glucose monomers of 160 to 1600. Amylopectin shows additionally 4% of α-1,6 linked glucose units, resulting in a branched structure, shorter polymer chains than amylose, and a molar mass of 10^7-10^8 g/mol (Durrani & Donald, 1995). The ratio of amylose/ amylopectin depends on the starch source and varies between 0.25 and 0.28 in general (Van Hung, Maeda, & Morita, 2006).

The functional properties of starch mainly depend on the chain length of amylose and amylopectin, as well as the ratio of starch polymers amylose and amylopectin. For instance, while amylose (AM) revealed a high iodine binding capacity, amylopectin (AP) shows a significantly lower ability to embed iodine molecules (only 0.38% w/w of iodine is embedded by total AP in potato solution) (Davis, Skrzypek, & Khan, 1994; Jackson, 2003). This knowledge is especially for detection methods elementary, as the SDmatic, where the extent of starch modification or amylose content is determined by the amount of embedded iodine molecules (Zhu, Jackson, Wehling, & Geera, 2008).

Thereby, the ratio of amylose and amylopectin and the molecular weight of polymers are related to the structure of starch chains, as the chain length distribution. Amylopectin molecular weight, for instance, rises with elevated ratio of amylopectin short chains in rice starches (Li, Wen, Wang, & Sun, 2018).

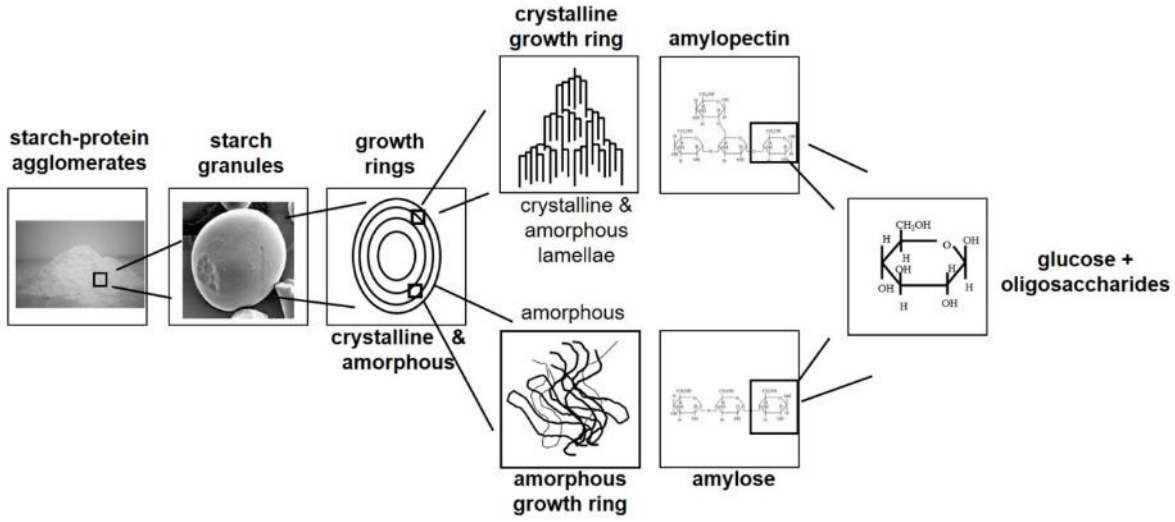

Figure 1.3: Schematic illustration of the structural constitution of starch (modified from (S. Jakobi, Jekle, & Becker, 2018)**)**

Furthermore, alterations of the amylose content in grains affect the molecular weight of amylopectin and amylose, as shown on different rice starches by Li, Wen, Wang, & Sun. The relation was the following: the higher the amylose content found in the study, the lower the molecular weight of amylose and amylopectin (Li et al., 2018). To describe the structure of amylopectin, a cluster model is often used, which differentiate amylopectin branches into A, B and C chain(s), as displayed in Figure 1.4:

1. A chains are connected by B chains
2. B chains shows bonds with A chains, other B chains or the C chain
3. The C chain occurs only once per molecule and has a reducing end (Peat, Whelan, & Thomas, 1952).

The special arrangement of starch (predominantly amylopectin) chains leads to a semi-crystalline order of starch, which is further differentiated into alternating crystalline and amorphous lamella with a periodicity of 9-10 nm. Crystalline lamella consists mainly of amylopectin, whereby branching points of amylopectin, as well as amylose, are likely located in the amorphous lamella. Lamella itself form either ellipsoidal blocklets of 50-500 nm, which are further arranged to crystalline growth rings, or blocklets of 20-50 nm,

which are further arranged to semi-crystalline growth rings. The arrangement leads to the formation of A or B-type crystallites or a mixed type of A and B crystallites, which depends on the origin of the starch plant, and determines the functional properties of starch among other things by the inclusion of water molecules into starch unit cells (Donald, Kato, Perry, & Waigh, 2001; Imberty & Perez, 1988; Sanderson, Daniels, Donald, Blennow, & Engelsen, 2006).

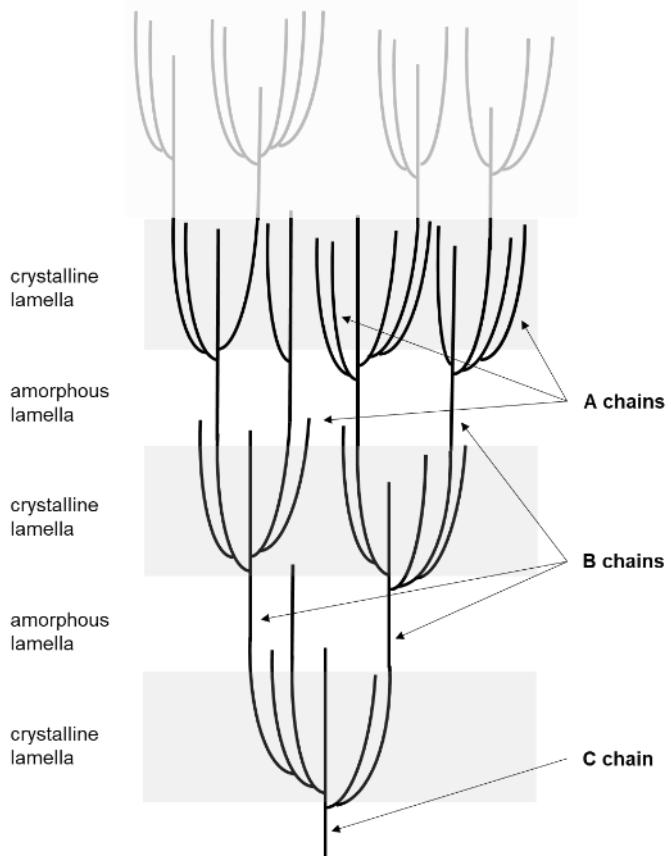

Figure 1.4: Amylopectin arrangement forming crystalline and amorphous lamella in starch granules

The combination of alternating growth rings results in highly organized starch granules. The supramolecular structure of starch granules can vary between spherical, ellipsoid to polyhedral and provide guidance with respect to the origin of starch. While corn starch displayed a predominantly homogenous fraction of polyhedral, round, sharply edged granules of a diameter of 10 – 25 µm, for instance, wheat starches consist a bimodal composition of spherical B granules ranging in diameter from 2 – 15 µm and lenticular shaped A granules of a diameter of 30 – 40 µm (Gallant, Bouchet, & Baldwin, 1997; Pérez & Bertoft, 2010; Tegge, 2004). Finally, starch granules are combined into particles in flours and embedded in a protein matrix. Thus, the mean volume particle size of wheat flours is about 90 µm (S. Jakobi et al., 2018).

The complex arrangement and constitution of starch and the presence of further components in cereals, which depend on the origin of starch source, are predominant factors, influencing the functional properties of starch based matrices: the size and constitution of blocklets, amylopectin branch chain-length, presence of pores and furthermore the granule size determine the accessibility for water and endogenous

enzymes in cereal matrices and, consequently, the enzymatic degradation of starches (James & Whistler, 2009; Srichuwong et al., 2017; Tang, Mitsunaga, & Kawamura, 2006; Zhang, Venkatachalam, & Hamaker, 2008). Besides, enzymatical starch degradation is also controlled by enzyme inhibitors, associated protein matrix (Srichuwong et al., 2017) and further minor components, as lipophilic components, proteins, minerals or phosphor-containing substances.

Determination of starch functionality - challenges

To analyze the impact of different modification techniques and selected chemicals on starch functionality, researchers used extracted starches, which are commonly known as ‘native starch’. The term ‘native’, however, is misleading: the so called ‘native’ starches are obtained in industrial processes by the extraction of starch-containing plants with aqueous /sodium chloride solution, whereby, the starch molecular weight distribution is altered by the removal of minor components or dextrin (Pauly, Pareyt, De Brier, Fierens, & Delcour, 2012). These alterations affect the thickening/ gelling and recrystallization properties of starch pastes, in turn. Thus, starches obtained by extraction process are not identically with starches, which are present in starch-containing materials, as flours and grains. Consequently, a systematic enlightenment of mechanism of isolated starch, acting as hydrocolloid, is hampered.

Thus, a lot of investigations of starch functionalities were performed in complex materials, as flours, although a systematic and fundamental elucidation of starch alterations is complicated due to the presence of further plant components. Proteins, as gluten polymers, extensively interact with water at room temperatures, binding a multiple amount of their weight of water. On the other hand, starch water interactions take place at the surface of starch granules at room temperature, restricting solely a low percentage amount of water. Thereby, starch granules are nearly completely insoluble in water, which is referred to their semi-crystalline, highly-ordered structure (Jane, 2009). Through the specific weight of starch granules of 1.6 g cm^{-3} (Dengate, Baruch, & Meredith, 1978), starch granules tend to sediment in aqueous suspensions at rest. To enable a permanent exchange of water on the surface of starch and a uniform distribution of starch in wheat flour suspensions a constant mixing must additionally be ensured, however, this can result in constitutional changes of gluten by the development of a protein network in wheat matrices. Thus, new approaches have

to be found to ensure a precise and valid assignment of the constitution and functional characteristics of starches in complex materials with interfering substances.

Functional characteristic of starch in complex materials

The ubiquitous occurrence and the comparatively easy extraction of starch from plant cells make starch beneficial for the application in baking products, dairies, sweets and frozen products (Altemimi, 2018). Especially, the thickening function during heating of starchy suspension and the gelling properties during subsequent cooling, allow the application of starch in several food products. When aqueous starch solutions are heated, starch granules start to swell, whereby starch granules are penetrated by water, leading to a volume expansion and a weight gain of granules. Exceeding a specific temperature, granular structure of starch is disrupted, leading to the loss of crystalline structure and consequently reduction in crystalline order. Starch granules begin to crack and break, so that starch molecules, especially amylose, leach into the aqueous phase, which facilitates a further swelling of starch granules. This intensifies the disruption of crystalline parts and loss in birefringence (James & Whistler, 2009; Muñoz, Pedreschi, Leiva, & Aguilera, 2015). Swelling of starch granules and the enhanced water binding result in a rise in viscosity (Cornell, 2004) and the phenomena of starch gelatinization/ pasting occurs (compare Figure 1.1). The gelatinization/ pasting of wheat starch suspensions typically takes places at temperatures around 60-90 °C, depending on the analyzed wheat variety, the modification of starch during production/ processing and the presence of interfering substances (R. Kumar & Khatkar, 2017; Tan, 2014). Already the (physical) modification of starch during grinding of grains significantly affects the gelatinization characteristic by altering starch structures and thereby, the interaction of starch with water. The possible modification of further cereal polymers, as gluten, during processing and associated changes in starch polymer interaction and hydration are additional aggravating factors in the elucidation of the relation of starch constitution and hydration properties.

The extent of paste formation is determined by the type of used starch. The swelling of starch granules is inhibited by higher amylose/ amylopectin ratio resulting in a reduced paste viscosity (Schirmer, Höchstötter, Jekle, Arendt, & Becker, 2013). Thus, functional properties in dough and bread can be controlled by the appropriate selection of starches (Blazek & Copeland, 2008). Amylopectin initially retain the crystalline

structure in starch granules. Only, when starch is exposed for a longer period to temperature above gelatinization/ pasting in aqueous solutions, granular structure and crystallinity is destroyed.

This allows to partially adjust desired temperatures of gelatinization, the extent of crystal melting and further functional properties of the resulting starch pastes by a specific selection or modification of starch (Ariyantoro, Katsuno, & Nishizu, 2018). Wheat starches form pastes above 67 °C, whereby pasting temperature of potato starches is above 62°C, for instance. The resulting pastes of potato starch is less rigid than pastes of corn starch due to the different polymer composition and polymer arrangement. Furthermore, the composition determines the gel development of starch suspensions, which takes place, when a starchy suspension is cooled down, so that a re-arrangement of starch polymers (recrystallization/ retrogradation) can occur. Thereby, typical textures (as crumb structure) are formed, which show a viscoelastic behavior (compare Figure 1.1). Hence, mechanical properties of resulting pastes/ gels and the sensory of thick or gelled systems are mainly determined by the ratio of amylose/ amylopectin, due to difference in polymer functionality (Arvisenet, Bail, Voilley, & Cayot, 2002; Gunaratne & Corke, 2016; Kaufman, Wilson, Bean, Herald, & Shi, 2015): due to the linear structure, amylose polymers show a high solubility with good gelling properties. On the other hand, amylopectin molecules are responsible for the firming of baked goods (Aguilera & Stanley, 1999; Kong & Singh, 2011). In the last decades, modification techniques were developed, which allow the industry to modify the starch functionality during mixing, heating and cooling of starch based materials within limits.

1.3 Types of starch modification

The origin of starch determines the ratio of amylose/ amylopectin, its molecular weight (distribution), the morphology of starch granules and consequently the functionality of non-modified starches (Abebe, Collar, & Ronda, 2015; Goering, Fritts, & Eslick, 1973; Gunaratne & Corke, 2016; Jobling, 2004; Kaptso et al., 2015; Kaufman et al., 2015), resulting in different application fields of starches.

Thereby, amylopectin forms stable solutions in cold water, if it is able to immigrate into aqueous phase, while the linear polymer amylose is not/ less soluble in water

(Mukerjea & Robyt, 2010). Thus, textural properties of starchy systems are tried to be controlled by a combination of amylose and amylopectin, as well as the selection of appropriate starches. However, there are still limitations in the variations of functional properties of native starches (Cornell, 2004).

To overcome these limits and to achieve, for instance, a high paste stability at higher temperatures or a high freeze-thaw stability, modification techniques (chemical, physical or enzymatic) have to be applied (Jane, 2009; T. Y. Liu, Ma, Yu, Shi, & Xue, 2011; Singh, A. V. ; Nath, L. K. ; Singh, 2010). Acetylation of starch is used to achieve a higher solubility, swelling power and viscosity, and succinylation increases the water binding capacity of starches (Ariyantoro et al., 2018). Thus, deficiencies of native starches can be solved (Tharanathan, 2005). The swelling behavior, pasting properties, as well as digestibility after a chemical modification depend on the structural constitution of used starches (as amylose content) (Shen et al., 2019). Consequently, even for a chemical modification, the structural constitution of starch must be understood and controlled. Although the chemical modification is a widely used method for changing functional properties of food starches, it shows great disadvantages, concerning the declaration (European Parliament, 2008) and environmental compatibility. Additionally, chemical residuals can retain in modified starches and transferred into the food product.

Thus, other modification techniques are favored, as an enzymatic hydrolysis or a physical modification of starch. Enzymatic modification techniques are used to modify starches and flours during the processing and within the food matrix, for instance by using a fermentation process of 24 h with *Lactobacillus amylovorus* (Cho, Lee, & Eun, 2019). Although enzymes are widely used in the baking industry, fluctuations of starch properties make a targeted application of enzymes challenging, since enzymatic kinetic depends immensely on the accessibility of starch granules, which is affected by physical forces acting upon starch.

Physical modification of starch has several economic and ecological advantageous. Beside the fact of being a cheap modification technique, since it gets along without the usage of expensive chemical treatments, physically modified starches are considered as food ingredients and do not have to be declared as food additives (Zia-ud-Din,

Xiong, & Fei, 2017). Since the percentage of consumers, who try to avoid food containing additives for nutritional reasons is growing, physical modification offers economic benefits compared to chemically modified starches to achieve desired functional characteristics of products. Especially in ‘traditional’ food, as baked goods, consumers expect and prefer clean label products. Thus, industrial and scientific interest is on the physical modification of starch, preferably in the raw material ‘flour’ to avoid an expensive modification techniques and prior extraction processes.

Physical treatment of cereals (so called physical modification) can lead to an increase in accessibility of starch, alterations of starch crystallinity and pasting properties, as well as changes in visco-elastic properties of wheat based systems (Khunae, Tran, & Sirivongpaisal, 2007; Knutson, 1990; Krueger, Walker, Knutson, & Inglett, 1987), resulting in modified texture and specific volume of baked goods. A targeted physical treatment during processing of grains, for instance during grinding, could therefore represent a promising approach to alter starch functionality. Therefore, the effects of physical forces on starch constitution must be elucidated in order to understand structure-function relations.

1.4 Grinding – physical modification of cereal biopolymers

The physical modification during grinding represents a powerful tool to modify the starch (and possibly gluten) functionality and to achieve the required hydration characteristic of flours. White flours are produced from cereal grains by separating the endosperm (starch-containing part of grain kernels) from the surrounding, high-fiber bran and aleurone layers, which is achieved in a multi-step grinding process. Grinding is a highly industrialized process, in which grains are broken/ degraded after several pre-treatments, as the adaption of moisture content and cleaning, in a roller mill. Afterwards bran particles and other, low density particles are separated by sieving from the starch-enriched fraction. A further grinding step in a roller mill with reduced grinding gap and a separating process of the starch-enriched fraction leads to the production of purified flour fraction with desired particle size distribution (Posner & Hibbs, 2005). Figure 1.5 displays the effect of ball milling on the morphology of wheat flour particles.

Alterations of the starch constitution and functional properties of starches in flours depend notably on the mill type, as shown by ball ground and cryogenically ground

starches: while ball grinding partially destroyed the crystalline and double-helical structure of starch, resulting in a reduced gelatinization enthalpy (Huang, Xie, Chen, Lu, & Tong, 2008; T. Y. Liu et al., 2011; Martínez-Bustos, López-Soto, San Martín-Martínez, Zazueta-Morales, & Velez-Medina, 2007), reduced starch crystallinity is also achieved by cryogenic grinding, without affecting gelatinization enthalpy (Dhital et al., 2011). Furthermore, ball grinding is known to alter microscopic structures of starch, as particle size reduction, which increases the surface of starch and leads to a rise in water absorption and water solubility (Martínez-Bustos et al., 2007). Beside alterations of hydration properties, differences in an enzymatic starch hydrolysis of rice flours were evident after grinding by jet mills or hammer mills (Lee, Shim, Goh, Mok, & Puligundla, 2019). Furthermore, a strong correlation was found between apparent amylose content and cold-water solubility of cassava starch. Since the apparent amylose content, in turn, is modified by grinding, this represents an effective possibility to modify cold water properties of starch (Huang et al., 2008). Extrusion of banana starches showed a fragmentation of amylopectin and formation of slowly digestible starch (Roman, Gomez, Hamaker, & Martinez, 2019). The control of starch digestion has a great importance for food products, containing a high amount of fast available carbohydrates (as baking products), since it significantly affects the nutritive value of food.

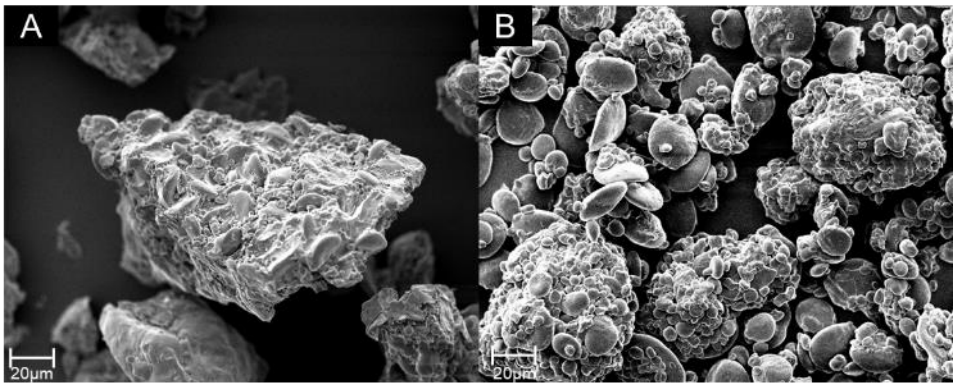

Figure 1.5: Wheat flour particle degradation analyzed by scanning electron microscopy (SEM) (A) reference wheat flour (B) subsequently modified wheat flour in ball mill at 600 rpm/ 5min

Many relevant changes in starch functionality are traced back to changes in the particle size distribution of wheat flours. These alterations of the wheat flour particle size can be achieved by an uncontrolled breaking of single starch particles out of starch-protein agglomerate (flours particles) or the fragmentation of starch granules itself. Latter is especially realized in high-energy mills and ball mills, which can alter the starch

granules size and granule surface properties of pure starch (Liu et al., 2011; Martínez-Bustos et al., 2007).

To determine relevant structural alterations of pure starch or starch in complex flour matrices, a broad range of starch and flour modifications were performed in the last decades. Jet grinding, for instance, reduced the particle size of wheat flours from d_{50} < 75 µm to d_{50} < 12 µm and decreased the gelatinization enthalpy, while no changes of peak molecular weight occurred (determined by HPSEC–RI). The modification provoked the formation of harder and more sticky dough (Lazaridou, Vouris, Zoumpoulakis, & Biliaderis, 2018). Transformation of crystalline parts into amorphous parts accompanied with a modification of starch granules from a smooth to an abrasive surface was also determined, when whole wheat flour or maize starch were modified using a superfine or ball mill (He et al., 2014; Niu, Zhang, Jia, & Zhao, 2017). On the other hand, flours produced on a Buhler laboratory mill or Brabender senior mill caused a rise in starch accessibility and the fragmentation of starch granules, but no changes in relative crystallinity, although pasting properties were altered (Yu et al., 2015). The differences in starch/ flour modification and resulting functionality after grinding processes on different mills hamper a direct correlation of starch constitution and functionality. Thus, a targeted modification failed so far.

The reasons for uncertainties and unknowingness regarding the effects of different grinding types on constitution and functional characteristics of starches and flours, are found in the variation of several influencing factors. The extent and range of introduced alterations of starch/ flour particles depend mainly on (1) the constitution of starch and the presence of further flour components, as well as on (2) the used mill/ applied forces:

(1) A high ratio of amylose/ amylopectin counteracts the molecular degradation during grinding, which is referred to the role of amylose as plasticizer in starch granules (Dhital et al., 2011). Amylose shows a more flexible behavior due to its amorphous constitution, whereby amylopectin has a rigid characteristic explainable by the greater molecular weight, as amylose. Thereby, the crystalline structure of amylopectin is more sensitive to a mechanical destruction (Liu et al., 2017). Further detailed interrelationships of other starch structures in complex wheat flours matrices, and the impact of grinding on wheat flour hydration are still insufficiently understood, which is

connected to the fact, that the role of the wheat polymer ‘gluten’ in grinding processes has not been sufficiently scientifically researched, yet, although it is the second main polymer in quantity in wheat flours.

(2) Studies on different mills determined a significant influence of the type of mill on the morphology, crystallinity and molecular constitution of starch and the functionality of cereal biopolymers (Lee et al., 2019), which is referred to the presence of different physical forces (various mechanical/ thermal forces) during the grinding procedure. Besides, the extent of those forces is essential for the degree of polymer modification. For jet ground flours, the particle size distribution was mainly influenced by the feed rate: the less the feed rate was set, the more pronounced the particle size reduction was (Lazaridou et al., 2018). For ball or planetary mills significant correlations were found between the reduction of gelatinization temperature or starch crystallinity and the increase in grinding time or grinding energy, respectively (González, Loubes, & Tolaba, 2018; Huang et al., 2008). Thus, the modifications of starch constitution do strongly depend on grinding parameters, however, these parameters vary significantly in different studies. As a result, the comparison and transfer of results is hardly possible.

Especially industrial grinding processes combine mechanical and thermal forces during grinding (Dhital et al., 2011). Temperatures up to 85°C were detected, when a planetary mill was used, for instance, in a high energy mode (González et al., 2018). A differentiation between mechanical and thermal forces is indispensable. Studies applying ‘exclusively’ mechanical forces during the grinding processes, as in cryogenic mills, indicate, that starch degradation is a mechanically induced process (Dhital et al., 2011). Other studies focused on the effects of exclusively thermal forces, as present in drying processes of starch based products. Malumba, Massaux, Deroanne, Masimango, & Béra demonstrated 2009 an increase in rigidity with high drying-temperatures of wet ground starch granules, reducing a swelling of dried starch. Latter affects the pasting characteristic and mechanical gel properties (Malumba, Massaux, Deroanne, Masimango, & Béra, 2009). In conventional grinding processes, a clear allocation of the consequences of mechanical and thermal forces on starch constitution and the resulting alterations of the hydration properties were not carried out, yet. Thereby, the impact of the thermal part as well as synergistic effects are in general

ignored in conventional grinding procedures, although thermal forces can affect starch functionality, as mentioned before.

To produce wheat based products, a clear analysis of introduced changes of wheat flour polymers is necessary to predict and moreover control the properties of dough and baked goods. The modifications of flours during grinding are almost completely referred to a modification of starch and the analysis of gluten functionality is often neglected. Thereby, gluten itself can be affected by an intense heat development or the presence of mechanical forces altering the high molecular structure, as tertiary structure, of gluten polymers. Additionally, gluten functionality could be affected by altered interactions of modified starch and (modified) gluten polymers. Due to the simultaneous occurrence of starch and gluten polymers in wheat flours, the distinction of modifying forces during grinding (mechanical, thermal or thermo-mechanical) on starch or gluten polymers was not possible, so far.

1.5 Thesis Outline

Raw material fluctuations of wheat flours complicate the production standardized baking products. To achieve consistent properties of wheat flours in compliance with the consumers requirements of additive free products, the milling industry uses two strategies: grains/ flours can be mixed to compensate grain/ flour fluctuations (Miskelly & Suter, 2017) or a defined physical modification of grains/ flours can be applied during grinding. Both methods provide the opportunity to optimize the hydration and gelatinization properties to the desired purpose. The physical modification of starch in flours can be a low-cost tool, moreover, which enables a targeted functionalization of wheat polymers, especially starch, (Liu, C.; Liu, Lin; Li, Limin; Hao, Chunming; Zheng & Bian, Ke; Zhang, Jie; Wang, 2015), on one condition that structure function relations of wheat biopolymers are known. Hence, the knowledge of the dependency of constitution of flour polymers and their functional properties are essential for a targeted functionalization of flours in baking industry.

Although relations of starch constitution and resulting functional changes are well-known for chemical modifications (for instance esterification) (Hong, Zeng, Brennan, Brennan, & Han, 2016; Tian, Chen, Chen, Yang, & Wang, 2018), structure function relations of physically modified flours are incompletely analyzed, arising from defective

experimental designs and a poor interpretation of data of previous experiments. Most studies focused on single structural alterations, correlating determined changes of the starch constitution to the hydration properties of obtained flours. In this way, false correlations could be drawn, because no distinction between causal correlation and side-effects of the grinding process was possible. Furthermore, former research dealt primary with the modification of starch during physical modification of wheat flour, neglecting a potential modification of gluten polymers (Hackenberg, Jekle, & Becker, 2018). Many functional properties of dough, as dough strength and elasticity, are based on the composition and content of gluten (Dhaka & Khatkar, 2015; Shewry, Halford, Belton, & Tatham, 2002; Žilić, 2013). If alterations of dough functionality occurred after grinding, changes were explained by the effect of modified starch on gluten network development during kneading, for instance by a reduced hydration of gluten (Hackenberg, Jekle, et al., 2018). It seems to be an accepted fact, that gluten polymers do not change during grinding due its more flexible constitution causing changes of dough functionality. Based on this assumption, it was excluded, that potentially modified gluten polymers affect the gelatinization/ pasting characteristic of dough or flour suspensions, which is in general attributed to the starch behavior. Thereby, it is known, that starch pasting is influenced not only by the amount (Jekle, Mühlberger, & Becker, 2016), but furthermore by the type of gluten polymers (Chen, Deng, Wu, Tian, & Xie, 2010).

The challenges in a systematic analysis of physically modified flours is that grinding of flours is a highly variable process including different temperature profiles and the application of different mechanical forces. This evokes alterations on different structural level of starch (nanoscopic, microscopic and macroscopic), potentially modifies gluten functionality and the morphology of flour particles, leading to various hydration and gelatinization characteristics of flours (Kijima et al., 2015; C. S. Kumar, Malleshi, & Bhattacharya, 2008; Ngamnikom & Songsermpong, 2011; Nishita, K. D.; Bean, 1982). Consequently, research results are often poorly comparable, and a knowledge-driven grinding process fails, since an in-depth understanding of the impact of structural changes of starch polymers (and gluten polymers) during grinding of flours on the functional changes is missing. New approaches using model dough, combining starch polymers and gluten polymers in varying ratios and functionalities, enable a more specific investigation of the interplay and mutual interference of starch and gluten

(Jekle et al., 2016). This methodical approach was hitherto rarely introduced for physically modified cereal polymers. Thus, the allocation of modified flour and dough properties to either modified starch or modified gluten polymers failed, so far.

The physical modification of starch, gluten and wheat flours during grinding can be seen as a tool to elucidate the impact of a modification of starch on molecular and microscopic scale on the hydration properties of flours. Additionally, this facilitates to investigate interaction effects of starch and gluten polymers in more detail and enable to clarify if gluten is modified during grinding of wheat flours. In combination with a detailed distinction of the impact of thermal and mechanical forces on starch/ flour modification, this knowledge would allow a targeted control of the grinding procedure.

Based on these requirements for a targeted physical modification of wheat flours, the thesis is structured as follows:

1. Alterations of starch constitution, affecting the properties of dough and texture of baked goods, are critically reviewed including
    a. the determination of required dough properties to produce low-density baked goods
    b. the identification of starch structures, which determine the required dough properties
2. A distinction of the effects of different physical forces, occurring during grinding, on wheat flour polymers was made containing
    a. the proof of a modification of gluten polymers and its potential influence on hydration and gelatinization/ pasting of starch (in model dough)
    b. a precise differentiation into a mechanical and thermal flour modification enabling a targeted control of grinding processes and the selection of appropriate grindings for a physical flour modification.

1.6 Methods

Modification procedures

Aim of this work was to investigate changes in hydration properties of wheat starch, wheat gluten and wheat flour caused by different physical treatments. The evoked wide range of physical changes – mechanical, mechanical-thermal and thermal – were achieved by different grinders and the thermal modification (13 min) using a baking oven. The ultra-centrifugal grinder (ZM 200, Retsch, Haan, Germany) combined mechanical forces (shear and impact forces) and thermal forces (temperature rise during grinding up to 70 °C). To exclude heat induced alteration, sample modification was additionally performed in a cryogenic ball grinder (Retsch, Haan, Germany). To determine precisely the type of mechanical force altering starch-gluten constitution and functionality, a self-constructed high-pressure device (at the Chair of Technical Microbiology of the Technical University of Munich) was used, where exclusively pressure was applied on the samples (up to 600 MPa). A temperature induced modification was excluded.

Analytical methods

Methods used for analytical sample characterization are summarized in Table 1.1. The functional characteristic of flour hydration, as well as hydration of flour components, prior and after modification procedures was monitored using the water solvent retention capacity (SRC, AACC Method 56-11.02) method containing exclusively the solvent ‘distilled water’ (hereinafter called WRC). Reasons for choosing solely water for the analysis was the better comparison of the solvent retention between polymers or polymer mixtures, as present in wheat flours. Variations for single polymers, for instance lactic acid SRC, would provide good results for the analysis of exclusively gluten (especially glutenin fraction), however, would be less suitable for the starch analysis. Especially analysis of polymer mixtures, containing varying starch-gluten ratios, would lead to unknown effects in solvent absorption and consequently are not interpretable. The precise application and adaption of the AACC Method 56-11.02 method is described in the papers in chapter 2.3 (S. Jakobi et al., 2018). An adaption of the method was necessary since the subsequent high-pressure treatment of wheat flours provided only little amount of probe material. The suitability of the adaptation was reviewed, using non-modified wheat flours. With the adapted method higher total SRC values were gained, however, the slope and linear rise of SRC with increase in

starch modification degree (SMD) was constant. Thus, absolute values of the publication (chapter 2.3) are not directly comparable to the other publications (Sabina Jakobi, Jekle, & Becker, 2018).

Table 1.1 Applied methods for analytical characterization of wheat flour, wheat starch and gluten

Analysis	Method	Applied in chapters
Protein content	AACC 46-12.01 (conversion factor 5.7)	2.3, 2.5
Ash content	AACC 08-12	2.3, 2.5
Moisture content	AACC 44-40	2.3, 2.4, 2.5, 2.6
Particle size	Static light scattering	2.3, 2.4, 2.5
Total starch	Total starch assay kit (Megazyme Ltd)	2.3, 2.5
Amylose/ Amylopectin content	Amylose/amylopectin assay kit (Megazyme Ltd)	2.3, 2.4, 2.5
Glucose, maltose and maltotriose content	High-performance anion exchange chromatography with electron detector	2.3, 2.5
Hydrodynamic radius of amylose and amylopectin	Size exclusion chromatography (SEC)	2.6
Starch modification degree (starch damage)	AACC Method 76-31.01	2.3, 2.4, 2.5, 2.6
Gelatinization behavior	Differential-scanning-calorimeter (DSC)	2.3, 2.4, 2.5, 2.6
Water mobility	Nuclear magnetic resonance (NMR) spectroscopy	2.6
Water Solvent retention capacity (SRC or WRC)	AACC Method 56-11.02	2.3, 2.4, 2.5, 2.6

The SRC method determines the global water retention and thus global water-polymer interaction of the sample, which are suitable results for industrial and technological application. The elucidation of local water-polymer shifts (shifts between different

polymer structures or water shifts between polymers), however, necessitates the usage of Nuclear magnetic resonance (NMR) spectroscopy (topspin 3.2, Bruker Corporation, Billerica, USA) to measure the water mobility. Therefore, model dough, containing 46.25 % water, consisting of reference/ modified starch and/ or reference/ modified gluten were measured, and the water distribution analyzed. This approach allowed, the exact determination of the local water binding of polymers prior and after modification in an ultra-centrifugal grinding (Paulik, Wen Yu, et al., 2019).

Prior the conduction of the PhD studies, the starch modification degree (SMD), which is a quality parameter of flours and does often correlate with the hydration properties of flours, determined using an amperometric (AACC Method 76-33.01, Damaged Starch) and an enzymatic (AACC Method 76-31.01, Damaged starch) method. The results showed that the intense treatment of starch containing materials (wheat flours) only provided a limited increase of the amperometrically measured SMD, while the enzymatically measured SMD still further increased. This could possibly be referred to the severe destruction of the helical structure of amylose, which is responsible for the iodine absorption. A poor iodine absorption, as caused by the destruction of amylose, resulted in a low SMD value. Although the SDmatic method (amperometric) is fast and cheap and thus more often used in milling industry, authors decided to use the enzymatic SMD method, since the method is suitable for a wider physical treatment range. For the enzymatic analysis of the SMD the starch damage assay kit of Megazyme was used (Paulik, Jekle, Becker, et al., 2019). The testing principles is based in the hydrolysis of damaged and hydrated starch granules by fungal α-amylase to maltosaccharides and dextrins. Thus, damaged starch samples get soluble, while undamaged starch stays almost native and insoluble. The further treatment with amyloglucosidase leads to a complete degradation of soluble starch breakdown products to glucose, which can specifically be measured with a glucose oxidase/peroxidase reagent mixture (Megazyme Assay Procedure, 2018).

The combination of analysis methods allowed the allocation of structural changes and the evoked modifications of polymer hydration and in general functionality.

2 Results

2.1 Results (thesis publications)

A review: reverse approach to analyze the impact of starch modification on the inflation and gas holding properties of wheat-based matrices (page 31-39)

Differentiating between causal relations and simple correlations is a challenge in applied sciences. In case of physically modified wheat starch, a variety of structural changes of starch are known to be altered by physical forces, however, structural alterations are often of subordinate importance to forecast functionality of starch based flours on gas forming kinetics and gas holding capacity of wheat flours. Causal relations between physically modified starch structures and matrix functionalities were pointed out by using a reverse approach. Thereby, focus was first on relevant dough characteristics to receive a satisfactory gas holding capacity. Secondly, responsible starch structures were identified, before the impact of physical modification on the relevant dough functionalities are considered. Early gelatinization (caused by a facilitated hydration) and lowered viscosity in combination with an altered gluten network formation cause an impaired formation of an open-cell foam, which is probably achieved by the molecular degradation and granular disruption of starch.

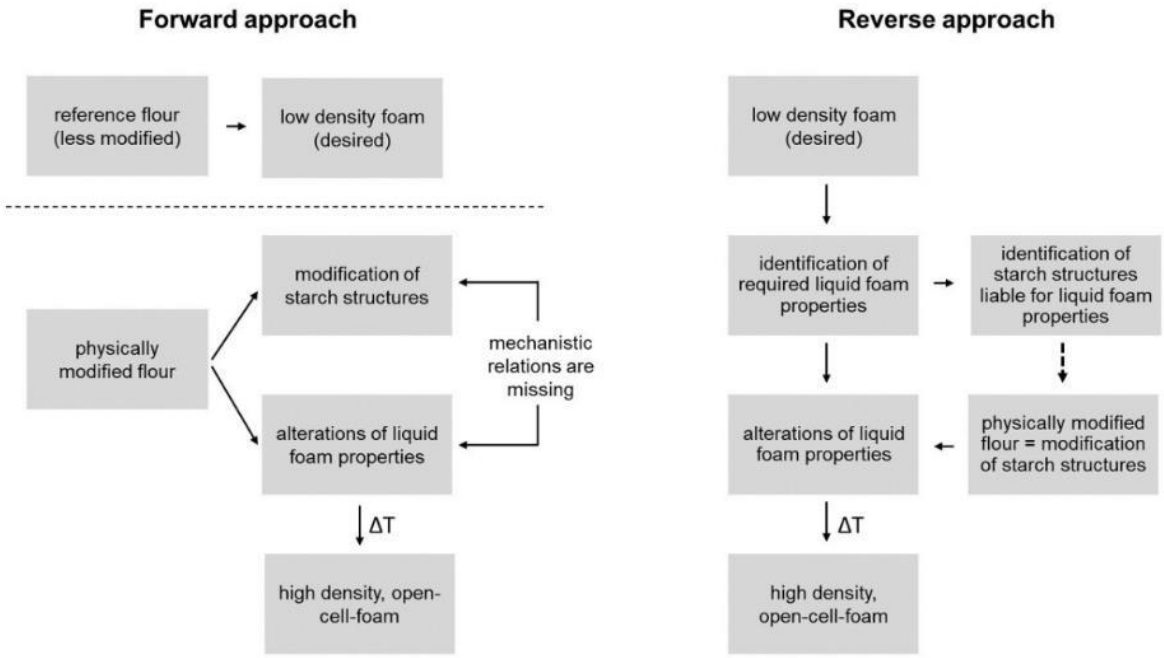

Figure 2.1: Former, forward approach and new, reverse approach to elucidate the impact of starch modification on flour functionality

Contributions

The doctoral candidate created the structure of the review article and conducted the literature research. Structure of the article was critically discussed and improved with co-authors. All authors critically reviewed the article and contributed significantly to the innovative approach and concept of this article.

Direct link between specific structural levels of starch and hydration properties (page 40 – 47)

Physical treatments of flours, as high-pressure treatments, extrusion or grinding cause a modification of starch (formerly known as starch damage), which result in an enhanced water retention capacity and facilitated hydration of physically modified flours. Although a lot of analysis were performed, structural alterations of starch causing rise in hydration properties could not be determined so far. Using two different mills and grinding principles (impact mill and cryogenic mill), various structural alterations on nanoscopic, molecular and microscopic level of wheat flour could be achieved. The water retention capacity of modified flours linearly raised with the grinding time in the cryogenic mill or rotation speed in the impact mill, thus with the rise in supply of energy. For cryogenically ground flours, a linear correlation of water retention and destruction in crystallinity was proven, however, no linear correlation between reduction in crystallinity and flour hydration was noticed for the ground flours in impact mill. It could therefore be concluded that formation of amorphous starch due to the destruction in crystallinity did not favor the enhanced water retention of flours. Differences in grinding procedures could be caused by different physical forces. Since for both mills a linear correlation of WRC and starch modification degree was found, it was evident, that starch accessibility and not flour particle accessibility attributed to altered hydration properties.

Contributions

The doctoral candidate created the design, carried out analysis, checked and evaluated data, and drafted and revised the article. Profound discussions with co-authors improved the conception of the study and interpretation of the data.

Mechanically and Thermally Induced Degradation and Modification of Cereal Biopolymers during Grinding (page 48 – 60)

Differences in structural constitution of wheat flours and their functionality can be induced by the grinding procedure and are often referred to varying mechanical forces during grinding. A thermally induced modification of flour functionality is ignored, since temperature rise during grinding is regarded to be poor and due to the low moisture content in flours, no alterations of starch or proteins are expected. The comparison of a non-cooled impact mill, which results in temperatures during the grinding procedure above 60 °C, and a temperature-controlled impact mill (temperatures stayed under 60 °C) demonstrated no differences between both grinding procedures regarding the particle size distribution or hydration properties of flours. Mechanical forces during grinding are therefore mainly responsible in controlling the flour functionality, as the hydration properties. Exclusively dry, thermal forces up to 110 °C led to a rise in gelatinization onset of wheat flour, although gelatinization onset of the gelling agent 'pure wheat starch' kept constant. Further studies should be performed to reveal to which extent a gluten modification is responsible for altered hydration properties of physically modified wheat flours.

Contributions

The doctoral candidate created the design of the study, carried out analysis, interpreted data, performed statistical analysis, as well as drafted and revised the article. Co-authors critically revised the design of the work and supported the evaluation and interpretation of the data.

High-pressure treatment of non-hydrated flour affects structural characteristic and hydration (page 61 – 70)

Wheat grains and wheat flours functionality are mainly affected by the mechanical forces impact, shear and pressure during the grinding procedure. Specially, high-pressure forces become beside the grinding process more important in food industry due to the preservation effects on food matrices. Former studies often dealt with high-pressure treatments on systems containing moisture contents above 20%. Effects of high-pressure treatments on low moisture matrices, as non-hydrated wheat flours, have hardly been studied. High-pressure treated wheat flour for 10 min up to 600 MPa or at 150 MPa up to 20 min resulted in structural flour changes on nanoscopic,

molecular and microscopic scale. Amount of total starch, as well as relative amylopectin content in total starch declined about 11-13% as well as 12% linearly for enhanced pressure level or sigmoidal for pressure time, respectively. Furthermore, pressure treatment led to a reduction in gelatinization enthalpy and a rise in water retention capacity of flours by 11.0 ± 0.6%. Consequently, even low moisture conditions as present in wheat flour of 14.64 ± 027% enable a pressure-induced modification of wheat flour. Thus, modification of starch structures should be monitored during pressure treatments, beside the desired preservation effects.

Contributions

The doctoral candidate designed the experiments, carried out analysis, checked and evaluated data, performed statistical analysis, as well as drafted and revised the article. Co-authors substantially contribute to the interpretation of the data and approved the article and significant contributions to the article by critically reviewing and improving the article draft.

Characterizing the impact of starch and gluten-induced alterations on gelatinization behavior of physically modified model dough (page 71 – 77)

Grinding of flours causes a modification of starch, which is known to affect the gelatinization and pasting properties of flours and the gluten network formation during kneading. However, less is known about the impact of possibly modified gluten on the pasting and gelatinization characteristics of flours. Using model dough consisting of reference starch/ physically modified starch with reference gluten/ physically modified gluten, the influence of modified gluten under limited water conditions (46.25 %) could be elucidated. Ground starch showed a reduced gelatinization enthalpy, which is known from previous experiments. Modification of gluten did not affect the pasting onset (beginning of rise in viscosity, inflection point of tan δ) or gelatinization onset (beginning of destruction of crystal parts, intersection of the tangent and base line at the left side of the gelatinization peak) of reference or modified starch (starch-gluten = 50 : 50 (m/m)). However, modified gluten altered gelatinization enthalpies of model dough containing reference starch or modified starch. The gelatinization enthalpy of dough predominantly depends on the amount of added water, since transformation of crystalline to amorphous structures require the presence of water, and furthermore the

crystallinity of starch. NMR experiments proved a tighter water binding of modified gluten, which can lead to a shift of water from starch polymers to gluten polymers. Despite no global changes of the water content in the model dough were present in this investigation, the local water shift to gluten polymers can reduce the available amount of water for the starch gelatinization and pasting. Consequently, the reduced gelatinization enthalpy of physically modified starch – gluten matrices is evoked by the destruction of starch crystallinity and additionally by a tighter water binding of modified gluten. Thus, functional flour changes are not exclusively based on modified starch but can also be caused by modified gluten polymers.

Contributions

The doctoral candidate created the design of the experiments, evaluated data, performed statistical analysis, as well as drafted and revised the article. Co-authors supported the acquisition of data and their interpretation and revised the article critically and significantly improved the informative value of the article.

2.2 A review: reverse approach to analyze the impact of starch modification on the inflation and gas holding properties of wheat-based matrices

Trends in Food Science & Technology 91 (2019) 231–239

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

A review: Reverse approach to analyze the impact of starch modification on the inflation and gas holding properties of wheat-based matrices

Sabina Paulik, Mario Jekle*, Thomas Becker

Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology and Process Engineering, Weihenstephaner Steig 20, 85354 Freising, Germany

A B S T R A C T

Background: A variety of analysis methods exist to quantify structural changes of starch and to determine starch modification degree. However, analyzed structural alterations are often of subordinate importance to predict implications of physical starch modification caused by grinding on the inflation kinetics and gas holding capacity of starch-gluten matrices or effects are not examined for the gas stabilization in dough and crumbs.
Scope and approach: The lack of systematic approaches to create a direct link between physically modified starch structures by grinding processes and their impact on gas holding capacity in liquid and solid cereal, food foams resulted in an imprecise specification of the term starch modification in baking flours. This review uses a reverse approach to identify mechanistic relations between physically modified starch structures and the inflation as well as gas stabilization of wheat-based matrices. For this purpose, firstly, relevant dough functionalities and influential starch structures to receive a satisfactory gas holding capacity are considered. Secondly, the impact of structural starch modifications on those relevant dough functionalities are discussed.
Key findings and conclusion: The reverse approach helped to identify relevant physical modifications of starch enabling the reduction of the present analysis methods of functionalized flours during grinding. Moreover, the knowledge of the relevant starch structures provides a clear specification of functional mechanisms of starch in physically modified in baking flours. Thus, a purposive selection of baking flours and a controlled physical starch modification are enabled.

1. Introduction

Many investigations were performed to establish new modification techniques of starch in order to expand the field of application, to achieve a targeted modification and thus functionality of starch or flours (López, Zaritzky, & García, 2010; Masina et al., 2017; Tharanathan, 2005; Wilpiszewska & Spychaj, 2007; Zia-ud-Din, Xiong, & Fei, 2017).

Chemical, physical and enzymatic modification treatments are often used in food industry, especially baking industry, when high paste and freeze-thaw stabilities are presumed. (Jay-lin Jane, 2009; T. Y. Liu, Ma, Yu, Shi, & Xue, 2011; Singh, A. V.; Nath, L. K.; Singh, 2010) to overcome deficiencies of natives starches (Tharanathan, 2005). An enzymatic modification for instance by using amylases can result in the formation of low molecular weight fragments altering retrogradation and thus reducing crumb firmness during staling. Since the precise functionality, however, is not fully understood and types of usable enzymes are poor, the field of application is limited. Specially chemical modification of starches provide a wide range of possible applications, since manifold functionalities, as a quick gelling and altered swelling behavior, can be created by introducing functional groups by etherification, esterification or acid-hydrolysis of starch (Ariyantoro, Katsuno, & Nishizu, 2018; Bao & Bergman, 2004; Shen et al., 2019). Thus, physical modification methods get in the scope of interest (BeMiller & Huber, 2015; Haghayegh, G.; Schoenlechner, 2011; Wolf, 2010), since chemically modified starches have great disadvantages of declaration obligation and the remain of by-products of the processes in the final product (Chiu & Solarek, 2009; FAO, 2000). Physical modified flours have substantial advantages, as an enhanced water binding. In baking industry, however, flours with a low starch modification degree are preferred for the production of baked goods (Dexter, Preston, Martin, & Gander, 1994; Kindelspire, Glover, Caffé-Treml, & Krishnan, 2015; Schlesinger, 1964), since the usage of modified starches led to deteriorations, as the reduction of the specific volume, of the resulting crumbs (Makinde & Akinoso, 2014; Mongi et al., 2012; Mudgil, Barak, & Khatkar, 2016; Nwosu & Onwurah, 2014).

Thereby, the type of structural changes of starch during grinding and thus evoked effects on dough (liquid foam) processing and the product (open-cell foam) characteristic still remain unclear. Due to missing of structure-function links concerning the gas forming kinetics and gas holding capacity during the process, the selection of forecasting advices of starch modification degree is impossible (compare Fig. 1, forward approach). Consequently, a purposeful physical modification of baking flours by grinding processes fails and the design of new products follows the trial-and-error principle (see Fig. 1).

In this review, decisive structural changes of starch on processing

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

https://doi.org/10.1016/j.tifs.2019.07.031
Received 13 September 2018; Received in revised form 9 April 2019; Accepted 19 July 2019
Available online 24 July 2019

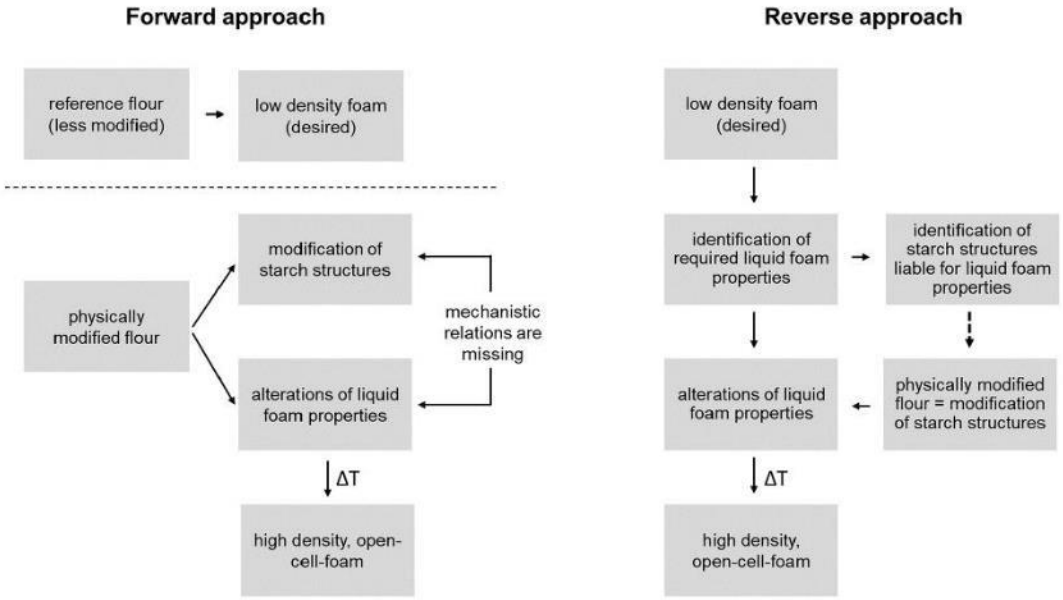

Fig. 1. Former, forward approach and new, reverse approach to elucidate the impact of starch modification on inflation and gas holding properties of wheat flours.

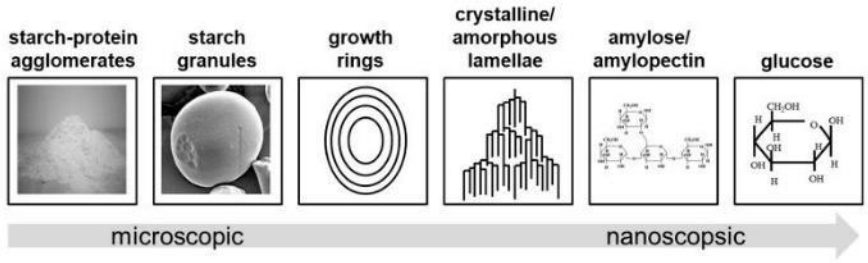

Fig. 2. Complex constitution of starch from microscopic to nanoscopic scale in wheat flour.

and product quality will be enlightened based on mechanical starch modification. The clarification of the underlying relations will allow to determine relevant analysis methods and to close the gap of a missing specification of physically modified flours for the usage in baking industry. For this purpose,

(1) requirements for flour and dough characteristics to receive low density products will be identified by considering the most relevant dough properties and the most important starch structures affecting liquid foams (= dough)
(2) foam destabilizing mechanisms of required dough properties, induced by the usage of physically modified starch/flours during grinding processes, will be discussed (Fig. 1).

Derived from this reverse-engineering approach, desired dough properties are known, and a targeted physical modification of starch can be used to produce starches with the required gas holding properties in wheat dough.

2. Constitution and physical modification of wheat starch and flours

Functional properties of flours and isolated starches are based on the chemical composition, the structural constitution and arrangement of starch copolymers amylose and amylopectin (Codină, Zaharia, Ropciuc, & Dabija, 2017; Dias, da Rosa Zavareze, Spier, de Castro, & Gutkoski, 2010; J.; Jane et al., 1999; Kaur, Bala, Singh, & Rehal, 2011; I. A.; Wani, Sogi, Wani, Gill, & Shivhare, 2010). Starch is arranged in starch granules, which can be further divided in wheat flour into A and B granules, which differ in size and shape (Zeng, Li, Gao, & Ru, 2011; Zhang et al., 2017). For wheat flours, the larger, ovoid shaped A granules account for about 90% of total volume distribution of starch, whereby the smaller, circular B granules represent a small proportion of the volume distribution, however, a major proportion of the total number. The constitution of A and B granules are characteristic for sources of starch (Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003). In wheat flours, starch granules are surrounded by proteins and embedded in a protein matrix forming flour particles. Surface proteins and the inclusion of minor components in native starches and flour particles, as lipophilic components, proteins, minerals or phosphor-containing substances, determine the functionality of native starches and flours, as the technological behavior during heating and cooling (retrogradation and recrystallization) (Karim et al., 2007; W.; Li et al., 2016; Oyeyinka, Singh, Venter, & Amonsou, 2017; Srichuwong et al., 2017). The distinction in the copolymer of A granules and B granules composition (A granules exhibit higher amylose contents than B granules do) result in a varying behavior during thermal treatments: B granules gelatinize at higher temperatures, while A granules exhibit higher gelatinization enthalpies (Geera, Nelson, Souza, & Huber, 2006). Granules itself consist of alternating amorphous and semi crystalline

rings, called growth rings (Pilling, E.; Smith, 2003), whereby semi crystalline growth rings contain alternating amorphous and crystalline lamellae (J. H. Li, Vasanthan, Hoover, & Rossnagel, 2003). Amylose and amylopectin comprise of the glycosidically linked building element α-D-glucose (Tegge, 1984) Fig. 2.

Although the modification of minor and major components of flours during grinding are known to alter the flour functionality (Paulik et al., 2019), modification of starch shows the main impact on modified flour functionality after grinding. Physical forces during milling and grinding are divided into mechanical and thermal forces and depend on the used grinder: ball-mills show predominantly shear and impact forces (mechanical) and noticeable heat development, hammer-mills contain mainly impact forces (mechanical) and a severe heat development (thermal), while cryogenic mills, which can be a special kind of ball-mill, modify starch by shear and impact forces in a cryogenic atmosphere (thermal) (Austin, 2004; El-Eskandarany & El-Eskandarany, 2001; Huang, Pan, & McCormick, 1997). Today's state of science gives the conclusion, that modification of pure starch or starch in cereal flours is achieved during grinding and milling processes on each structural level of starch, ranging from a particle size reduction, changes in morphology and crystallinity to a molecular degradation of starch polymers and the formation of low molecular weight dextrin. Thereby, the source of starch and consequently its constitution plays an important role in the modification process. The structural modification, in turn, causes technological changes of starches (Banafa, 2004; Chen, J.-J.; Lii, C.-Y.; Lu, 2003; Devi, Fibrianto, Torley, & Bhandari, 2009; Dhital, Shrestha, Flanagan, Hasjim, & Gidley, 2011; Dhital, Shrestha, & Gidley, 2010; He et al., 2014; Kim, Y; Suzuki, T.; Hagiwara, T.; Yamaji, I.; Takai, 2001; Moraes, Alves, & Franco, 2013; Tran et al., 2011).

Thus, this review focuses on grinding processes inducing changes on all presented starch structures and its technological modification. Despite the great amount of research conducted in determining microscopic and macroscopic changes of starch after physical modification of flours, differentiation between relevant and non-relevant structural alteration of flour biopolymers, especially starch, on the dough properties regarding gas holding capacity was rarely performed. Thus, significant functional dough properties (hereinafter called liquid foam properties) for receiving low density crumbs (open-cell foams) must be defined to relate modification of starch with changes of inflation and gas holding properties.

3. Requirements for flour and dough characteristics to produce low-density open-cell foams

Wheat bread doughs are food foams, which consist of impeded gas (closed cells) in a starch-gluten matrix (Gibson & Ashby, 1997). During proofing and baking steps, those foams exhibit an expansion leading to an open-cell structure (Hosomi, Nishio, Matsumoto, & Mita, 1992). The density of open-cell foams is determined by the interplay of gas forming kinetics and the gas holding capacity kneading, fermentation and baking (Mills, Wilde, Salt, & Skeggs, 2003; Visireddy, Rayas-Duarte, Mcglynn, Bowser, & Payton, 2008). Different material properties are required during kneading and fermentation (isotherm), as well as heating (thermal ramp), since significant transformation of starch matrices occur during the different processing steps (compare Table 1).

Depending on the processing step in the baking industry, gas uptake or gas formation by/in the starch-gluten matrices follow different kinetics leading to variations in matrix stresses. During kneading, density of doughs is reduced due to the mechanical insertion of air and the formation of CO_2 by yeast or chemical leavening agents resulting in a fast expansion of doughs and the need of a high gas holding capacity. During the fermentation (isothermal), exclusively biological gas formation leads to a slow gas entry. During baking (temperature ramp) further fermentation and thermal expansion of gas cells result in an accelerated expansion of liquid and solid foams. Thus, specific matrix characteristics are required during diverse processing steps, which are separately discussed in the following chapters.

3.1. Influence of dough viscosity on the gas holding capacity of wheat-based foams (isothermal)

Gas holding capacity of liquid foams mainly determines the density of the crumbs after heating of common wheat-based matrices (He, H.; Hoseney, 1991; Visireddy et al., 2008). Although the gas holding capacity of a matrix depends on the partial pressure and surface properties of gas bubbles (surface lamellae and surface tension) as well as the viscosity of the surrounding matrix (Kinsella, 1981; Marinova et al., 2009; Sroan, 2007), in food industry, gas holding capacity is often solely controlled by the viscosity of the matrix, as shown on a whey protein matrix by Tan, Chin, Yusof, Taip, & Abdullah (Tan, Chin, Yusof, Taip, & Abdullah, 2015). Thereby, a high viscosity of doughs reduces the ascent of cells (Trinh, 2013) and the presence of naturally occurring and added biopolymers contribute to the gas holding capacity of foams. In rye doughs, for example, non-starch polysaccharides (soluble arabinoxylan) increase the dough viscosity probably promoting a better gas holding capacity of doughs (Delcour, J. A.; Vanhamel, S.; Hoseney, 1991). Due to the addition of hydrocolloids, as HPMC (hydroxypropyl methylcellulose), CMC (carboxymethyl cellulose) or xanthan, the viscosity is heightened and furthermore viscoelastic properties of gluten are mimic in gluten-free, starch-based system, as reviewed by Anton & Artfield and Saha & Bhattacharya (Anton & Artfield, 2008; Saha & Bhattacharya, 2010). However, the rise in dough consistency of starch-based matrices by the addition of hydrocolloids as bran can also result in an impaired gas holding capacity (Föste et al., 2014), when gas bubbles are destructed by bran particles. Thus, not only the presence of hydrocolloids is crucial for the gas holding capacity of doughs and the density of the product, but also the type of used hydrocolloids. Changes in the composition of the recipe by the exchange or addition of hydrocolloids, can in turn, also affects the gas forming kinetics in doughs.

3.2. Inflation kinetics - availability of fermentable sugars and other limited substances

Several methods exist to foam up starch-gluten-based matrices, as doughs and crumbs. Beside the mechanical aeration of gluten-free, rice-based doughs (Elgeti, Yu, Stüttgen, Jekle, & Becker, 2017), inflation during baking is needed to create low density, open-cell foams. Gas formation during baking is achieved by using yeast or chemical leavening agents (Verheyen, Albrecht, Elgeti, Jekle, & Becker, 2015; Verheyen, Jekle, & Becker, 2014). The application of yeast is

Table 1
The density of open-cell foams is determined by the gas holding capacity and the inflation kinetics during the processing.

density and specific volume of open-cell foams (breads) determined by		
gas holding capacity during kneading/fermentation	**Inflation kinetics** during kneading/fermentation and heating	**gas holding capacity** during heating
• **mechanical properties** (Rosell & Collar, 2009)	• **availability of substrate** (Doescher, L. C.; Hoseney, R. C.; Milliken, G. A.; Rubenthaler, 1987)	• **pasting onset** (Soulaka & Morrison, 1985)
• **protein network** (Amjid et al., 2013)	• **water and ethanol evaporation**	• **consistency** (Rosell & Collar, 2009; Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008)

advantageous, since inflation of dough also result in an aroma formation and consequently aroma intensive products. However, at least a low amount of flour endogenous sugars are required and/or sugar are additionally added, which can hydrolyzed by yeast, to achieve positive effects on the density of the product (Doescher, L. C.; Hoseney, R. C.; Milliken, G. A.; Rubenthaler, 1987). Endogenous sugars are synthesized by the hydrolysis of enzymatically accessible starch by exogenous or endogenous amylases. Since even non modified flours (so called native flours) contain a low amount of physically modified starch, low accessibility of starch to an enzymatic hydrolysis is always present ranging between 0.7% for normal corn starch and 1.66% for wheat flours (X. Liu et al., 2017; Sharif Hossen et al., 13AD; S. Wang, Yu, Xin, Wang, & Copeland, 2017). Due to grinding procedures of flours, accessibility for an enzymatic digestion rises of physically modified starch in wheat flour (Jakobi, Jekle, & Becker, 2018b). Consequently, if sugars are the limiting factor for the gas production of yeast-leavened breads, increase in substrate by adding exogenous amylases or sugars could theoretically elevate the gas production.

Investigations performed on the gassing power of different cereals, however, indicated other conclusions: although wheat and corn showed the highest gassing power, followed by rye flour and finally rice, corn-based products had a smaller loaf volume than wheat breads (He, H.; Hoseney, 1991). Thus, in this study, the gassing power was not an appropriate tool to predict the gas volume in the product. A limited influence of starch accessibility on the product quality was also investigated on rice-gluten-doughs. Thereby, loafs prepared of physically modified starches showed a reduced loaf volume (Araki et al., 2009). Consequently, beside the extent of CO_2 formation, the capacity of doughs and breads holding gas (stability) should be monitored. Drapron and Godon noticed, that a possible higher tendency of doughs and bread to collapse, when prepared out of physically modified starch (Drapron, R.; Godon, 1987), which could be due to differences in the gas forming kinetics (Verheyen et al., 2015). These contrary findings demonstrate that other factors than the gas forming kinetics are significantly responsible for the crumb density.

3.3. Gas holding capacity during thermal transition (heating)

Viscosity Gas stabilizing properties are especially important in doughs during heating processes, where a restructuration of cereal biopolymers and the transformation of the liquid foam (dough) to an open-cell, solid foam (crumb) occurs, since fast and extent gas formation/expansion takes place during oven rise, which necessitates a high resistance to extension and reinforcement of the matrix. Thereby, rise in viscosity of starch during heating is seen as a major mechanism to achieve a high gas holding capacity in wheat dough (Dobraszczyk, 2004), especially for matrices where less polymeric gluten network exists to retain expanding air nuclei, as shown on starch-gluten blends with varying gluten content (Wilderjans et al., 2008).

During heat treatment, starch undergo a phase transition: amorphous parts of starch granules (amorphous growth rings) absorb water causing the swelling of amorphous growth rings and the disruption of crystalline areas of starch enhancing a water uptake again in turn. The solvent retention capacity (in cold water) of non-modified, native wheat flour is between 70 and 80% depending on the analyzed wheat variety (Barak, Mudgil, & Khatkar, 2014). During heating, water uptake of starch significantly rises promoting the formation of a solid-structured crumb during baking. The extended water uptake during pasting causes a swelling of starch granules and elevates amylose leaching. Leached amylose forms an alternative network leading to an increase in the viscosity of starch-containing suspensions improving the gas holding capacity of the matrix (Iannace & Park, 2016; Wilderjans et al., 2008).

Onset of pasting: The time of starch pasting (rise in viscosity) in cereal foams depends on the composition of the matrix and is elevated with the presence of sugars, sugar alcohols, and lipids (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016; Perry & Donald, 2002; Spies, R. D.; Hoseney, 1982). For gluten-free matrices (= starch-based matrices), which do not contain a polymeric, visco-elastic network with a satisfying gas holding capacity, an early pasting is desired. Thus, gas bubble buoyancy and coalescence, which occur especially during the fast, thermal expansion of gas bubbles during baking, can be restricted (Elgeti, Peng, Jekle, & Becker, 2017).

If a stabilizing network as available, late pasting is aspired resulting in higher loaf volumes for gluten containing starchy systems (Soulaka & Morrison, 1985). Thereby, the occurring phenomena of strain hardening in starch-gluten matrices prevent gas cell walls from failure, gas bubble coalescence and consequently rupture by increasing the stability of cell walls (Dobraszczyk, D. J.; Ainsworth, P.; Ibanoglu, S.; Bouchon, 2006). To prove the impact of pasting time on the gas holding capacity of doughs, Kusunose et al. prepared artificial doughs from wheat gluten and starches from wheat, potato and tapioca. The researcher showed, that an early gelatinized starch is hindering to achieve a high expansion of dough matrices (Kusunose, Fujii, & Matsumoto, 1999).

To capture the impact of physical modification on the transformations of starch during heating, as pasting (rise in viscosity) and gelatinization (transformation of crystallin parts into amorphous), fundamental measurements as rheometer (Park, Ibáñez, Zhong, & Shoemaker, 2007), DSC (differential scanning calorimetry)and NMR (Mendes et al., 1996) are widely used. Specially, combinations of methods, as imaging (CLSM) and thermo-analyses (DSC) provide detailed information of structural conformation of wheat starch copolymers (Markus Schirmer, Jekle, & Becker, 2011). However, interactions between cereal polymers, which affect the analysis methods and thus the informative value, are often neglected, as discussed in the next chapter.

4. Foam destabilizing mechanisms induced by physically modified starch

4.1. Impact of modified starch on dough viscosity in isothermal and heating processes

High viscous matrices can stabilize gas cells by reducing buoyancy and coalescence of gas. Due to the increased accessibility of physically modified starches, water uptake of modified starches rises in comparison to native starch under isothermal conditions. Consequently, at a constant water addition an enhanced dough viscosity and resistance of wheat dough during kneading is measured (Hackenberg, Verheyen, Jekle, & Becker, 2017). Thereby, rise in water retention of starch and enhanced dough viscosity can be reduced to an altered morphology of starch/flour particles measured by particle size distribution (de la Hera, Gomez, & Rosell, 2013; Jakobi, Jekle, & Becker, 2018a). Thus, physical modification of flours on microscopic scale, which is achieved especially during grinding, can increase dough viscosity during kneading and fermentation. However, breakdown and peak viscosity (viscosity during heat treatment) of modified triticale flour, modified jicama starch or cassava starch decreased with increase in grinding time (León, Barrera, Pérez, Ribotta, & Rosell, 2006; Martínez-Bustos, Ló Pez-Soto, San Martín-Martínez, Zazueta-Morales, & Velez-Medina, 2006) (Chen, J.-J.; Lii, C.-Y.; Lu, 2003) were observed, which was referred to nanoscopic changes of starch, as the increase in amylose content (A. A. Wani et al., 2012).

In general, a particular amylopectin degradation is favored during physical treatments due to the higher branching degree and higher molecular weight (Devi et al., 2009; Dziedzic, S.Z., Kearsley, 1995; Han, X.-Z.; Campanella, O. H.; Mix, N. C.; Hamaker, 2002; Tester, Patel, & Harding, 2006), resulting in the reduction of amylopectin content to amylose-like fragments and relative rise in amylose. This reduction of amylopectin is known to reduce the paste viscosities, since amylopectin is primary responsible for the development of high paste viscosities. Furthermore, amylose molecules led to an inhibition of extensive

granule swelling lowering significantly the paste viscosity (Guo et al., 2003; M.; Schirmer, Höchstötter, Jekle, Arendt, & Becker, 2013). Thus, physical modification of starch should decrease paste viscosity due to the shift in amylose/amylopectin ratio from a nanoscopic view.

However, depending on the applied forces, degradation of amylose in wheat and rice starch is possible or even favored, as noticed by Colonna et al., Tamaki et al. and Tran et al. (Colonna, P.; Doublier, J. L.; Melcion, J. P.; deMonredon, F.; Mercier, 1984; Tamaki, Hisamatsu, Teranishi, & Yamada, 1997; Tran et al., 2011). These results illustrate, that the type of physical treatment significantly affects the structural alteration of starch and thus matrix functionality by degrading amylose and amylopectin. The consideration of exclusively the amylose/amylopectin content could result in a misleading correlation of paste viscosity and amylose content. The total reduction of starch content could additionally explain the reduced paste viscosity.

To summarize, physical modification during grinding favors a reduction in dough viscosity, which in turn is responsible for an impaired gas stabilization.

4.2. Impact of physically modified starches on pasting onset and peak temperature

The preparation of low-density, wheat-based baking products requires a late gelatinization of starch. The onset temperature, as well as peak temperature depend on the type of starch and the processing of starch (Kusunose et al., 1999; M.; Schirmer et al., 2013; Sharma, Yadav, Singh, & Tomar, 2015). Increased gelatinization temperature was noticed for thermally, which could be due to a restructuring of the crystalline parts of starch and thus a higher temperature stability of crystals or possibly due to changes of surface properties of tuber and root starch, which prevent swelling of starch (Gunaratne & Hoover, 2002). It has to be clarified, if a temperature-induced restructuration of crystalline parts of starch occur during grinding processes, as well.

However, considering the state of science, mechanical modification of pure starch or wheat flours cause a particle size reduction resulting in a facilitated amylose leaching (Moisio, Forssell, Partanen, Damerau, & Hill, 2015). Beside the degree of amylose leaching, it is known, that the content of amylose is highly positive correlated to the pasting onset, since water uptake of starch granules is reduced resulting in a rise in pasting temperature in non-modified starch systems (de la Hera et al., 2013; Lii, Tsai, & Tseng, 1996; Varavinit, Shobsngob, Varanyanond, Chinachoti, & Naivikul, 2003). However, since modification of starch result only in a relative rise in amylose/amylopectin ratio, changes in total amount of amylose are unlike to cause changes in pasting onset, rather the particle size reduction during grinding has to be considered.

For cryogenically milled rice starch, no raised gelatinization temperature due to amylose leaching was detectable (Dhital et al., 2011), probably due to an extreme disruption of starch granules during grinding (Dhital et al., 2010). Disruption of granules would favor the hydration of starch and, moreover, result in an early pasting of starches.

Beside the modification of polymers, mechanical treatments, as grinding or high-pressure processes, of flours/starch led to a fragmentation of starch polymers into smaller carbohydrates (Dhital et al., 2011; Jakobi et al., 2018b). It is assumed, that those low molecular weight dextrin are able to retard the gelatinization onset of potato starch (Kim, C. S.; Walker, 1992), since the delay in gelatinization of starch correlates with the increase in the polymeric degree (glucose → maltose → maltotriose) (Spies, R. D.; Hoseney, 1982) and the formation of short chain fragments of starch was already proven for ball-milled flours (Tamaki et al., 1997). However, it remains unclear, if the formation of maltodextrins occurs due to a mechanical disruption of starch chains, due to the temperature rises during grinding causing an enhanced enzymatic activity and enzymatic hydrolysis of starch or due to a facilitated leaching of already existing low molecular weight dextrins from the starch granules into the surrounding matrix. Furthermore, the effects of endogenous maltodextrins on pasting onset is questionable, since concentration of maltodextrins after grinding is low, in comparison to studies with added sugars.

In summary, the granular disruption of starch and degradation of amylopectin predominantly evokes a timely pasting of starch affecting the gas holding capacity of starchy matrices.

5. Theory of physically modified starch affecting gluten network formation in wheat flour-based matrices

Wheat flours and derived doughs have unique processing properties due to the technological functionality of wheat proteins, more precise gluten. Gluten proteins are a heterogeneous mixture of polymers accounting for 80% of proteins occurring in wheat flour. They can be divided, according to their behavior of solubility, into gliadins and glutenins, whereby monomeric gliadin proteins, which show intramolecular disulphide bonds, contribute to a viscous behavior, and polymeric glutenin fraction, which show intermolecular bonds, contribute to an elastic behavior of starch-gluten matrices (Veraverbeke & Delcour, 2002; Wieser, 2007). Physical treatments of gluten – especially thermal treatments – cause changes in gluten surface properties, gluten aggregation, decreased protein solubility, gluten network strength but improved gelation ability (Jeanjean, M. F.; Damidaux, R.; Feillet, 1980; Pommet, Morel, Redl, & Guilbert, 2004; J.-S.; Wang, Zhao, Yang, Jiang, & Chun, 2007), which can negatively affect the gas holding capacity. In those mentioned studies, isolated gluten was exposed to physical forces neglecting the stabilizing impact of a starchy matrices - starch is able to absorb mechanical and thermal shocks - on protein denaturation. Other studies were performed to elucidate the impact of thermal or mechanical treatments on high complex, heterogeneous wheat flour matrices (Guerrieri & Cerletti, 1996; J. Mann, Schiedt, Baumann, Conde-Petit, & Vilgis, 2014; Neill, Al-Muhtaseb, & Magee, 2012). However, since starch polymers is altered by the grinding procedure, starch-gluten interactions (Julia Mann, Schiedt, Baumann, Conde-Petit, & Vilgis, 2014) and thus gluten functionality can be affected.

For the preparation of high volume wheat bread, dough elasticity (elastic modulus) plays an important role (Amjid et al., 2013) explainable by the development of a gluten network, which is responsible for the gas retention and thus foam stabilization during proofing (Hui, Y. H.; Meunier-Goddik, L.; Josephsen, J.; Nip, W.-K.; Stanfield, 2004). To achieve a gluten network with high gas holding characteristic, kneading procedure has to be monitored precisely:

Kneading time and speed significantly affect the dough formation, as visualized by scanning electron microscopic images by Codina and Mioneasa. Higher kneading speed of 250 rpm in Mixolab or kneading energy up till ~120 kJ/kg led to homogenous gluten structures and decrease in tan δ (G"/G') (Codina, G. G.; Mioneasa, 2013; Contamine, A. S.; Abecassis, J.; Morel, M.-H.; Vergnes, B.; Verel, 1995). However, a low kneading speed in Mixolab of 80 or 160 rpm favored the formation of structural inhomogeneous doughs and an insufficient hydration of flour particles (Codina, G. G.; Mioneasa, 2013). Since hydration of flour particles is also referred to granular changes starch, strong interactions of starch-gluten are expected. Inadequate kneading result later in collapse of foam systems (Kilborn, R. H.; Tipples, 1972). Furthermore, a positive dependency between kneading speed as well as time and dough consistency was recognized (Skeggs, P. K.; Kingswood, 1981). These findings allow the derivation of two critical aspects for dough development: kneading speed AND introduced work should be in between a critical range that varies with flour and kneading geometry (Kilborn, R. H.; Tipples, 1972).

The clear effects of kneading parameter on dough development can mainly be attributed to the impact of starch granules on network formation. During kneading, starch granules force the extension of gluten proteins and the formation of new interactions of protein polymers in wheat dough (Provost, Colabroy, Kelly, & Wallert, 2016). Thereby, the source of starch and consequently granule size and distribution strongly affect viscosity of starch-gluten matrices (Petrofsky & Hoseney, 1995),

which is altered by physical modification procedures. Furthermore, interactions between gluten and starch polymers influence dough matrix functionality (Jekle, M., Mühlberger, K., Becker, 2016), as illustrated on artificial dough systems, where Schiedt, Baumann, Conde-Petit, & Vilgis noticed an increase in elastic modulus (G') by raising the starch content up to 80% (Schiedt, Baumann, Conde-Petit, & Vilgis, 2013). Thus, starch granules do not act just as functionless filler particles in gluten network, but rather as tools for the extension of protein polymers to polymer strains.

Modified protein network formation

It is often postulated that during grinding procedures protein structure are not affected and consequently, decrease in specific bread volume is correlated to the modification of starch (granules) (Barrera et al., 2007). Starch-gluten interactions, which decisively codetermine the functionality of wheat doughs were often insufficiently considered in physical modification processes. However, the analysis is complicated, since modified gluten network formation occurs in the presence of physically modified starch, as indicated by Hackenberg, Jekle, & Becker (Hackenberg, Jekle, & Becker, 2018):

Physical, especially mechanical, modification of flours causes the reduction of flour particles size, as well as the formation of micro cracks and the removal of surface proteins (Baldwin, Adler, Davies, & Melia, 1995; Tian, J.-Z.; Yao, Y.; Shen, S.-S.; Zheng, X.-L.; Liu, C.; Han, 2015). These alterations facilitate the water binding of physically modified starches (in flours), for instance after grinding, which function as hydrocolloids, resulting in a faster and enhanced hydration in several cereal starch matrices, as wheat flour, as well as corn, rice and potato starch (Ali et al., 2014; Baldwin et al., 1995; de la Hera et al., 2013; Herceg et al., 2010). The modified hydration can provoke an insufficient hydration of the proteins gliadin and glutenin, which is a prerequisite for sufficient gluten network formation (Eduardo, Svanberg, & Ahrné, 2016; Hackenberg et al., 2018).

The impact of hydrocolloids and consequently of a modified hydration of the system on gluten functionality was investigated in detail by Bàrcenas, O-Keller, & Rosell. The addition of low concentration of HPMC, arabic gum, or pectin resulted in a decrease of the elastic modulus G' at 25 °C and a weakened gluten network at proofing temperatures of doughs (Bàrcenas, O-Keller, & Rosell, 2009). This behavior is explainable by the loop and train model of Belton. During low hydration of gluten proteins, interchain hydrogen bonds between glutamine dominate leading to a β-spiral structures (Belton et al., 1995). But, at higher water amounts above 37% w/w and consequently upon hydration of gluten, gluten proteins change conformation partly to β-sheet and a rise in chain mobility. This explains changes in gluten behavior as a rise in elasticity (Belton, 2005; Belton et al., 1995; Laskowski, J.; Pożyło, 2004), without evoking chemical-structural changes of gluten polymers.

To summarize, the modified gluten network formation in complex cereal matrices can strongly be attributed to granular changes of starch altering the water distribution within liquid foams (doughs). In future, research should question the functional changes of gluten due to altered hydration properties of starch.

6. Focus on gas holding capacity enables a specification of structural and functional alterations of physically modified starches in dough

Physical modification of starch by mechanical and thermal forces during milling and grinding is tried to be avoided due to the negative consequences on doughs and breads during processing (Araki et al., 2009). In case of the production of highly inflated wheat breads, the following specification for the baking industry for physically modified flours is provided, which is divided into a functional (1) and a structural (2) part:

(1) Modification of starch polymer chains, crystallinity and granular structure evoke an impaired gluten network formation, a timely starch pasting and a reduction in dough viscosity causing a decreased gas holding capacity of wheat doughs during fermentation and thermal transformation
(2) As main factors, molecular degradation of starch, which leads to a reduced viscosity and timely starch pasting. Granule disruption facilitates early pasting and evokes a higher starch hydration leading to an impaired gluten network formation.

Structural alteration and functional mechanism of physically modified flours by grinding: Physically modified flours are characterized by an intense disruption of starch-gluten agglomerates causing an enhanced swelling, therefore premature gelatinization during processing of doughs, and a reduced viscosity during baking. Early gelatinization and lowered viscosity in combination with an altered gluten network formation result in a modified gas holding capacity and consequently impaired formation of an open-cell foam.

7. Conclusion and outlook

The structuration of research findings regarding the modification of starch during grinding has shown that a reduced gas holding capacity of ground (physically modified) flours can be referred to specific alterations of starch constitution due to the grinding procedure. Ground starch can also affect gluten functionality due to the enhanced swelling and hydration of starch granules. If altered protein functionality can exclusively be referred to modified starch in wheat dough made of ground wheat flours, analysis methods can be simplified focusing on exclusively on starch in baking industry. Studies on separate physical gluten or starch modification (including thermal and mechanical forces) would provide essential knowledge about modified flour functionalities. Beside investigations on the gas holding capacity, research should further focus on the impact of starch, modified during grinding, on the staling process of the baked goods. Since alterations of starch granules and starch content affect the hydration and gelatinization of starch, effects on recrystallization of starch are expected, which determine the quality of baked goods during storage. In future, a fundamental rethink concerning the usage of physically modified starch achieved by grinding procedures should be performed to exploit the advantages of grinding processes to produce target flour qualities.

Acknowledgements

This IGF Project of the FEI (18679N) is supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

References

Ali, R., Khan, M. S., Sayeed, S. A., Ahmed, R., Saeed, G., & Mobin, L. (2014). Relationship of damaged starch with some physicochemical parameters in assessment of wheat flour quality. *Pakistan Journal of Botany, 46*(6), 2217–2225.

Amjid, M. R., Shehzad, A., Hussain, S., Shabbir, M. A., Khan, M. R., & Shoaib, M. (2013). A comprehensive review on wheat flour dough rheology. *Pakistan Journal of Food Sciences, 23*(2s), 105–123. Retrieved from http://psfst.com/_jpd_fstr/9db9d2eac8ae428e46637a216c96feec.pdf.

Anton, A. A., & Artfield, S. D. (2008). Hydrocolloids in gluten-free breads: A review. *International Journal of Food Sciences and Nutrition, 59*(1), 11–23. https://doi.org/10.1080/09637480701625630.

Araki, E., Ikeda, T. M., Ashida, K., Takata, K., Yanaka, M., & Iida, S. (2009). Effects of rice flour properties on specific loaf volume of one-loaf bread made from rice flour with wheat vital gluten. *Food Science and Technology Research, 15*(4), 439–448. https://doi.org/10.3136/fstr.15.439.

Ariyantoro, A. R., Katsuno, N., & Nishizu, T. (2018). Effects of dual modification with succinylation and annealing on physicochemical, thermal and morphological properties of corn starch. *Foods, 7*(9), 133. https://doi.org/10.3390/foods7090133.

Austin, L. G. (2004). A preliminary simulation model for fine grinding in high speed hammer mills. *Powder Technology, 143–144*, 240–252. https://doi.org/10.1016/J.

POWTEC.2004.04.017.
Baldwin, P. M., Adler, J., Davies, M. C., & Melia, C. D. (1995). Starch damage Part 1: Characterisation of granule damage in ball-milled potato starch study by SEM. *Starch - Stärke, 47*(7), 247–251. https://doi.org/10.1002/star.19950470702.
Banafa, W. (2004, April 26). *The effect of impact - milling and air classification on composition and processing qualities of wheat flours.* Retrieved from https://depositonce.tu-berlin.de/handle/11303/1133.
Bao, J., & Bergman, C. J. (2004). The functionality of rice starch. *Starch in Food, 258–294*https://doi.org/10.1533/9781855739093.2.258.
Barak, S., Mudgil, D., & Khatkar, B. S. (2014). Effect of flour particle size and damaged starch on the quality of cookies. *Journal of Food Science and Technology, 51*(7), 1342–1348. https://doi.org/10.1007/s13197-012-0627-x.
Bàrcenas, M. E., O-Keller, J. D. la, & Rosell, C. M. (2009). Influence of different hydrocolloids on major wheat dough components (gluten and starch). *Journal of Food Engineering, 94*(3–4), 241–247. https://doi.org/10.1016/j.jfoodeng.2009.03.012.
Barrera, G. N., Prez, G. T., Ribotta, P. D., Len, A. E., Pérez, G. T., Ribotta, P. D., et al. (2007). Influence of damaged starch on cookie and bread-making quality. *European Food Research and Technology, 225*(1), 1–7. https://doi.org/10.1007/s00217-006-0374-1.
Belton, P. S. (2005). New approaches to study the molecular basis of the mechanical properties of gluten. *Journal of Cereal Science, 41*(2), 203–211. https://doi.org/10.1016/j.jcs.2004.06.003.
Belton, P. S., Colquhoun, I. J., Grant, A., Wellner, N., Field, J. M., Shewry, P. R., et al. (1995). FTIR and NMR studies on the hydration of a high-M(r) subunit of glutenin. *International Journal of Biological Macromolecules, 17*(2), 74–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7547718.
BeMiller, J. N., & Huber, K. C. (2015). Physical modification of food starch functionalities. *Annual Review of Food Science and Technology, 6*(1), 19–69. https://doi.org/10.1146/annurev-food-022814-015552.
Chen, J.-J., Lii, C.-Y., & Lu, S. (2003). Physicochemical and morphological analyses on damaged rice starches. *Journal of Food and Drug Analysis, 11*(4), 283–289.
Chiu, C., & Solarek, D. (2009). *Starch. Starch.* Elsevierhttps://doi.org/10.1016/B978-0-12-746275-2.00017-3.
Codina, G. G., & Mioneasa, S. (2013). Influence of mixing speed on dough microstructure and rheology. *Food Technology and Biotechnology, 51*(4), 509–519.
Codină, G. G., Zaharia, D., Ropciuc, S., & Dabija, A. (2017). The EuroBiotech Journal Influence of magnesium gluconate salt addition on mixing, pasting and fermentation properties of dough. *The EuroBiotech Journal, 222*(3)https://doi.org/10.24190/ISSN2564-615X/2017/03.04.
Colonna, P., Doublier, J. L., Melcion, J. P., deMonredon, F., & Mercier, C. (1984). Extrusion cooking and drum drying of wheat starch. I. Physical and macromolecular modifications. *Cereal Chemistry, 61*(6), 538–543.
Contamine, A. S., Abecassis, J., Morel, M.-H., Vergnes, B., & Verel, A. (1995). Effect of mixing conditions on the quality of dough and biscuits. *Cereal Chemistry, 72*, 516–522.
Delcour, J. A., Vanhamel, S., & Hoseney, R. C. (1991). Physicochemical and functional properties of rye nonstarch polysaccharides. II. Impact of a fraction containing water-soluble pentosans and proteins on gluten-starch loaf volumes. *Cereal Chemistry, 68*, 72–76.
Devi, A. F., Fibrianto, K., Torley, P. J., & Bhandari, B. (2009). Physical properties of cryomilled rice starch. *Journal of Cereal Science, 49*(2), 278–284. https://doi.org/10.1016/j.jcs.2008.11.005.
Dexter, J. E., Preston, K. R., Martin, D. G., & Gander, E. J. (1994). The effects of protein content and starch damage on the physical dough properties and bread-making quality of Canadian durum wheat. *Journal of Cereal Science, 20*(2), 139–151. https://doi.org/10.1006/jcrs.1994.1054.
Dhital, S., Shrestha, A. K., Flanagan, B. M., Hasjim, J., & Gidley, M. J. (2011). Cryo-milling of starch granules leads to differential effects on molecular size and conformation. *Carbohydrate Polymers, 84*(3), 1133–1140. https://doi.org/10.1016/j.carbpol.2011.01.002.
Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Effect of cryo-milling on starches: Functionality and digestibility. *Food Hydrocolloids, 24*(2–3), 152–163. https://doi.org/10.1016/j.foodhyd.2009.08.013.
Dias, A. R. G., da Rosa Zavareze, E., Spier, F., de Castro, L. A. S., & Gutkoski, L. C. (2010). Effects of annealing on the physicochemical properties and enzymatic susceptibility of rice starches with different amylose contents. *Food Chemistry, 123*(3), 711–719. https://doi.org/10.1016/j.foodchem.2010.05.040.
Dobraszcyk, D. J., Ainsworth, P., Ibanoglu, S., & Bouchon, P. (2006). Baking, extrusion and frying. *Food processing handbook*.
Dobraszczyk, B. J. (2004). The physics of baking: Rheological and polymer molecular structure–function relationships in breadmaking. *Journal of Non-newtonian Fluid Mechanics, 124*(1–3), 61–69. https://doi.org/10.1016/J.JNNFM.2004.07.014.
Doescher, L. C., Hoseney, R. C., Milliken, G. A., & Rubenthaler, G. L. (1987). A study of wheat flour fermentation , Journal of Agroalimentary Processes and Technologies. *Cereal Chemistry, 64*, 163–167.
Drapron, R., & Godon, B. (1987). Enzymes and their role in cereal technology. In D. S. C. Kruger, & J. E. Lineback (Eds.). *American association of cereal chemists (pp. 281–324). St. Paul*.
Dziedzic, S. Z., & Kearsley, M. W. (1995). In M. W. Kearsley, & S. Z. Dziedzic (Eds.). *Handbook of starch hydrolysis products and their derivatives*Boston, MA: Springer UShttps://doi.org/10.1007/978-1-4615-2159-4.
Eduardo, M., Svanberg, U., & Ahrné, L. (2016). Effect of hydrocolloids and emulsifiers on the shelf-life of composite cassava-maize-wheat bread after storage. *Food Science & Nutrition, 4*(4), 636–644. https://doi.org/10.1002/fsn3.326.
El-Eskandarany, M. S., & El-Eskandarany, M. S. (2001). 6. Mechanically induced gas-solid reaction. *Mechanical alloying* (pp. 94–117). William Andrew Publishing. https://doi.org/10.1016/B978-081551462-6.50008-1.
Elgeti, D., Peng, L., Jekle, M., & Becker, T. (2017). Foam stabilization during processing of starch-based dough systems. *Innovative Food Science & Emerging Technologies, 39*, 267–274. https://doi.org/10.1016/j.ifset.2016.12.012.
Elgeti, D., Yu, L., Stüttgen, A., Jekle, M., & Becker, T. (2017). Interrelation between mechanical and biological aeration in starch-based gluten-free dough systems. *Journal of Cereal Science, 76*, 28–34. https://doi.org/10.1016/j.jcs.2017.05.008.
FAO (2000). *Modified starches.* Retrieved January 1, 2017, from ftp://ftp.fao.org/codex/meetings/CCFL/ccfl28/fl00_08e.pdf.
Föste, M., Nordlohne, S. D., Elgeti, D., Linden, M. H., Heinz, V., Jekle, M., et al. (2014). Impact of quinoa bran on gluten-free dough and bread characteristics. *European Food Research and Technology, 239*(5), 767–775. https://doi.org/10.1007/s00217-014-2269-x.
Geera, B. P., Nelson, J. E., Souza, E., & Huber, K. C. (2006). Composition and properties of A- and B-type starch granules of wild-type, partial waxy, and waxy soft wheat. *Cereal Chemistry Journal, 83*(5), 551–557. https://doi.org/10.1094/CC-83-0551.
Gibson, L. J., & Ashby, M. F. (1997). *Cellular solids.* Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9781139878326.
Guerrieri, N., & Cerletti, P. (1996). Effect of high-temperature short-time treatment of wheat flour on gluten vitality and structure. *Cereal Chemistry, 73*, 375–378. Retrieved from https://www.aaccnet.org/publications/cc/backissues/1996/Documents/73_375.pdf.
Gunaratne, A., & Hoover, R. (2002). Effect of heat–moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydrate Polymers, 49*(4), 425–437. https://doi.org/10.1016/S0144-8617(01)00354-X.
Guo, G., Jackson, D. S., Graybosch, R. A., Jackson, D. S., Graybosch, R. A., & Parkhurst, A. M. (2003). Asian salted noodle quality: Impact of amylose content adjustments using waxy wheat flour. *Cereal Chemistry, 80*(4), 437–445. Retrieved from http://digitalcommons.unl.edu/foodsciefacpub.
Hackenberg, S., Jekle, M., & Becker, T. (2018). Mechanical wheat flour modification and its effect on protein network structure and dough rheology. *Food Chemistry, 248*, 296–303. https://doi.org/10.1016/J.FOODCHEM.2017.12.054.
Hackenberg, S., Verheyen, C., Jekle, M., & Becker, T. (2017). Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. *European Food Research and Technology, 243*(2)https://doi.org/10.1007/s00217-016-2743-8.
Haghayegh, G., & Schoenlechner, R. (2011). Physically modified starches: A review. *Journal of Food, Agriculture & Environment, 9*(1), 27–29.
Han, X.-Z., Campanella, O. H., Mix, N. C., & Hamaker, B. R. (2002). Consequence of starch damage on rheological properties of maize starch pastes. *Cereal Chemistry, 79*(6), 897–901.
He, H., & Hoseney, R. C. (1991). Gas retention of different cereal flours. *Cereal Chemistry, 68*, 334–336.
He, S., Qin, Y., Walid, E., Li, L., Cui, J., & Ma, Y. (2014). Effect of ball-milling on the physicochemical properties of maize starch. *Biotechnology Reports, 3*, 54–59. https://doi.org/10.1016/J.BTRE.2014.06.004.
de la Hera, E., Gomez, M., & Rosell, C. M. (2013). Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties. *Carbohydrate Polymers, 98*(1), 421–427. https://doi.org/10.1016/j.carbpol.2013.06.002.
Herceg, I. L., Režek Jambrak, A., Šubarić, D., Brnčić, M., Rimac Brnčić, S., Badanjak, M., et al. (2010). Texture and pasting properties of ultrasonically treated corn starch. *Czech Journal of Food Sciences, 28*(2), 83–93. Retrieved from http://agriculturejournals.cz/publicFiles/18878.pdf.
Horstmann, S. W., Belz, M. C. E., Heitmann, M., Zannini, E., & Arendt, E. K. (2016). Fundamental study on the impact of gluten-free starches on the quality of gluten-free model breads. *Foods, 5*(2)https://doi.org/10.3390/foods5020030.
Hosomi, K., Nishio, K., Matsumoto, H., & Mita, T. (1992). Behavior of surface membrane of yeasted dough during expansion in a cylinder. *Nippon Shokuhin Kogyo Gakkaishi, 39*(1296), 1156–1162. Retrieved from https://www.jstage.jst.go.jp/article/nskkk1962/39/12/39_12_1156/_pdf.
Huang, H., Pan, J., & McCormick, P. G. (1997). Prediction of impact forces in a vibratory ball mill using an inverse technique. *International Journal of Impact Engineering, 19*(2), 117–126. https://doi.org/10.1016/S0734-743X(96)00018-8.
Hui, Y. H., Meunier-Goddik, L., Josephsen, J., Nip, W.-K., & Stanfield, P. S. (2004). *Handbook of food and beverage fermentation technology.* Marcel Dekker. Retrieved from https://www.crcpress.com/Handbook-of-Food-and-Beverage-Fermentation-Technology/Hui-Meunier-Goddik-Josephsen-Nip-Stanfield/p/book/9780203913550.
Iannace, S., & Park, C. B. (2016). *Biofoams: Science and applications of bio-based cellular and porous materials.* CRC Press Book.
Jakobi, S., Jekle, M., & Becker, T. (2018a). Direct link between specific structural levels of starch and hydration properties. *Carbohydrate Polymers, 181*(1), 159–166. https://doi.org/10.1016/J.CARBPOL.2017.10.062.
Jakobi, S., Jekle, M., & Becker, T. (2018b). High-pressure treatment of non-hydrated flour affects structural characteristics and hydration. *Foods, 7*(5), 78. https://doi.org/10.3390/foods7050078.
Jane, J. (2009). Structural features of starch granules II. *Starch*, 193–236. https://doi.org/10.1016/B978-0-12-746275-2.00006-9.
Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch 1. *Cereal Chemistry, 76*(5), 629–637. https://doi.org/10.1094/CCHEM.1999.76.5.629.
Jeanjean, M. F., Damidaux, R., & Feillet, P. (1980). Effect of heat treatment on protein solubility and viscoelastic properties of wheat gluten. *Cereal Chemistry, 57*, 325–331.
Jekle, M., Mühlberger, K., & Becker, T. (2016). Starch-gluten interactions during gelatinization and its functionality in dough like model systems. *Food Hydrocolloids, 54*, 196–201. https://doi.org/10.1016/j.foodhyd.2015.10.005.

Karim, A. A., Toon, L. C., Lee, V. P. L., Ong, W. Y., Fazilah, A., & Noda, T. (2007). Effects of phosphorus contents on the gelatinization and retrogradation of potato starch. *Journal of Food Science, 72*(2), C132–C138. https://doi.org/10.1111/j.1750-3841.2006.00251.x.

Kaur, A., Bala, R., Singh, B., & Rehal, J. (2011). Effect of replacement of sodium chloride with mineral salts on rheological characteristics of wheat flour. *American Journal of Food Technology, 6*(8), 674–684. https://doi.org/10.3923/ajft.2011.674.684.

Kilborn, R. H., & Tipples, K. H. (1972). Factors affect ing mechanical dough development. I. Effect of mixing intensity and work input. *Cereal Chemistry, 49*, 34–47.

Kim, Y., Suzuki, T., Hagiwara, T., Yamaji, I., & Takai, R. (2001). Enthalpy relaxation and glass to rubber transition of amorphous potato starch formed by ball-milling. *Carbohydrate Polymers, 46*(1), 1–6. https://doi.org/10.1016/S0144-8617(00)00274-5.

Kim, C. S., & Walker, C. E. (1992). Effects of sugars and emulsifiers an starch gelatinization evaluated by differential scanning calorimetry. *Cereal Chemistry, 69*(2), 2012–2017.

Kindelspire, J. Y., Glover, K. D., Caffé-Treml, M., & Krishnan, P. G. (2015). Dough strain hardening properties as indicators of baking performance. *Cereal Chemistry Journal, 92*(3), 293–301. https://doi.org/10.1094/CCHEM-12-13-0249-R.

Kinsella, J. E. (1981). Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chemistry, 7*(4), 273–288. https://doi.org/10.1016/0308-8146(81)90033-9.

Kusunose, C., Fujii, T., & Matsumoto, H. (1999). Role of starch granules in controlling expansion of dough during baking. *Cereal Chemistry Journal, 76*(6), 920–924. https://doi.org/10.1094/CCHEM.1999.76.6.920.

Laskowski, J., & Pożyło, R. (2004). Influence of starch damage extent in wheat flour on rheological (alveograph) properties of dough. *Acta Agrophysica, 4*(2), 373–380.

León, A. E., Barrera, G. N., Pérez, G. T., Ribotta, P. D., & Rosell, C. M. (2006). Effect of damaged starch levels on flour-thermal behaviour and bread staling. *European Food Research and Technology, 224*(2), 187–192. https://doi.org/10.1007/s00217-006-0297-x.

Lii, C.-Y., Tsai, M.-L., & Tseng, K.-H. (1996). Effect of amylose content on the rheological property of rice starch'. *Cereal Chemistry, 73*(4), 415–420. Retrieved from http://www.aaccnet.org/publications/cc/backissues/1996/documents/73_415.pdf.

Liu, T. Y., Ma, Y., Yu, S. F., Shi, J., & Xue, S. (2011). The effect of ball milling treatment on structure and porosity of maize starch granule. *Innovative Food Science & Emerging Technologies, 12*(4), 586–593. https://doi.org/10.1016/J.IFSET.2011.06.009.

Liu, X., Xiao, X., Liu, P., Yu, L., Li, M., Zhou, S., et al. (2017). Shear degradation of corn starches with different amylose contents. *Food Hydrocolloids, 66*, 199–205. https://doi.org/10.1016/J.FOODHYD.2016.11.023.

Li, J. H., Vasanthan, T., Hoover, R., & Rossnagel, B. G. (2003). Starch from hull-less barley: Ultrastructure and distribution of granule-bound proteins. *Cereal Chemistry Journal, 80*(5), 524–532. https://doi.org/10.1094/CCHEM.2003.80.5.524.

Li, W., Wu, G., Luo, Q., Jiang, H., Zheng, J., Ouyang, S., et al. (2016). Effects of removal of surface proteins on physicochemical and structural properties of A- and B-starch isolated from normal and waxy wheat. *Journal of Food Science and Technology, 53*(6), 2673–2685. https://doi.org/10.1007/s13197-016-2239-3.

López, O. V., Zaritzky, N. E., & García, M. A. (2010). Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity. *Journal of Food Engineering, 100*(1), 160–168. https://doi.org/10.1016/j.jfoodeng.2010.03.041.

Makinde, F. M., & Akinoso, R. (2014). Physical, nutritional and sensory qualities of bread samples made with wheat and black sesame (Sesamum indicum Linn) flours. *International Food Research Journal, 21*(4), 1635–1640. Retrieved from http://www.ifrj.upm.edu.my.

Mann, J., Schiedt, B., Baumann, A., Conde-Petit, B., & Vilgis, T. A. (2014a). Effect of heat treatment on wheat dough rheology and wheat protein solubility. *Food Science and Technology International, 20*(5), 341–351. https://doi.org/10.1177/1082013213488381.

Marinova, K. G., Basheva, E. S., Nenova, B., Temelska, M., Mirarefi, A. Y., Campbell, B., et al. (2009). Physico-chemical factors controlling the foamability and foam stability of milk proteins: Sodium caseinate and whey protein concentrates. *Food Hydrocolloids, 23*(7), 1864–1876. https://doi.org/10.1016/J.FOODHYD.2009.03.003.

Martínez-Bustos, F., Ló Pez-Soto, M., San Martín-Martínez, E., Zazueta-Morales, J. J., & Velez-Medina, J. J. (2006). *Effects of high energy milling on some functional properties of jicama starch (Pachyrrhizus erosus L. Urban) and cassava starch (Manihot esculenta Crantz)*. https://doi.org/10.1016/j.jfoodeng.2005.10.043.

Masina, N., Choonara, Y. E., Kumar, P., du Toit, L. C., Govender, M., Indermun, S., et al. (2017). A review of the chemical modification techniques of starch. *Carbohydrate Polymers, 157*, 1226–1236. https://doi.org/10.1016/j.carbpol.2016.09.094.

Mendes, C. E., Silva, D., Ciacco, C. F., Barberis, G. E., Solano, W. M. R., & Rettori, C. (1996). Starch gelatinization measured by pulsed nuclear magnetic resonance. *Cereal Chemistry, 73*(3), 297–301. Retrieved from https://www.aaccnet.org/publications/cc/backissues/1996/Documents/73_297.pdf.

Mills, E. N. C., Wilde, P. J., Salt, L. J., & Skeggs, P. (2003). Bubble formation and stabilization in bread dough. *Food and Bioproducts Processing, 81*(3), 189–193. https://doi.org/10.1205/096030803322437956.

Moisio, T., Forssell, P., Partanen, R., Damerau, A., & Hill, S. E. (2015). Reorganisation of starch, proteins and lipids in extrusion of oats. *Journal of Cereal Science, 64*, 48–55. https://doi.org/10.1016/j.jcs.2015.04.001.

Mongi, R., Ndabikunze, B., Chove, B., Mamiro, P., Ruhembe, C., & Ntwenya, J. (2012). African journal of food, agriculture, nutrition, and development. *African Journal of Food, Agriculture, Nutrition and Development, 11*(7), 5586–5599. Retrieved from https://www.ajol.info/index.php/ajfand/article/view/74283.

Moraes, J., Alves, F. S., & Franco, C. M. L. (2013). Effect of ball milling on structural and physicochemical characteristics of cassava and Peruvian carrot starches. *Starch - Stärke, 65*(3–4), 200–209. https://doi.org/10.1002/star.201200059.

Mudgil, D., Barak, S., & Khatkar, B. S. (2016). Optimization of bread firmness, specific loaf volume and sensory acceptability of bread with soluble fiber and different water levels. *Journal of Cereal Science, 70*, 186–191. https://doi.org/10.1016/j.jcs.2016.06.009.

Neill, G., Al-Muhtaseb, A. H., & Magee, T. R. A. (2012). Optimisation of time/temperature treatment, for heat treated soft wheat flour. *Journal of Food Engineering, 113*(3), 422–426. https://doi.org/10.1016/j.jfoodeng.2012.06.019.

Nwosu, U. L., & Onwurah, C. O. (2014). Physical characteristics and sensory quality of bread produced from wheat/African oil bean flour blends. *African Journal of Food Science, 8*(6), 351–355. https://doi.org/10.5897/AJFS2013.1079.

Oyeyinka, S. A., Singh, S., Venter, S. L., & Amonsou, E. O. (2017). Effect of lipid types on complexation and some physicochemical properties of Bambara groundnut starch. *Starch - Stärke, 69*(3–4), 1600158. https://doi.org/10.1002/star.201600158.

Park, I., Ibáñez, A. M., Zhong, F., & Shoemaker, C. F. (2007). Gelatinization and pasting properties of waxy and non-waxy rice starches. *Starch - Stärke, 59*(8), 388–396. https://doi.org/10.1002/STAR.200600570.

Paulik, S., Jekle, M., & Becker, T. (2019). Mechanically and thermally induced degradation and modification of cereal biopolymers during grinding. *Polymers, 11*(3), 448. https://doi.org/10.3390/polym11030448.

Perry, P., & Donald, A. (2002). The effect of sugars on the gelatinisation of starch. *Carbohydrate Polymers, 49*(2), 155–165. https://doi.org/10.1016/S0144-8617(01)00324-1.

Petrofsky, K. E., & Hoseney, R. C. (1995). Rheological properties of dough made with starch and gluten from several cereal sources'. *Cereal Chemistry, 72*(1), 53–58. Retrieved from https://pdfs.semanticscholar.org/c67c/ede1d74d490f8877103d113f86861ba90690.pdf.

Pilling, E., & Smith, A. M. (2003). Growth ring formation in the starch granules of potato tubers. *Plant Physiology, 132*(1), 365–371.

Pommet, M., Morel, M.-H., Redl, A., & Guilbert, S. (2004). Aggregation and degradation of plasticized wheat gluten during thermo-mechanical treatments, as monitored by rheological and biochemical changes. *Polymer, 45*(20), 6853–6860. https://doi.org/10.1016/j.polymer.2004.07.076.

Provost, J. J., Colabroy, K. L., Kelly, B. S., & Wallert, M. (2016). *The science of cooking: Understanding the biology and chemistry behind food and cooking*.

Rosell, C. M., & Collar, C. (2009). Effect of temperature and consistency on wheat dough performance. *International Journal of Food Science & Technology, 44*(3), 493–502. https://doi.org/10.1111/j.1365-2621.2008.01758.x.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. *Journal of Food Science and Technology, 47*(6), 587–597. https://doi.org/10.1007/s13197-010-0162-6.

Schiedt, B., Baumann, A., Conde-Petit, B., & Vilgis, T. A. (2013). Short- and long-range interactions governing the viscoelastic properties during wheat dough and model dough development. *Journal of Texture Studies, 44*(4), 317–332. https://doi.org/10.1111/jtxs.12027.

Schirmer, M., Höchstötter, A., Jekle, M., Arendt, E., & Becker, T. (2013). Physicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio. *Food Hydrocolloids, 32*(1), 52–63. https://doi.org/10.1016/j.foodhyd.2012.11.032.

Schirmer, M., Jekle, M., & Becker, T. (2011). Quantification in starch microstructure as a function of baking time. *Procedia Food Science, 1*, 145–152. https://doi.org/10.1016/j.profoo.2011.09.023.

Schlesinger, J. (1964). Results of ball-milling buhler experimentally milled hard winter wheat flour. *Cereal Chemistry, 41*(4), 465–473. Retrieved from http://www.aaccnet.org/publications/cc/backissues/1964/Pages/Number4.aspx.

Sharif Hossen, M., Sotome, I., Takenaka, M., Isobe, S., Nakajima, M., Shimizu, N., et al. Ultra-fine pulverization of rice: Effects on hydration properties and enzymatic hydrolysis. Japan Journal of Food Engineering, 14(1), 37-46. Retrieved from https://www.jstage.jst.go.jp/article/jsfe/14/1/14_37/_pdf.

Sharma, M., Yadav, D. N., Singh, A. K., & Tomar, S. K. (2015). Rheological and functional properties of heat moisture treated pearl millet starch. *Journal of Food Science and Technology, 52*(10), 6502–6510. https://doi.org/10.1007/s13197-015-1735-1.

Shen, Y., Zhang, N., Xu, Y., Huang, J., Yuan, M., Wu, D., et al. (2019). Physicochemical properties of hydroxypropylated and cross-linked rice starches differential in amylose content. *International Journal of Biological Macromolecules, 128*, 775–781. https://doi.org/10.1016/J.IJBIOMAC.2019.01.194.

Singh, A. V., Nath, L. K., & Singh, A. (2010). Pharmaceutical, food and non-food applications of modified starches: A critical review. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 9*(7), 1214–1221. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20103366090.

Singh, N., Singh, J., Kaur, L., Singh Sodhi, N., & Singh Gill, B. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry, 81*(2), 219–231. https://doi.org/10.1016/S0308-8146(02)00416-8.

Skeggs, P. K., & Kingswood, K. (1981). Mechanical dough development—pilot scale studies. *Cereal Chemistry, 58*, 256–260.

Soulaka, A. B., & Morrison, W. R. (1985). The bread baking quality of six wheat starches differing in composition and physical properties. *Journal of the Science of Food and Agriculture, 36*(8), 719–727. https://doi.org/10.1002/jsfa.2740360812.

Spies, R. D., & Hoseney, R. C. (1982). Effects of sugars on starch gelatinization. *Cereal Chemistry, 59*(2), 128–131.

Srichuwong, S., Curti, D., Austin, S., King, R., Lamothe, L., & Gloria-Hernandez, H. (2017). Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. *Food Chemistry, 233*, 1–10. https://doi.org/10.1016/J.FOODCHEM.2017.04.019.

Sroan, B. S. (2007). *Mechanism of gas cell stability in bread making*. Kansas State University.

Retrieved from http://krex.k-state.edu/dspace/handle/2097/338.
Tamaki, S., Hisamatsu, M., Teranishi, K., & Yamada, T. (1997). Structural change of wheat starch granule by ball-mill treatment. *Journal of Applied Glycoscience, 44*(4), 505–513. https://doi.org/10.11541/jag1994.44.505.
Tan, M. C., Chin, N. L., Yusof, Y. A., Taip, F. S., & Abdullah, J. (2015). Characterisation of improved foam aeration and rheological properties of ultrasonically treated whey protein suspension. *International Dairy Journal, 43*, 7–14. https://doi.org/10.1016/j.idairyj.2014.09.013.
Tegge, G. (1984). *Stärke und Stärkederivate.* Hamburg: Behr's Verlag.
Tester, R. F., Patel, T., & Harding, S. E. (2006). Damaged starch characterisation by ultracentrifugation. *Carbohydrate Research, 341*(1), 130–137. https://doi.org/10.1016/j.carres.2005.10.018.
Tharanathan, R. N. (2005). Starch ? Value addition by modification. *Critical Reviews in Food Science and Nutrition, 45*(5), 371–384. https://doi.org/10.1080/10408390590967702.
Tian, J.-Z., Yao, Y., Shen, S.-S., Zheng, X.-L., Liu, C., & Han, X.-X. (2015). Twin-screw extrusion of rice flour without a die: Effect of barrel temperature and screw speed on extrusion and extrudate characteristics. *Journal of Henan University of Technology (Natural Science Edition, 04*.
Tran, T. T. B., Shelat, K. J., Tang, D., Li, E., Gilbert, R. G., & Hasjim, J. (2011). Milling of rice grains. The degradation on three structural levels of starch in rice flour can be independently controlled during grinding. *Journal of Agricultural and Food Chemistry, 59*(8), 3964–3973. https://doi.org/10.1021/jf105021r.
Varavinit, S., Shobsngob, S., Varanyanond, W., Chinachoti, P., & Naivikul, O. (2003). Effect of amylose content on gelatinization, retrogradation and pasting properties of flours from different cultivars of Thai rice. *Starch - Stärke, 55*(9), 410–415. https://doi.org/10.1002/star.200300185.
Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition, 42*(3), 179–208. https://doi.org/10.1080/10408690290825510.
Verheyen, C., Albrecht, A., Elgeti, D., Jekle, M., & Becker, T. (2015). Impact of gas formation kinetics on dough development and bread quality. *Food Research International, 76*, 860–866. https://doi.org/10.1016/j.foodres.2015.08.013.
Verheyen, C., Jekle, M., & Becker, T. (2014). Effects of Saccharomyces cerevisiae on the structural kinetics of wheat dough during fermentation. *LWT - Food Science and Technology, 58*(1), 194–202. https://doi.org/10.1016/j.lwt.2014.02.050.
Visireddy, H., Rayas-Duarte, P., Mcglynn, W., Bowser, T., & Payton, M. E. (2008). *Dough fermentation properties as a function of physical and chemical changes.* Retrieved from https://shareok.org/bitstream/handle/11244/8885/Visireddy_okstate_0664M_11662.pdf?sequence=1.
Wang, S., Yu, J., Xin, Q., Wang, S., & Copeland, L. (2017). Effects of starch damage and yeast fermentation on acrylamide formation in bread. *Food Control, 73*, 230–236. https://doi.org/10.1016/J.FOODCONT.2016.08.002.
Wang, J.-S., Zhao, M.-M., Yang, X.-Q., Jiang, Y.-M., & Chun, C. (2007). Gelation behavior of wheat gluten by heat treatment followed by transglutaminase cross-linking reaction. *Food Hydrocolloids, 21*(2), 174–179. https://doi.org/10.1016/j.foodhyd.2006.03.006.
Wani, A. A., Singh, P., Shah, M. A., Schweiggert-Weisz, U., Gul, K., & Wani, I. A. (2012). Rice starch diversity: Effects on structural, morphological, thermal, and physicochemical properties-A review. *Comprehensive Reviews in Food Science and Food Safety, 11*(5), 417–436. https://doi.org/10.1111/j.1541-4337.2012.00193.x.
Wani, I. A., Sogi, D. S., Wani, A. A., Gill, B. S., & Shivhare, U. S. (2010). Physico-chemical properties of starches from Indian kidney bean (Phaseolus vulgaris) cultivars. *International Journal of Food Science & Technology, 45*(10), 2176–2185. https://doi.org/10.1111/j.1365-2621.2010.02379.x.
Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*(2), 115–119. https://doi.org/10.1016/j.fm.2006.07.004.
Wilderjans, E., Pareyt, B., Goesaert, H., Brijs, K., & Delcour, J. A. (2008). The role of gluten in a pound cake system: A model approach based on gluten–starch blends. *Food Chemistry, 110*(4), 909–915. https://doi.org/10.1016/j.foodchem.2008.02.079.
Wilpiszewska, K., & Spychaj, T. (2007). Chemical modification of starch with hexamethylene diisocyanate derivatives. *Carbohydrate Polymers, 70*(3), 334–340. https://doi.org/10.1016/j.carbpol.2007.04.023.
Wolf, B. (2010). Polysaccharide functionality through extrusion processing. *Current Opinion in Colloid & Interface Science, 15*(1–2), 50–54. https://doi.org/10.1016/j.cocis.2009.11.011.
Zeng, J., Li, G., Gao, H., & Ru, Z. (2011). Comparison of A and B starch granules from three wheat varieties. *Molecules, 16*(12), 10570–10591. https://doi.org/10.3390/molecules161210570.
Zhang, K., Lu, Q., Colonna, P., Valle, G. Della, Lourdin, D., & Zhou, H. (2017). Physicochemical properties of A- and B-type granules of wheat starch and effects on the quality of wheat-based noodle. *International Journal of Food Engineering, 13*(7), 161–169. https://doi.org/10.1515/ijfe-2016-0437.
Zia-ud-Din, Xiong, H., & Fei, P. (2017). Physical and chemical modification of starches: A review. *Critical Reviews in Food Science and Nutrition, 57*(12), 2691–2705. https://doi.org/10.1080/10408398.2015.1087379.

2.3 Direct link between specific structural levels of starch and hydration properties

Carbohydrate Polymers 181 (2018) 159–166

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Research paper

Direct link between specific structural levels of starch and hydration properties

Sabina Jakobi, Mario Jekle*, Thomas Becker

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

ARTICLE INFO

Keywords:
starch modification
flour functionality
water retention capacity
grinding
damaged starch
milling

ABSTRACT

Enhanced water retention capacity (WRC) of physically modified flours is often attributed to a particle size reduction, neglecting potential influences of altered amylose/amylopectin ratio and crystallinity of starch. Therefore, this study aims to investigate the impact of altered amylose/amylopectin ratio and crystallinity on WRC by modifying wheat flour using a cryogenic (CG) and an ultra-centrifugal grinder (UCG). With increased grinding time in CG and rotation speed in UCG, WRC rose linearly. Neither for CG nor for UCG modified flours a linear dependency of amylose/amylopectin ratio on WRC was detected. Additionally, no linear relation between crystallinity and WRC of UCG modified flours was determined ($R^2 = 0.03$). For both modifying methods, however, linear dependency of WRC and starch modification degree was found. Through this systemic approach it was proven that WRC of modified flours can exclusively be attributed to alterations of surface properties.

1. Introduction

During grinding, wheat grain (*Triticum aestivum* L.) is exposed to high mechanical and thermal forces affecting structural characteristic of cereal biopolymers. Especially changes in starch are known amongst others to be responsible for an increase in water binding capacity altering dough processing properties (Hackenberg, Verheyen, Jekle, & Becker, 2016; Majzoobi et al., 2011; Zhang, Ding, Ndeurumi, Wang, & Feng, 2015) and bread texture and sensory.

SMD – starch modification degree (formerly known as starch damage) – is a key value for the grinding industry analyzing mechanically modified flours (Ali et al., 2014; León, Barrera, Pérez, Ribotta, & Rosell, 2006; Lin & Czuchajowska, 1996; Rakszegi et al., 2010). Frequently used methods determining SMD are the rapid, amperometric detection (AACC 76-33.01) based on the enhanced iodine absorption of modified starches and the enzymatic method (AACC 76-30.02) analyzing the amount of enzymatically hydrolysable starch by fungal amylase. Especially enzymatically determined SMD correlates well with water retention capacity (Ali et al., 2014). It can therefore be concluded that modification of starch lead to improved accessibility for amylases and as well for water.

Although several studies were performed in starch modification, changes in amylose/amylopectin ratio (singular structure) and crystallinity (lamellar structure) of mechanically modified starches resulting in rise of water binding capacity of flours are still insufficiently understood. To achieve a better understanding for enhanced water retention capacity, complex starch structure has to be taken into consideration. Tran et al. classified 2011 starch into six structural levels. A modified schematic illustration is presented in Fig. 1, with accompanying determination methods for each structural level. Starch modification degree (SMD) could occur on each structural level. Several studies displayed a decline in gelatinization onset temperature and enthalpy by mechanical treatment of flours by altering crystalline structures (level 3 and 4) (Dhital, Shrestha, Flanagan, Hasjim, & Gidley, 2011; Hasjim, Li, & Dhital, 2013; Homer, Kelly, & Day, 2014; López, Zaritzky, & García, 2010; Morrison, Tester, & Gidley, 1994). Decrease in amylopectin content after mechanical treatment permits to draw indirect conclusions about destruction of crystallinity since crystalline lamellae are formed by amylopectin (Diop, Li, Chen, & Xie, 2012).

In general rise in WRC is attributed to an increase in specific surface area of starch caused by a reduction in flour particle size (Barak, Mudgil, & Khatkar, 2014), the formation of micro cracks (El-Porai, Salama, Sharaf, Hegazy, & Gadallah, 2013) or the removal of an outer protein and lipid layer of the granules (Debet & Gidley, 2006; Kulp and Ponte, 2000). However, linear correlations between WRC and changes on amylose/amylopectin ratio (singular) and crystallinity (lamellar) of

Abbreviations: WRC, water retention capacity; CG, cryogenic grinder; UCG, ultra-centrifugal grinder; SMD, starch modification degree; PSD, particle size distribution; SEM, scanning electron microscopy; AFM, atomic force microscopy; TEM, transmission electron microscopy; DSC, differential-scanning-calorimetry; TMA, thermomechanical analysis; FFF-MALLS, field-flow-fractionation with MALLS detection; HPAEC-ED, high-performance anion exchange chromatography with electron detector; ΔH, gelatinization enthalpy

* Corresponding author.
E-mail addresses: mjekle@tum.de, info@jekle.de (M. Jekle).

http://dx.doi.org/10.1016/j.carbpol.2017.10.062
Received 13 June 2017; Received in revised form 13 October 2017; Accepted 18 October 2017
Available online 19 October 2017
0144-8617/

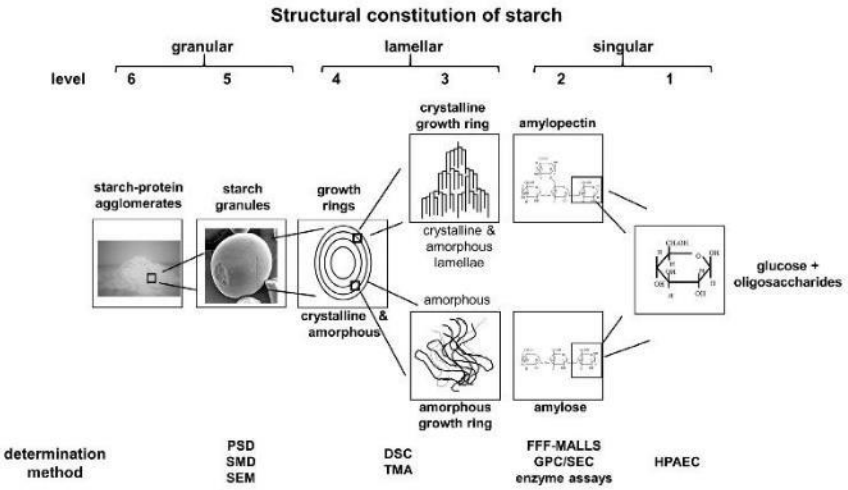

Fig. 1. Overview of the structural constitution of starches and methods determining mechanical modification. PSD = particle size distribution by light scattering, SMD = starch modification degree, SEM = scanning electron microscopy, AFM = atomic force microscopy, TEM = transmission electron microscopy, DSC = differential-scanning-calorimetry, TMA = thermomechanical analysis (Homer et al., 2014), FFF-MALLS = field-flow-fractionation with MALLS detection, GPC/SEC = gel permeation chromatography (GPC) and size exclusion chromatography (SEC); Enzyme assay = amylose/amylopectin assay kit by Megazyme, HPAEC = high performance anion exchange chromatography. Determination methods PSD, SMD, DSC, enzyme assays and HPAEC were used in the current study.

physically modified flours were also monitored. Focusing on modification of singular and lamellar starch structure and skipping analysis of altered granule morphology after mechanical modification can result in a mistaken correlation of lower structural levels with functional properties of the starch. Nonetheless, to the best of our knowledge, it couldn't be proven until know that modifications of starch on singular or lamellar scale are independent from the hydration properties of starch.

To evidence clear allocation of rise in WRC to changes in starch morphology (level 5), specific grinders were used to cause a variety of variations on different structural levels of starch. The ultra-centrifugal grinder is known to have a major effect on particle size distribution (Moreira, Chenlo, Arufe, & Rubinos, 2015) caused by impact and shear forces. Beside the mechanically induced flour alterations, modifications could occur due to heat development during grinding. To exclude heat denaturation of proteins or starch, a cryogenic grinder was used to apply merely mechanical changes of starch structures and hydration properties.

This approach provides the opportunity to create various alterations of starch on singular and lamellar scale with similar hydration properties. Thus, causal relations between starch constitution and hydration properties of flours should be available.

2. Experimental

2.1. Raw materials

Pure variety wheat grain 'Akteur' was donated by Kunstmühle (Buchloe, Germany) and grinded in a roller mill and sifted by Rosenmühle GmbH (Ergolding, Germany) to achieve the origin flour. Moisture content of the origin and processed flours were evaluated according to the approved method of the AACC 44-40 (2000). Protein content was 10.55 ± 0.03% db flours (according to Kjeldahl method AACC 46-12.01, conversion factor 5.7), ash content 0.67 ± 0.02 g/100 g (AACC 08-12), and total starch content 77.66 ± 0.78 g/100 g db (determined with 'Total starch enzyme kit' by Megazyme International Ireland Ltd., Wicklow, Ireland)

2.2. Grinding processes

The aim of the sample preparation step was to generate a broad range of mechanically modified samples but avoiding raw material fluctuations. Wheat flour was cryogenically ground using cryogenic grinder with 12 mm ceramic grinding ball (Retsch, Haan, Germany). The samples (3.0 ± 0.5 g) undergo a 5 min precooling step in the grinding jar (20 mL), followed by grinding step with varied grinding times of 0, 1, 5, 10, 20 min. A frequency of the steel impactor of 25 $\frac{1}{s}$ during the main grinding and 5 $\frac{1}{s}$ during the precooling was applied. Temperature during grinding was constantly under −180 °C. In the ultra-centrifugal grinder ZM 200 using 250 µm and 500 µm sieve (Retsch, Haan, Germany) flour modification was generated by the exposure of the flour to high shear and impact forces. Flour temperature after grinding did not exceed 70 °C. The modified flours were hermetically sealed and stored at 20 °C ± 2 °C in the dark until the following analysis. All grinding procedures were performed at least three times to achieve triplicates (n ≥ 3) and analyzed according to the following methods.

2.3. Particle size analysis

Grinding procedures were evaluated by the determination of the particle size distribution before and after subsequent grinding of the flour (Table 1) by static light scattering using Mastersizer 3000 (Aero S Unit, Malvern Instruments Ltd, Worcestershire, UK). The dry dispersion unit enabled the measurement of modified flours excluding a particle hydration. To calculate the particle size, measurement principle 'Mie Theory' with a refractive index of 1.54 and the general purpose mode was used. The obscuration for the measurements ranged between 2-8%. All measurements were performed in triplicates.

2.4. Glucose, maltose and maltotriose content

Glucose and maltose content was determined using HPAEC-ED (high-performance anion exchange chromatography with electron detector, Dionex, Germering, Germany). Flours were dissolved in a methanol-water (1:1) solution (1 g/8 mL solvent), filtered (0.45 µm syringe filters) and stored at −18 °C ± 1 °C before measurement. Calibration was performed once per week.

2.5. Relative amylose and amylopectin content

Relative amylose content was determined by amylose/amylopectin assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland).

Table 1
Particle size distribution (PSD) of cryogenic (0–20 min) and ultra-centrifugal grinded (0–18000 rpm; 250 µm or 500 µm mesh screen) wheat flours represented by D-values for volume distribution.

Cryogenic grinding	$D_{3,10}$ (µm)	$D_{3,50}$ (µm)	$D_{3,90}$ (µm)
0 min	23.5 ± 0.1	87.5 ± 0.8	180 ± 5.3
1 min	11.3 ± 0.1	53.7 ± 0.6	135 ± 4.9
5 min	10.8 ± 0.3	47.9 ± 2.8	115 ± 5.3
10 min	7.2 ± 0.1	39.6 ± 0.4	114 ± 2.7
15 min	3.6 ± 0.1	20.8 ± 1.2	85.9 ± 7.7
20 min	3.4 ± 0.1	18.9 ± 0.5	72.7 ± 7.3
Ultra-centrifugal grinding	$D_{3,10}$ (µm)	$D_{3,50}$ (µm)	$D_{3,90}$ (µm)
250 µm, 0 rpm	23.5 ± 0.1	87.5 ± 0.8	180 ± 5.3
250 µm, 6000 rpm	15.5 ± 0.3	65.7 ± 2.3	163 ± 3.6
250 µm, 12000 rpm	10.6 ± 0.2	36.8 ± 1.6	106 ± 3.4
250 µm, 18000 rpm	8.5 ± 0.7	26.4 ± 1.17	74.5 ± 3.4
500 µm, 6000 rpm	16.9 ± 0.4	69.3 ± 1.9	170 ± 5.0
500 µm, 12000 rpm	15.2 ± 0.2	63.2 ± 1.2	158 ± 1.4
500 µm, 18000 rpm	13.0 ± 0.7	52.3 ± 3.3	142 ± 7.2

$D_{3,10}$, $D_{3,50}$, $D_{3,90}$: Intercepts for 10%, 50%, 90% of the cumulative volume of the flour sample, respectively.

Thereby, amylose is solved in concanavalin A (ConA), whereby the insoluble amylopectin builds a precipitate with ConA. The supernatant containing amylose is hydrolyzed to glucose, which was detected according total starch method.

2.6. Crystallinity

Crystalline properties of flours were measured indirectly using a differential-scanning-calorimeter (DSC) (Pyris Diamond, Perkin Elmer, Waltham, USA) equipped with an Intracooler 2P cooling system. The DSC was calibrated with indium and n-decan for a temperature range from −30 to +150 °C. For measuring gelatinization enthalpy, aluminum pans were filled with 20–35 mg samples (flour: water ratio = 1:3) and hermetically sealed. Samples were hold for 2 min at 25 °C, then run at 10 K/min to 95 °C in a nitrogen atmosphere. The enthalpy (ΔH) of starch gelatinization was calculated from the area of the peak endotherm referred to the dry mass of the suspension.

2.7. Starch modification degree (SMD)

Starch modification degree was determined in accordance with the approved method AACC 76-31 using an enzyme assay kit (Starch damage, Megazyme International Ltd., Ireland). The method determines the percentage of starch granules (on 14% moisture basis) in flour able to be hydrolyzed by fungal alpha-amylase. SMD was calculated with the following equation:

$$SMD = \Delta E * F * 60 * (1/1000) * (100/W) * (162/180) \quad (1)$$

with SMD = starch modification degree (%), ΔE = absorbance (reaction) read against the reagent blank, F = (150 (µg of glucose))/(absorbance of 150 µg of glucose), W = sample weight (mg)

2.8. Water retention capacity

The water retention capacity (WRC) of the samples was determined in accordance with the approved method of the AACC 56–11.02 for ultra-centrifugal grinded wheat flour samples. For cryogenically grinded flours, WRC method had to be modified due to very small amount of flour samples. Weight of flour samples was reduced from 5.00 g ± 0.05 g to 1 g ± 0.01 g and corresponding water addition from 25.00 g ± 0.05 g to 5.00 g ± 0.01 g. WRC was calculated as the weight of the water hold by wheat flour samples after 10 min of centrifugation at 1000 g.

2.9. Statistical analysis

The statistical analysis was performed with JMP® Pro (Version 12.2.0, JMP Software, © SAS Institute Inc). To estimate the proportion of variation in the response, which can be traced to the model, coefficient of multiple determination (R^2) is calculated as (sum of squares (model))/(sum of squares (total)).

3. Results and Discussion

3.1. Particle size reduction of modified flours

Two different grinder types were used to generate a high variance of singular and lamellar modifications. In ultra-centrifugal grinder (UCG) modification of flour can occur mechanically by *impact* and *shear forces* and potentially simultaneous by heat induced changes of starch and protein. During cryogenic grinding (CG) heat damages of the flour are excluded by using liquid nitrogen. The mechanical modification of flours is caused by *impact* and *attrition forces*. Changes in the particle size distribution due to the grinding procedure are demonstrated in Table 1.

Subsequent grinding of the wheat flour resulted in a constant decrease in particle size with increase in grinding time for cryogenic grinding and rise in rotation speed for ultra-centrifugal grinder. Grinding for 20 min in cryogenic grinder resulted a a reduction of particle size from $D_{3,50}$ 87.5 ± 0.8 µm to $D_{3,50}$ 18.9 ± 0.5 µm (20 min grinding). In ultra-centrifugal grinder, maximum reduction at highest rotation speed (18000 rpm) lead to $D_{3,50}$ values of 26.4 ± 1.17 µm and 52.3 ± 3.3 µm for 250 µm and 500 µm sieve, respectively. The more effective reduction in particle size during CG modification can be due to embrittlement in cryogenic grinder causing an easier disruption of flour agglomerates.

3.2. Effects of grinder type and grinding parameters on water retention capacity (WRC)

The amount of water held by the modified flours after centrifugation and drainage (WRC) increased with the severity of the grinding treatment (Fig. 2).

Ultra-centrifugal with 250 µm and 500 µm sieve and cryogenic grinding leads to a linear rise in water retention capacity of the reference flours (R^2 = 0.93; R^2 = 0.69; R^2 = 0.93 respectively). Consequently both grinding methods are suitable to cause structural modification of cereals (Fan; Hsu; Kobayashi, 1976; Fox, Visser, Skov, Meijering, & Manley, 2014) and changes in the hydration properties.

However, the water retention capacity of CG (for 20 min) and UCG (12000 rpm, 250 µm) modified flour were comparable (WRC ~ 77–78%) (Fig. 2), although CG modification caused significantly lower PSD than UCG: CG modification for 20 min caused a reduction of the medium volume intercept ($D_{3,50}$) to 18.9 ± 0.5 µm, whereby UCG modification (250 µm, 12000 rpm) decreased $D_{3,50}$ to 36.8 ± 1.6 µm. This implies a dependency of the hydration properties of modified flours on other starch structures, then the particle size. This fact made both grinding procedures suitable for the examination of the influence of starch structure modification on the hydration properties and highlights the importance of the starch structure analysis to elucidates the causes for raised water retention capacity.

To examine if changes in molecular structure of starch, more precisely in the covalent constitution of singular starch chains give rise to increasing WRC of modified flours, formation of low-molecular weight fragments (glucose, maltose) and fragmentation of amyloase and amylopectin was analysed.

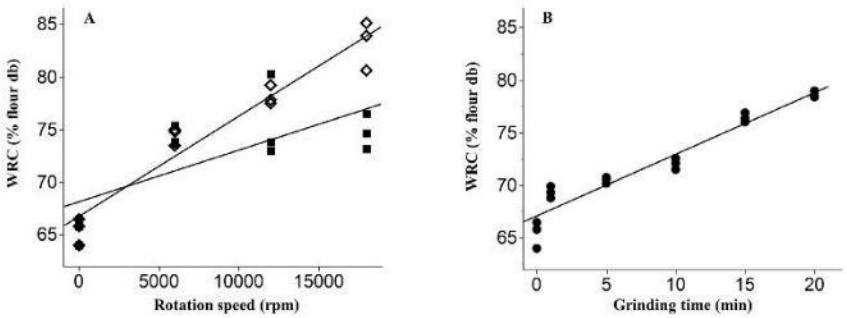

Fig. 2. Influence of grinding type and parameters on water retention capacity of the flours (A) ultra-centrifugal grinded (◊ 250 µm mesh screen ■ 500 µm mesh screen) and (B) cryogenic grinded.

3.3. Interrelation between maltose content and flour hydration

High-performance anion exchange chromatography was used to analyze relations between the formation of low molecular weight α-glucans and rise in water retention capacity of flours. Glucose and maltotriose content didn't increase signifacntly with enhanced rotations speed or grinding time (data not shown). Maltose content rised by grinding of the flour (Fig. 3A, B), however, for both grinders no linear interrelation of maltose content and WRC was found (R^2 = 0.38; R^2 = 0.08 respectively). The formation of maltose can either be explained by an enhanced endogenous β-amylases activity or by the mechanical splitting of covalent bonds.

The former generally appears when substrate availability and/or temperature increases. The temperature rose during ultra-centrifugal grinding up to 70 °C using 250 µm mesh screen at 18000 rpm. In general, temperature rise up to temperature maximum result in an enhanced enzyme activity (Collins, Meuwis, Gerday, & Feller, 2003), in this case possibly β-amylases activity, and consequently maltose formation. But additionally other studies revealed, that a threshold of 27% moisture content must be exceeded in grains in order that enzymatic hydrolysis by amylases can occur (Drapron, 1985). Although an enzymatic driven formation of maltose is exluded for cryogenic grinding due to the low temperatures and the reduced amount of unfrozen water, rise in maltose was still detected after CG modification.

However, a mechanically induced preferred formation of maltose still seems to be unlikely from an energetic point of view. C–C bonds have a lower bonding energy in comparison to C–O and therefore cleavage of C–C should be favored. Preferred formation of maltose seems improbable. Due to several interaction of steric effects and chemical/ionic bounds in high moleculare weight molecules as amylose or amylopectin, reliable forecast of fraction points are hardly possible. Independently from the formation mechanism of maltose, a poor correlation of maltose and WRC demonstrate, that maltose formation is a side effect of grinding and didn't evoke rise in WRC itself.

Due to steric effects, a prefered fragmentation of the inner parts of starch chains of amylopectin, resulting in the formation of high molecular weight glucans, is expected. This would lead to an increased relative amylose content based on total starch content (level 2). Degradation of amylopectin would also result in the destruction of crystalline areas of starch (level 3 and 4), which, in turn could boost water accessibility into flour particles especially starch granules.

3.4. Interrelation between amylose/amylopectin ratio, crystallinity and flour hydration

Relative amylose content provides substantial information about the amylose/amylopectin ratio and therefore the degradation mechanisms of the starch biopolymers amylose and amylopectin.

For cryogenic grinding, with prolonged grinding time an increase of relative amylose content from 24.60 ± 0.66% to 28.86 ± 0.91% and a linear reduction in gelatinization enthalpy with prolonged grinding time up to 20 min (R^2 = 0.725) was noticed (data not shown). For cryogenically grinded flours, WRC showed only a poor linear correlation (R^2 = 0.394) to amylose/amylopectin ratio (Fig. 4, B), however percentage reduction in enthalpy showed a high linear correlation (R^2 = 0.841) with water retention capacity of the modified flours (Fig. 4, B). Both factors – reduction in enthalpy and increased amylose/amylopectin ratio – indicate a destruction of amylopectin.

Thereby amylopectin can be degraded to amylose-like fragments (α-1,4 glyosidic bonds) or to non-amylose, low molecular weight dextrin. Cleavage of amylopectin side chains would generate amylose-like fragments leading to a multiplied increase in amylose/amylopectin ratio, which impeded the elucidation of linear effect of amylopectin degradation and WRC. It is known from literature that starches with high amylopectin proportions exhibit high water retention capacities (Schirmer, Höchstötter, Jekle, Arendt, & Becker, 2013). Equally to maltose formation, percentage rise in relative amylose and percentage reduction in enthalpy is regarded as side effect of the grinding procedure.

Thereby, gelatinization enthalpy is determined as the energy uptake

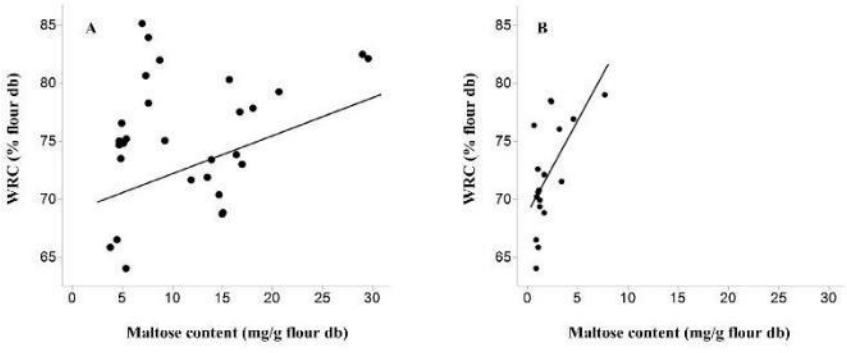

Fig. 3. Interrelation between maltose content and water retention capacity of the flours grinded by (A) ultra-centrifugal grinder (250 µm and 500 µm mesh screen) and (B) cryogenic grinder.

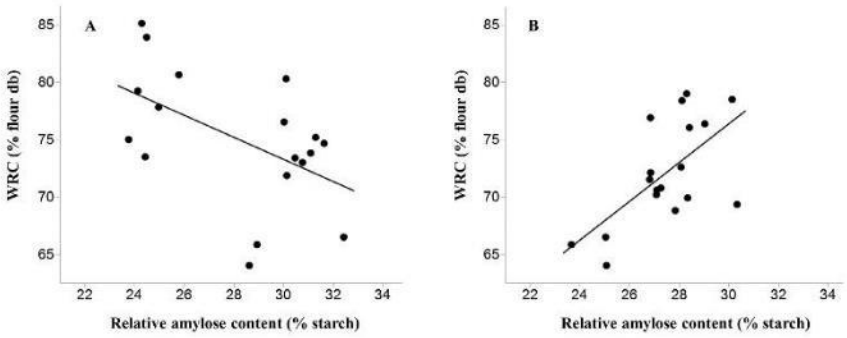

Fig. 4. Interrelation between relative amylose content and water retention capacity of flours grinded by (A) ultra-centrifugal grinder (250 µm and 500 µm mesh screen) and (B) cryogenic grinder.

of starch needed to go under a thermal transition (Cooper, 2000) and is dependent upon molecular and crystalline order of starch (Cooke & Gidley, 1992). Crystalline growth rings (level 4) are built up of crystalline and amorphous lamella (level 3) consisting predominantly of amylopectin (see introduction). Mechanical treatments of flours, especially in combination with low temperatures as occurring during cryogenic grinding, are known to cause a reduction in the crystallinity grade (Barrera, Leon, & Ribotta, 2011) leading to a decrease in gelatinization enthalpy (Dhital et al., 2011). Cause both grinding processes took place under glass transition temperature of starch, thus during the glassy state of starch, comparability of both grinding procedures is ensured (Zeleznak and Hoseney, 1987). Additionally, Dhital et al. measured a greater impact of cryogenic grinding on amylopectin than on amylose (Dhital et al., 2011). This is consistent with the measurement of the amylose/amylopectin ratio in this study. Increase in amylose/amylopectin ratio can occur by the primary destruction of amylopectin leading to a relative rise in amylose. This effect is strengthened when amylopectin degradation provokes the formation of fragments of the size of amylose resulting in a further climb in relative amylose content. An enhanced amylopectin destruction is expected since crystalline materials display generally a higher brittleness than amorphous materials (Dhital, Shrestha, & Gidley, 2010). Furthermore, high molecular weight and branching degree of amylopectin result in a high rigidity of the polymer and in turn favor a mechanical degradation of amylopectin for steric reasons (Dziedzic and Kearsley, 1995; Han, Campanella, Mix, & Hamaker, 2002; Li, Dhital, & Hasjim, 2014). Although rise in amylose/amylopectin ratio and decrease in gelatinization enthalpy are interrelated, solely a poor correlation of both parameters was noticed (R^2 = 0.482 date not shown) indicating that a loss in ordered structures doesn't need to be compulsory associated with a molecular degradation of starch biopolymers. Further research is necessary to explore the cryogenically forced breaking reactions of starch polymers to understand in detail the molecular modification mechanisms. For ultra-centrifugal grinded flours, only poor correlation of WRC and amylose content and furthermore of WRC and relative decrease of enthalpy was found (R^2 = 0.272, R^2 = 0.032, respectively) (Figs. 4 A; 5 A). For UCG modification, gelatinization enthalpy decreased in the same extent as by cryogenic grinding (up to 80%), however, WRC rise was much less pronounced (~5 instead of ~20%) after UCG treatment. Differences in the reduction of the gelatinization enthalpy and the increase in WRC between CG and UCG modified samples could be attributed to variance in heat exposure in both grinders. Qin et al. and Sun, Gong, Li, & Xiong established a decrease in gelatinization enthalpy after dry-heat treatments of starch (Qin et al., 2016; Sun et al., 2014). These findings permit the conclusion, that thermal effects can affect the structural constitution of starch and if so, thermally induced alterations of starch/flour differ from mechanically induced ones. Further research should focus on development of methods to distinguish solely mechanical, combined mechanical-thermal and solely thermal structural starch modifications and consequently functionality during grinding.

It is known from literature, that starch pasting leads to a reduction in crystallinity and simultaneously eases water penetration into the granules and enhances water absorption of starch (Park, Ibáñez, Zhong, & Shoemaker, 2007). Although grinding and pasting result in the reduction of crystalline areas of starch, both starch modifications are barely comparable cause grinding leads to an increase of amorphous state amongst other things by degradation of starch polymers at low water content and without heat exposure. To what extent reduced crystallinity itself enhances the water retention of flours, has to be determined in further studies.

However, as no causal relation of level 3 or 4 (relative amylose or gelatinization enthalpy) and water retention capacity was found in the analysis so far, focus was redirected on the surface properties and accessibility of flour agglomerate.

3.5. Interrelation between starch modification degree and flour hydration

A common parameter for the determination of occurring alterations of flours during grinding is the starch modification degree, which is based on the accessibility of starch for enzymatic hydrolysis. Thereby, modified starch is subjected to an amylolytic degradation of a defined period and subsequently formation of glucose is quantified. The higher

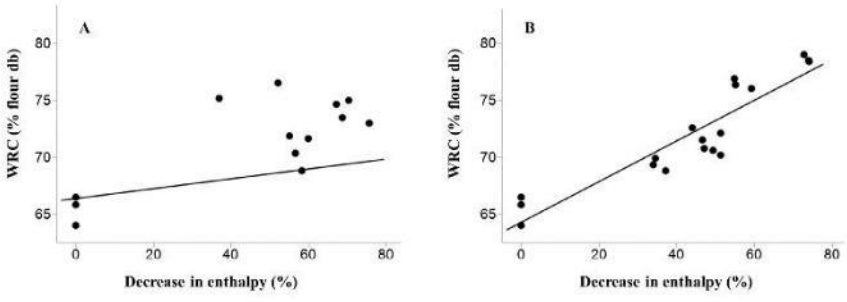

Fig. 5. Interrelation between percentage change in gelatinization enthalpy and water retention capacity of the flours grinded by (A) ultra-centrifugal grinder (250 µm and 500 µm mesh screen) and (B) cryogenic grinder.

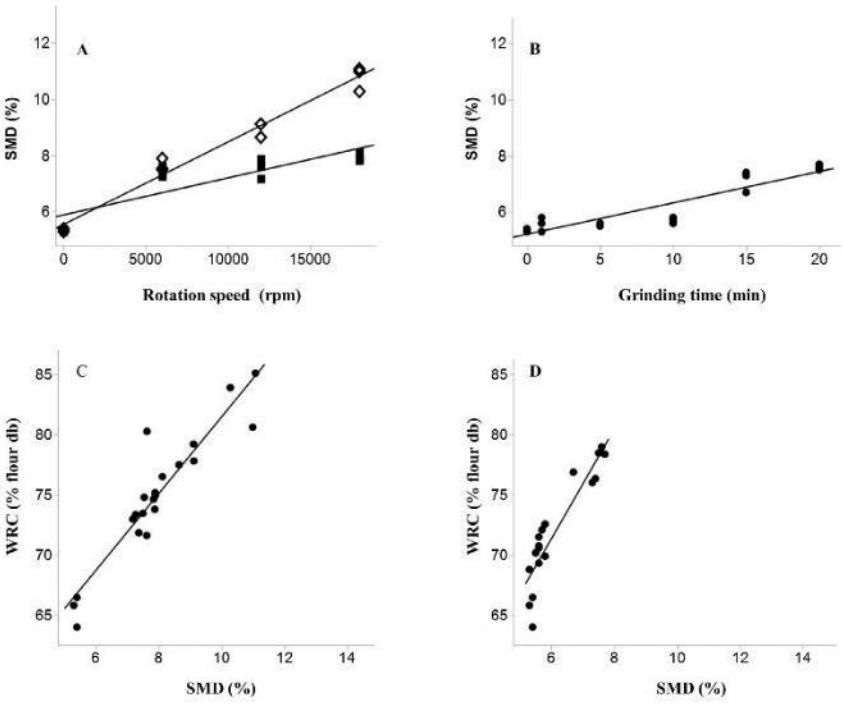

Fig. 6. Influence of grinding type and parameters on starch modification degree: A ultra-centrifugal grinded (◊ 250 µm mesh screen ■ 500 µm mesh screen); B cryogenic grinded and resulting impact on WRC: C ultra-centrifugal grinded (250 µm and 500 µm mesh screen); D cryogenic grinded flours.

the accessibility of flour particles is, the more glucose is formed and the higher the starch modification degree is.

Water retention capacity and mechanical starch modification degree rose significantly with enhanced grinding speed for UCG and grinding time for CG (compare Figs. 2 A/B and 6 A/B). Furthermore, WRC showed a high correlation to the starch modification degree for UCG and CG (R^2 = 0.865, R^2 = 0.834, respectively) as illustrated in Fig. 6C/D.

A relation of starch modification degree with water retention capacity, swelling of modified starch granules as well as cold-water soluble starch was also observed in other studies (Ali et al., 2014; Hackenberg et al., 2016; Hasjim et al., 2012; Tester & Morrison, 1994). High linear correlation between SMD and WRC is explainable due to the same underlying mechanisms of both analysis methods. WRC as well as SMD highly depend on the accessibility of starch either for water or for aqueous enzyme solutions. Subsequent grinding of wheat flour results in a rise in surface area of starch by the formation of micro cracks, particle size reduction and/or removal of surface proteins and lipids leading to a better accessibility of starch particle for hydration and/or enzymatic hydrolysis. The exact cause of rise in surface area is not definitely known or specific relationships identified yet. Further investigations have to be performed to elucidate altered surface characteristic of physically modified flours and to be able to control hydration properties during grinding.

By comparing the mechanically induced modifications on different structural levels by both grinding methods, it could be shown by exclusion procedures, that rise in WRC is not due to changes in molecular constitution of starch chains or starch crystallinity, but due to changes surface properties. The following table summarizes the new findings.

3.6. Merger of both mills and modifications on level 1 to 4

Wheat flours treated by different mechanical and thermal stresses in UCG and CG showed modification on all five analyzed structural levels of starch. To prove a (causal) relation, an existing high correlation of structural level and rise in WRC for both grinding methods is required (Table 2).

Table 2
Simultaneous, multivariate analysis of structural levels and water retention capacity for cryogenic and ultra-centrifugal grinded flours.

		coefficient of determination R^2		
Structural level	Correlation of functionality and starch structures	Simultaneous UCG and CG	UCG	CG
1	WRC (%)–maltose (mg/g)	0.164	0.076	0.384
2	WRC (%)– relative amylose content (%)	0.016	0.272	0.394
3-4	WRC (%)– decrease in relative enthalpy (%)	0.640	0.032	0.841
5	WRC (%)–SMD (%)	0.771	0.865	0.834

For UCG and CG modified flours, no linear correlation for maltose content or relative amylose content and WRC was detected. Although for cryogenic grinding a linear correlation of WRC and decrease in gelatinization enthalpy was noticed, this correlation could not be proven for UCG modified flours, respectively. Since one type of grinder doesn't confirm a dependency of WRC and reduction gelatinization enthalpy, changes in starch crystallinity don't cause rise in WRC, rather the modification of lamellar starch structure and as well singular structure is just a side effect. Therefore, relationships are misleading when used for interpretation of rise in hydration properties.

Simultaneously performed analysis of WRC and SMD for both

grinders showed a high correlation ($R^2 = 0.771$). This indicates that rise in WRC during grinding is due to a physical modification of the granular structure of starch or flour particles. As displayed by the D-values of the non-modified and modified flours, particle size significantly differs from the grinder type and grinding parameters. Particularly, distinctions of cryogenic and ultra-centrifugal grinded flours with comparable particle distribution and various hydration characteristics introduces a highly interesting field of research. The new insights of this study, that structure levels 1–4 (see Fig. 1) have solely a minor impact on flour hydration, in combination with the findings, that hydration properties do not solely depend on the particle size of the flour filling, provide the basis for a targeted flour modification. Further research should therefore focus on examining changes in surface properties of modified starches.

4. Conclusion

Physical treatments of flour cause complex structural modifications of the starch constitution and consequently starch functionality, as the water retention capacity (WRC) of flours. Without the possibility to influence specifically individual levels of starch, the determination of causal correlations of structural starch modification and altered flour functionality is challenging. In this study, two different grinding systems were used to create diverse structural modifications of starch to deduce similarities in functional alteration between individual starch level and hydration properties.

For subsequent ultra-centrifugal grinded flours, dependencies of WRC were neither visible on maltose content (level 1), amylose/amylopectin ratio (level 2) nor on the reduction in gelatinization enthalpy (indications on level 3 and 4). Although subsequent cryogenic grinded flours showed a linear correlation of gelatinization enthalpy and WRC, an evoked rise of WRC by the transformation of crystalline parts of starch into amorphous parts during grinding can be excluded.

Future work will be directed to assess the mechanisms of enhanced WRC, which significantly influences dough properties and yeast fermentation during leavening and baking. While many studies examined modification of flour or starch by different grinders, the lack in mechanistic connections of starch structure and resulting functionality remain unaffected.

Acknowledgements

This IGF Project of the FEI is supported via AiF (18679 N) within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

References

Ali, R., Khan, M. S., Sayeed, S. A., Ahmed, R., Saeed, G., & Mobin, L. (2014). Relationship of damaged starch with some physicochemical parameters in assessment of wheat flour quality. *Pakistan Journal of Botany, 46*(6), 2217–2225.

Barak, S., Mudgil, D., & Khatkar, B. S. (2014). Effect of flour particle size and damaged starch on the quality of cookies. *Journal of Food Science and Technology, 51*(7), 1342–1348. http://dx.doi.org/10.1007/s13197-012-0627-x.

Barrera, G. N., Leon, A. E., & Ribotta, P. D. (2011). Effects of damaged starch on wheat starch properties. *6th International CIGR Technical Symposium – Towards a Sustainable Food Chain: Food Process, Bioprocessing and Food Quality Management*.

Collins, T., Meuwis, M.-A., Gerday, C., & Feller, G. (2003). Activity, Stability and Flexibility in Glycosidases Adapted to Extreme Thermal Environments. *Journal of Molecular Biology, 328*(2), 419–428. http://dx.doi.org/10.1016/S0022-2836(03)00287-0.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: Origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112. http://dx.doi.org/10.1016/0008-6215(92)85063-6.

Cooper, A. (2000). *Differential scanning microcalorimetry. In Protein-ligand interactions: Hydrodynamics and Calorimetry: A Practical Approach.* Oxford University Press287–318.

Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers, 64*(3), 452–465. http://dx.doi.org/10.1016/j.carbpol.2005.12.011.

Dhital, S., Shrestha, A. K., Flanagan, B. M., Hasjim, J., & Gidley, M. J. (2011). Cryo-milling of starch granules leads to differential effects on molecular size and conformation. *Carbohydrate Polymers, 84*(3), 1133–1140. http://dx.doi.org/10.1016/j.carbpol.2011.01.002.

Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Effect of cryo-milling on starches: Functionality and digestibility. *Food Hydrocolloids, 24*(2-3), 152–163. http://dx.doi.org/10.1016/j.foodhyd.2009.08.013.

Diop, C. I. K., Li, H. L., Chen, P., & Xie, B. J. (2012). Properties of maize starch modified by ball milling in ethanol medium and low field NMR determination of the water molecular mobility in their gels. *Journal of Cereal Science, 56*(2), 321–331. http://dx.doi.org/10.1016/j.jcs.2012.03.002.

Drapron, R. (1985). *Enzyme Activity as a Function of Water Activity. In Properties of Water in Foods.* Dordrecht: Springer Netherlands171–190. http://dx.doi.org/10.1007/978-94-009-5103-7_11.

Dziedzic, S. Z., & Kearsley, M. W. (1995). Handbook of Starch Hydrolysis Products and their Derivatives. In M. W. Kearsley, & S. Z. Dziedzic (Eds.). *Handbook of Starch Hydrolysis Products and their Derivatives*Boston, MA: Springer US. http://dx.doi.org/10.1007/978-1-4615-2159-4.

El-Porai, E. S., Salama, A. E., Sharaf, A. M., Hegazy, A. I., & Gadallah, M. G. E. (2013). Effect of different milling processes on Egyptian wheat flour properties and pan bread quality. *Annals of Agricultural Sciences, 58*(1), 51–59. http://dx.doi.org/10.1016/j.aoas.2013.01.008.

Fan, L. T., Hsu, K. H., & Kobayashi, T. (1976). A Note on the Characterization of Cryomilled Wheat Flour by Scanning Electron Microscopy. *Cereal Chemistry, 53*, 979–981.

Fox, G., Visser, J., Skov, T., Meijering, I., & Manley, M. (2014). Effect of different analysis conditions on Rapid Visco Analyser malt viscograms in relation to malt of varying fermentability. *Journal of the Institute of Brewing, 120*(3), 183–192. http://dx.doi.org/10.1002/jib.137.

Hackenberg, S., Verheyen, C., Jekle, M., & Becker, T. (2016). Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. *European Food Research and Technology*, 1–10. http://dx.doi.org/10.1007/s00217-016-2743-8.

Han, X.-Z., Campanella, O. H., Mix, N. C., & Hamaker, B. R. (2002). Consequence of Starch Damage on Rheological Properties of Maize Starch Pastes. *Cereal Chemistry, 79*(6), 897–901.

Hasjim, J., Li, E., & Dhital, S. (2012). Milling of rice grains: The roles of starch structures in the solubility and swelling properties of rice flour. *Starch – Stärke, 64*(8), 631–645.

Hasjim, J., Li, E., & Dhital, S. (2013). Milling of rice grains: effects of starch/flour structures on gelatinization and pasting properties. *Carbohydrate Polymers, 92*(1), 682–690. http://dx.doi.org/10.1016/j.carbpol.2012.09.023.

Homer, S., Kelly, M., & Day, L. (2014). Determination of the thermo-mechanical properties in starch and starch/gluten systems at low moisture content – a comparison of DSC and TMA. *Carbohydrate Polymers, 108*, 1–9. http://dx.doi.org/10.1016/j.carbpol.2014.02.049.

Kulp, K., & Ponte, J. G. (2000). In J. G. Kulp, & K. Ponte (Eds.). *Handbook of Cereal Science and Technology*(Second Edition (2nd ed.)). Revised and Expanded.

León, A. E., Barrera, G. N., Pérez, G. T., Ribotta, P. D., & Rosell, C. M. (2006). Effect of damaged starch levels on flour-thermal behaviour and bread staling. *European Food Research and Technology, 224*(2), 187–192. http://dx.doi.org/10.1007/s00217-006-0297-x.

Li, E., Dhital, S., & Hasjim, J. (2014). Effects of grain milling on starch structures and flour/starch properties. *Starch – Stärke, 66*(1-2), 15–27. http://dx.doi.org/10.1002/star.201200224.

Lin (Washington S. U. P. W), P. Y., & Czuchajowska, Z. (1996). *Starch damage in soft wheats of the Pacific Northwest.* USA): Cereal Chemistry.

López, O. V., Zaritzky, N. E., & García, M. A. (2010). Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity. *Journal of Food Engineering, 100*(1), 160–168. http://dx.doi.org/10.1016/j.jfoodeng.2010.03.041.

Majzoobi, M., Radi, M., Farahnaky, a., Jamalian, J., Tongdang, T., & Mesbahi, G. (2011). Physicochemical properties of pre-gelatinized wheat starch produced by a twin drum drier. *Journal of Agricultural Science and Technology, 13*(2), 193–202.

Moreira, R., Chenlo, F., Arufe, S., & Rubinos, S. N. (2015). Physicochemical characterization of white, yellow and purple maize flours and rheological characterization of their doughs. *Journal of Food Science and Technology, 52*(12), 7954–7963. http://dx.doi.org/10.1007/s13197-015-1953-6.

Morrison, W. R., Tester, R. F., & Gidley, M. J. (1994). Properties of Damaged Starch Granules. II. Crystallinity, Molecular Order and Gelatinisation of Ball-milled Starches. *Journal of Cereal Science, 19*(3), 209–217. http://dx.doi.org/10.1006/jcrs.1994.1028.

Park, I., Ibáñez, A. M., Zhong, F., & Shoemaker, C. F. (2007). Gelatinization and Pasting Properties of Waxy and Non-waxy Rice Starches. *Starch - Stärke, 59*(8), 388–396. http://dx.doi.org/10.1002/STAR.200600570.

Qin, Y., Liu, C., Jiang, S., Cao, J., Xiong, L., & Sun, Q. (2016). Functional Properties of Glutinous Rice Flour by Dry-Heat Treatment. *PloS One, 11*(8), http://dx.doi.org/10.1371/journal.pone.0160371 e0160371.

Rakszegi, M., Bognár, Z., Li, Z., Békés, F., Láng, L., & Bedő, Z. (2010). Effect of Milling on the Starch Properties of Winter Wheat Genotypes. *Starch – Stärke, 62*(2), 115–122. http://dx.doi.org/10.1002/star.200900166.

Schirmer, M., Höchstötter, A., Jekle, M., Arendt, E., & Becker, T. (2013). hysicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio. *Food Hydrocolloids, 32*(1), 52–63. http://dx.doi.org/10.1016/j.foodhyd.2012.11.032.

Sun, Q., Gong, M., Li, Y., & Xiong, L. (2014). Effect of dry heat treatment on the physicochemical properties and structure of proso millet flour and starch. *Carbohydrate Polymers, 110*, 128–134. http://dx.doi.org/10.1016/j.carbpol.2014.03.090.

Tester, R. F., & Morrison, W. R. (1994). Properties of Damaged Starch Granules. V.

Composition and Swelling of Fractions of Wheat Starch in Water at Various Temperatures. *Journal of Cereal Science, 20*(2), 175–181. http://dx.doi.org/10.1006/jcrs.1994.1057.

Zeleznak, K. J., & Hoseney, R. C. (1987). The Glass Transition in Starch. *Cereal Chemistry, 64*, 121–124.

Zhang, W., Ding, W., Ndeurumi, K. H., Wang, Z., & Feng, Y. (2015). Effect of wet ball milling on physicochemical properties and crosslinking reaction performance of corn starch. *Starch – Stärke, 67*(11-12), 958–963. http://dx.doi.org/10.1002/star.201400251.

2.4 Mechanically and Thermally Induced Degradation and Modification of Cereal Biopolymers during Grinding

polymers

MDPI

Article

Mechanically and Thermally Induced Degradation and Modification of Cereal Biopolymers during Grinding

Sabina Paulik, Mario Jekle * and Thomas Becker

Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology and Process Engineering, 85354 Freising, Germany; sabina.jakobi@tum.de (S.P.); tb@tum.de (T.B.)
* Correspondence: mjekle@tum.de; Tel.: +49-8161-71-3669

Received: 15 February 2019; Accepted: 5 March 2019; Published: 8 March 2019

check for updates

Abstract: It is presumed that structural and functional alterations of biopolymers, which occur during grinding, are caused by a mechanical modification of polymers. As a result, thermally induced changes of flours are neglected. In this study, the impact of thermo-mechanical stress (TMS), as occurring during general grinding procedures, was further differentiated into thermal stress (TS) and mechanical stress (MS). For TS, native wheat flour, as well as the purified polymers of wheat—starch and gluten—were heated without water addition up to 110 °C. Isolated MS was applied in a temperature-controlled ultra-centrifugal grinder (UCG), whereby thermal and mechanical treatment (TMS) was simultaneously performed in a non-cooled UCG. TS starch (110 °C) and reference starch did not show differences in starch modification degree (2.53 ± 0.24 g/100 g and 2.73 ± 0.15 g/100 g, AACC 76-31), gelatinization onset (52.44 ± 0.14 °C and 52.73 ± 0.27 °C, differential scanning calorimetry (DSC)) and hydration properties (68.9 ± 0.8% dm and 75.8 ± 3.0%, AACC 56-11), respectively. However, TS led to an elevated gelatinization onset and a rise of water absorption of flours (Z-kneader) affecting the processing of cereal-based dough. No differences were visible between MS and TMS up to 18,000 rpm regarding hydration properties (65.0 ± 2.0% dm and 66.5 ± 0.3% dm, respectively). Consequently, mechanical forces are the main factor controlling the structural modification and functional properties of flours during grinding.

Keywords: milling; physical flour modification; grinding; wheat starch; starch damage; hydration properties

1. Introduction

Wheat flours display unique interactions of water, gluten proteins and starch fractions during dough preparation and bread making, which enable the production of porous foods such as white bread and cakes [1]. Wheat flour quality is often described by the degree of starch damage (hereinafter called the starch modification degree (SMD)) [2–4]. However, the term starch damage comprises poorly defined structural as well as functional modifications of flours, such as the water retention capacity [5], that occur during grinding. (Post)-Grinding or extrusion of cereals resulted in enhanced hydration properties of physically modified flours, which can lead to desired higher bread weight [6], but also to an adverse decrease in loaf volume [7,8] by creating altered dough properties [9]. A detailed elucidation of underlying mechanisms has not been completed yet, since different types of physical forces take place simultaneously during the modification process and affect biopolymer modification on the molecular, nanoscopic and microscopic scale.

The physical modification of cereal polymers can thereby occur under dry conditions or in the presence of water through the treatment of polymer suspensions (Figure 1, left).

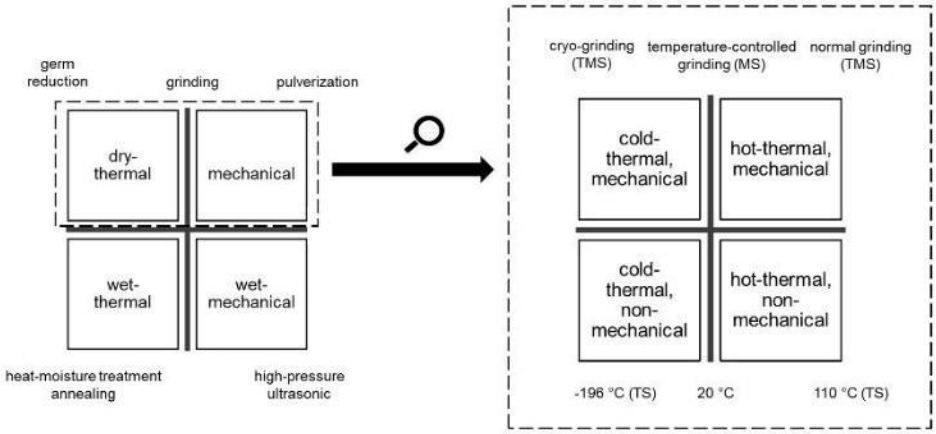

Figure 1. Physical modification of cereals; left: physical stresses in fluid and dry media, right: differentiation of physical stresses in dry media. TMS = thermal-mechanical stress, MS = mechanical stress.

Although a lot of research was related to physical starch modification in suspensions, as heat moisture treatments (HMT), annealing and ultrasonic treatments [10–15], only few studies have dealt with modification techniques, as high-pressure treatment, of cereal materials with low moisture content (≤14%) [16]. Thereby, the type of structural and consequently, functional characteristics of flours strongly depend on the modification technique and the parameters used. While HMT is known to increase the gelatinization onset and to decrease the gelatinization enthalpy with elevated moisture content of the sample, the size and surface of starch granules are not altered [11]. However, ultrasonically treated starch became porous and starch granules were disrupted after the treatment [14,15]. This complicates a clear definition of starch modification, since modifications occur on different structural levels of starch, which depends on the applied physical forces.

The most common physical modification process—the grinding procedure of cereals—combines thermal and mechanical forces [17]. Nevertheless, a sharp differentiation between a mechanically and thermally induced flour modification has not been made and flour modifications during grinding were generally referred to a mechanically induced modification of flours. Thereby, previously studies showed that prolonged heat-treated whole grains or flours exhibited changes in structural conformation and functionality [18,19], as protein denaturation [19] and partial gelatinization [20,21]. However, the effects of shorter dry heat treatments on flour characteristics, as applied during grinding, have not been analyzed so far, although the temperature of ground rice reached 75 °C and 88 °C for hammer mill and high-speed impact mill, respectively.

As a result, the distinction between thermally and mechanically induced alterations is a prerequisite to purposively modify the functional properties of the natural biopolymer starch during the grinding procedure. The physical modification during grinding presents a comparably cheap method and offers benefits compared to a chemical modification, since physically modified starch does not have to be declared and is free of chemical agents [22].

To enable the elucidation of the impact of isolated thermal and mechanical forces during grinding on polymer modification, flour (wheat, rye, rice) as well as gluten and wheat starch were subjected either to a thermal treatment, to a combined thermal and mechanical treatment (heat and impact/shear) in a grinder, or to a mechanical treatment (impact/shear) by a temperature-controlled grinding procedure. Subsequent analysis of hydration properties (WRC, AACC 56-11), starch modification degree (AACC 76-31), particle size distribution and characteristics of starch crystal melting provided information concerning the modification on structural and functional levels of cereal biopolymers. Dough made from (modified) flours were analyzed by a Z-kneader system to gain detailed knowledge

of correlations between flour treatments and functionality. The aim of the study was to differentiate between mechanical and thermal effects on flour during grinding in order to achieve a targeted modification of wheat flours or to standardize the grinding process. Based on this knowledge, physically modified flours or starches can be purposively deployed in the food industry and replace chemical modified starches.

2. Materials and Methods

2.1. Physical Treatments of Raw Materials

Pure variety wheat grain 'Akteur' (donated by Kunstmühle, Buchloe, Germany; ground by Rosenmühle GmbH, Ergolding, Germany;), wheat starch (Hamstarch A, Jaeckering Muehlen- und Naehrmittelwerke GmbH, Hamm, Germany) and gluten (Kroener-Staerke GmbH, Ibbenbueren, Germany) were used in this study.

Furthermore, selected experiments were performed on a second wheat flour variety (Rosenmühle GmbH, Ergolding, Germany), rice flour (Mueller's Muehle GmbH, Gelsenkirchen, Germany) and rye flour (Rosenmühle GmbH, Ergolding, Germany). Moisture content (AACC- 44-01) of reference samples was 11.96 ± 0.03%, 13.87 ± 0.06%, 9.50 ± 0.04%, 13.70 ± 0.02%, 12.49 ± 0.02% and 12.26 ± 0.06%, respectively. The substances were exposed to either mechanical stress (MS), thermo-mechanical-stress (TMS) or thermal stress (TS). To achieve a thermal modification, bio-materials were distributed in thin layers (<5 mm) on baking parchment and thermal stress (TS) was applied for 13 min at 20 °C, 40 °C, 65 °C, 80 °C, 95 °C, and 110 °C in a baking oven. Thermo-mechanical modification was achieved by a non-temperature controlled impact grinder (ZM 200, Retsch, Haan, Germany). Flour samples were ground (at room temperature) at rotational speeds of 0, 6000, 9000, 12,000, 15,000 and 18,000 rpm using a 250 µm mesh size sieve, resulting in extensive heat development and a temperature rise. Mechanical treatment (MS) of flours was achieved by using pre-cooled flour (−18 °C). The grinding procedure (0–18,000 rpm, 250 µm mesh size sieve) was performed in a cooled operational room (+ 4°C) for MS, so that flour temperature did not exceed 60 °C during mechanical treatment. After modification, samples were hermetically sealed and stored at 20 °C ± 2 °C in the dark until the following analysis. All modification procedures were performed in triplicates (n = 3).

2.2. Particle size Distribution

Changes in particle size were evaluated using Mastersizer 3000 (static light scattering, Aero S Unit, Malvern Instruments Ltd., Worcestershire, UK). The dry dispersion unit enabled the measurement of modified flours, gluten and starch excluding a particle hydration. To calculate the particle size, the measurement principle 'Mie Theory' with a refractive index of 1.54 and the general-purpose mode was applied.

2.3. Starch Modification Degree

The starch modification degree was measured by means of an enzyme assay kit (approved method AACC 76-31, starch damage, Megazyme International Ltd., Ireland). The method determines the percentage of starch granules (on 14% moisture basis) in flour and starch hydrolyzed amylases within a defined time range. Calculation was performed using the following Equation (1):

$$SMD = \Delta E \times F \times 90 \times (1/1000) \times (100/W) \times (162/180) \quad (1)$$

with SMD = starch modification degree (%), ΔE = absorbance (reaction) read against the reagent blank, F = (150 (µg of glucose))/(absorbance of 150 µg of glucose), W = sample weight (mg).

2.4. Starch Crystal Melting (Gelatinization Properties)

Determination of gelatinization properties of starch and flour samples were carried out using a differential scanning calorimeter (DSC) (Pyris Diamond, PerkinElmer, Waltham, MA, USA) equipped with an Intracooler 2P cooling system. Calibration was performed with indium and *n*-decan for a temperature range from +20 to +95 °C. Each sample was mixed with deionized water (flour: water ratio = 1:3), filled in DSC pans (20–35 mg) and hermetically sealed. After 2 min of equilibration at 25 °C, samples were heated to 95 °C at 10 K/min in a nitrogen atmosphere. The gelatinization onset (To), peak (Tp), and conclusion temperatures (Tc), and the enthalpy of gelatinization (ΔH) were determined using the Pyris software.

2.5. Water Retention Capacity (WRC)

Hydration properties of flour, gluten and starch were analyzed in accordance with the approved method of the AACC 56–11.02 (solvent retention capacity with water = water retention capacity) for reference and modified samples. WRC was calculated as the weight of the water hold by wheat flour samples after 10 min of centrifugation at 1000× g based on dry mass.

2.6. Dough Properties (Z-Kneading System)

Dough was with (thermally modified) wheat flour (100 parts) and distilled water. According to the AACC method 54.21 a torque measuring Z-kneader system (DoughLab; Perten Instruments, Germany) was used to determine the optimum water addition of flours. The optimum was reached when torque was between 480 and 520 FU (farinograph units).

2.7. Statistical Analysis

The statistical analysis was performed with JMP® Pro (Version 12.2.0, JMP Software, © SAS Institute Inc). To estimate the proportion of variation in the response, coefficient of multiple determination (R^2) was calculated as (sum of squares (model))/(sum of squares (total)).

3. Results and Discussion

3.1. Particle Size Distribution

Particle size distribution (PSD) of flours provides important knowledge to predict the functional behavior of modified flours. In general, wheat flours show a three-modal distribution: the first peak (<10 µm) is formed by small, spherical B starch granules, the second peak (around 10–41 µm) by disc-shaped A starch granules and the largest peak (41–300 µm) by starch granule-protein agglomerates [23], whereby 89%–98% of the particle volume distribution were allocated to the fractions ranging from 10–41 µm and 41–300 µm [23,24]. However, B granules represents the greatest fraction in the number distribution of wheat flours [25]. The value $D_{50,3}$ describes the medium, volume particle size of each flours and decreases triggered by an increase in the extent of certain physical treatments, such as high-pressure application [26]. In this study, an isolated thermal treatment of wheat flour or pure starch did not lead to alterations of the mean volume particle size of the samples (compare Figure 2A). However, the formation of gluten aggregates was observed, when temperatures exceeded 90 °C. These findings can be explained by the formation of disulphide bonds, as shown by investigations on the low moisture gluten of Weegels et al. [27].

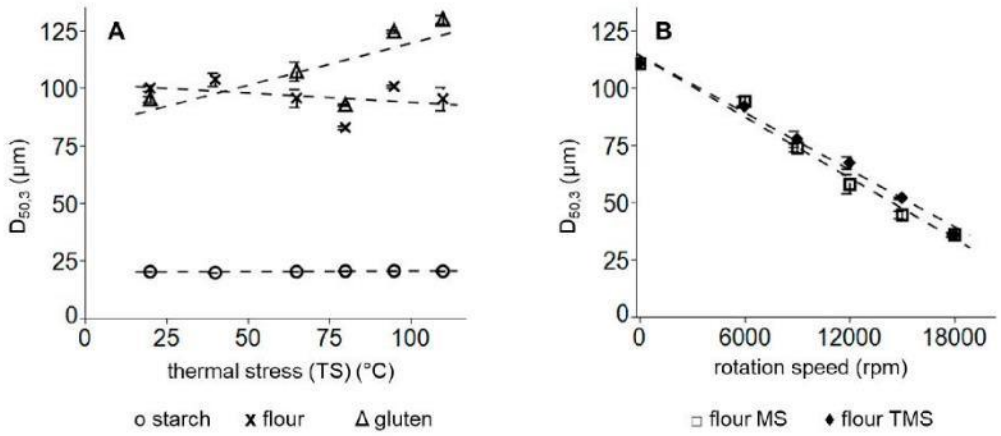

Figure 2. Mean particle size distribution after (**A**) exclusively thermal treatment of flour x, starch O and gluten Δ and (**B**) after mechanical treatment with thermal stress (TMS) ♦ or without thermal stress (MS) ≤ of wheat flours. Error bars represent standard deviations; $n = 3$.

To elucidate the impact of thermal stress in combination with mechanical stress on flour particle size, wheat flour was either subsequent ground in a temperature-controlled (isolated mechanical stress, MS)) or non-temperature-controlled (mechanical-thermal stress, TMS) impact grinder. The temperature development during MS and TMS processes is illustrated in Figure 3. During non-temperature-controlled TMS treatment, temperatures were raised above 60 °C, when high-rotation speeds were set. The usage of a precooled grinder and flours, however, impeded a global temperature increase above protein denaturation temperatures, which are stated at ~55 °C [28], although local peak temperatures above 60 °C could not be excluded during grinding.

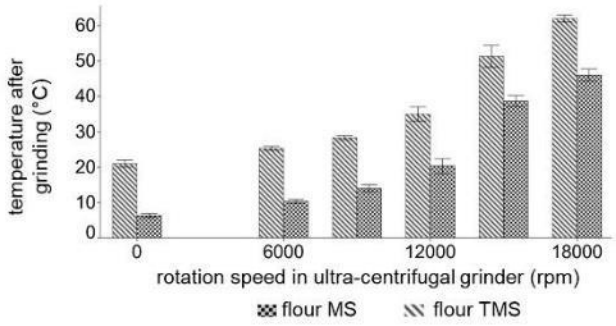

Figure 3. Mean flour temperature after the exposure of mechanical stress in a temperature-controlled impact grinder (flour MS) and after exposure of thermo-mechanical stress in a non-temperature-controlled ultra-centrifugal grinder (flour TMS. Error bars represent standard deviations; $n = 3$.

No differences between particle size distribution of exclusively mechanical stress (MS) and thermomechanical stress (TMS) were noticed (compare Figure 2B). Thus, the particle size reduction is a process driven by mechanical forces during grinding and it can be concluded that temperature development has no impact on particle size reduction during grinding.

3.2. Starch Modification Degree

Beside the particle size distribution of flours, the starch modification degree (SMD) is an important factor for the evaluation of flour quality, as is the baking capability. The measurement of the starch

modification degree (SMD) is based on the enzymatic digestibility of starch in flour particles by amylolytic enzymes. Thus, the accessibility and surface/volume ratio of particles significantly affect the SMD. The fragmentation of particles leads to the enlargement of the particle surface, which in turn promotes the digestion by enzymes [5,29,30]. This negative correlation between the particle size distribution and the starch modification degree (SMD) was also confirmed in the present study: while an exclusively thermal treatment of starch and flour did not result in an enhanced starch modification degree (SMD) and or the shift of PSD to smaller sizes (Figure 4A), mechanical treatments with/without thermal forces (MS and TMS) led to a decrease in particle size of wheat flours and an increase in SMD (compare Figure 4B), which is in agreement with other studies, analyzing the effects of grinding on starch modification and particle size [31,32].

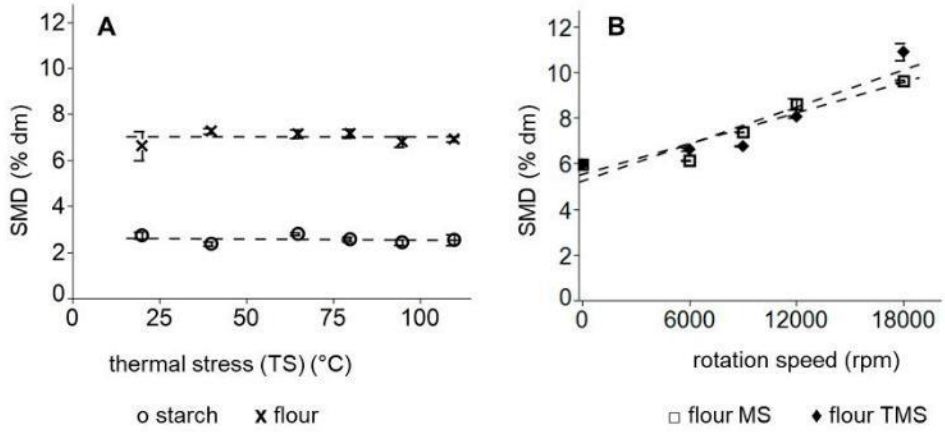

Figure 4. Mean starch modification degree after (A) exclusively thermal treatment of flour x and starch O and (B) after mechanical treatment with thermal stress (TMS) ♦ or without thermal stress (MS) ≤ of wheat flours. Error bars represent standard deviations; $n = 3$.

Isolated mechanical stress (MS) in temperature-controlled grinder did not lead to differences in SMD in comparison to thermo-mechanical stress (TMS) in non-temperature-controlled grinder at low rotation speed, where a low heat development took place. However, grinding in the ultra-centrifugal grinder at higher rotation speeds (18,000 rpm) resulted in a significantly lower SMD of mechanically stressed flours in comparison to thermo-mechanically stressed flours. Since the combination of thermal and mechanical forces during grinding caused a significantly higher starch modification degree (SMD) as isolated mechanical forces, TMS flours had a higher accessibility of starch for an enzymatic digestion, resulting in altered functional properties during fermentation.

Reasons for the enhanced enzymatic degradation (higher SMD) could be due to transformations in amorphous and the crystalline structures [33,34] caused by intense heat development during non-temperature-controlled grinding. DSC measurements were performed to determine changes in crystalline regions caused by thermal stress.

3.3. Gelatinization Properties

The recorded results of the transition of starch crystallites into an amorphous structure (gelatinization properties) of thermally stressed (TS) pure starch and wheat flour are summarized in Table 1. Both the gelatinization enthalpy (ΔH)—measure for the degree of starch crystallinity or rather the transition of crystal structure into amorphous structure—and the gelatinization onset—starting temperature of transition of crystalline into amorphous structure—are important parameters in predicting the functional properties of flours. In this present investigation, no continuous changes in gelatinization enthalpy of thermally treated flour or isolated starch were noticed with rise in

temperature of TS (Table S1), probably since the gelatinization process of starch requires a minimum water level (for corn starch $\geq$ 21%) at current process conditions, so that crystalline parts can be transformed into amorphous structures, which depends on treatment conditions prior to the analysis [35]. Unexpectedly, already pure, reference starch showed a lower gelatinization enthalpy (3.02 $\pm$ 0.01 J/g dm) than flour (5.63 $\pm$ 0.66 J/g dm). Reduced gelatinization enthalpy of isolated starch could be evoked by an exposure of starch to intensive heat and mechanical stress during the commercial extraction procedure, which causes the destruction of crystalline regions of starch. Furthermore, during starch extraction, A granules are predominantly extracted [36] showing a lower relative crystallinity than B granules [37].

Table 1. Mean ($\pm$ SD) gelatinization onset *T*onset of thermally treated (TS) wheat flour and wheat starch. SD = standard deviation; n = 3.

Thermal Treatment (°C)	T_{onset} Flour (°C)	T_{onset} Starch (°C)
+20	46.62 $\pm$ 1.70 ^{C}	52.73 $\pm$ 0.27 ^{A}
+40	46.92 $\pm$ 0.57 ^{B,C}	52.52 $\pm$ 0.18 ^{A}
+65	50.67 $\pm$ 1.98 ^{A,B,C}	52.24 $\pm$ 0.29 ^{A}
+80	51.97 $\pm$ 0.11 ^{A,B}	52.35 $\pm$ 0.13 ^{A}
+95	52.81 $\pm$ 0.46 ^{A}	52.31 $\pm$ 0.20 ^{A}
+110	52.01 $\pm$ 0.58 ^{A}	52.44 $\pm$ 0.14 ^{A}

Different letters demonstrate significant differences in columns.

However, a steady increase in the gelatinization onset was determined for thermally treated wheat flours (Table 1). Alterations of gelatinization temperature without changes of gelatinization enthalpy are also known from the common process HMT (heat moisture treatment). HMT takes place under low moisture conditions ($\leq$ 20%), leading to an increase in the thermal stability of cereal starches, too [38]. The extent of evoked alterations in gelatinization of starch depends on the heat treatment conditions, as well as the time and constitution of the material. The amylose/amylopectin ratio, the phosphorylation degree, as well as the moisture content of the suspension play an important role in the gelatinization process [35,39–41]. In contrast to the present findings, the formation of more stable crystalline structures after HMT [38] alters the gelatinization peak temperature T_{peak} of starches, however, T_{onset} seems to be less affected by heat-moisture treatments [38]. In particular, the low heating period in this present investigation in comparison to other studies [42,43] makes an increased gelatinization onset due to a restructuration of starch unlikely.

Furthermore, a rise in gelatinization onset was solely noticed for wheat flour, although the gelatinization onset of pure starch remained constant. Thus, a reorganization of crystalline parts of starch by heat treatment is unlikely. Rather, it is assumed that changes in gelatinization onset are due to alterations of water distribution between starch and gluten caused by a water barrier of denatured protein, as illustrated schematically in Figure 5. Functional behavior of starch in the matrix 'flour' differs from isolated starch. In flour, starch granules are partly isolated from water due to the formation of starch-gluten particles with a higher particle size diameter. The surface of starch granules is covered with small proteins, which significantly affect the overall properties of starch [44]. Those proteins (and lipids), as well as gluten proteins are known to decelerate digestion processes by acting as a natural barrier [45]. Heat treatment of proteins resulted in a formation of agglomerates, possibly due to a formation of disulphide bonds [27]. It is postulated that these newly formed bonds do strengthen protein cohesion, resulting in a merging of the protein cover layer and a more impeded water penetration. Consequently, water penetration is retarded, and higher temperatures are necessary to induce a water penetration into starch-gluten agglomerates. Nuclear magnetic resonance measurements should therefore be performed to finally show the water barrier effects of heat-treated proteins.

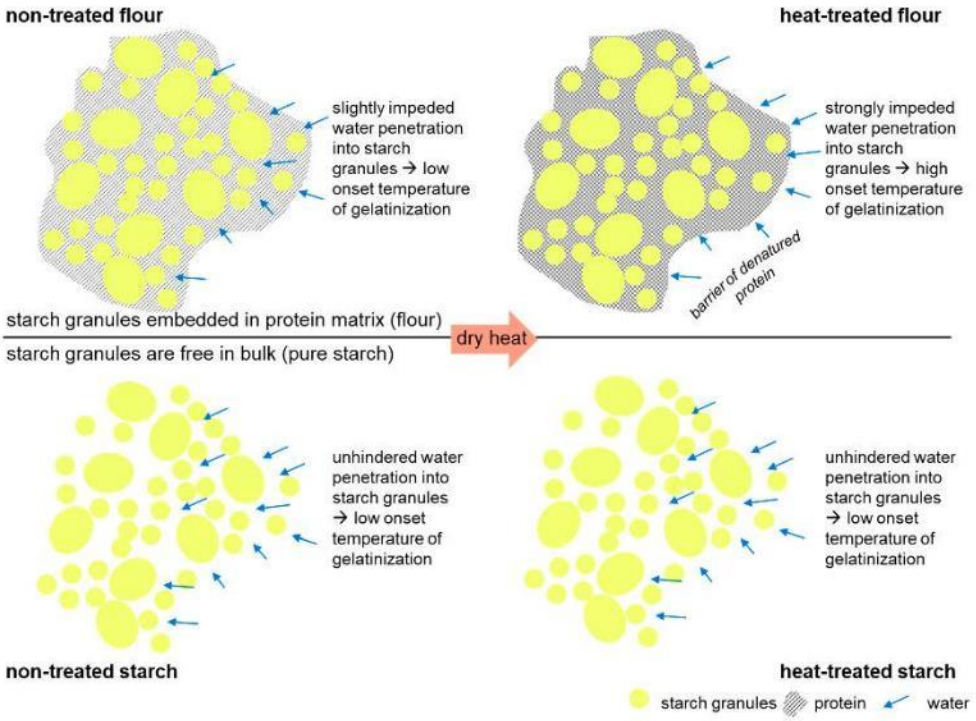

Figure 5. Schematic illustration of the formation of a protein barrier in heat treated wheat matrices and resulting water shielding effect.

On the other hand, starch granules of pure starch are freely accessible for water, since proteins were removed during the extraction of pure wheat starch. Consequently, heat treatment does not affect the gelatinization onset of starch. To investigate in more detail changes in polymer functionality due to the different physical treatments, water retention capacity (WRC) and dough functionality were analyzed.

3.4. Functional Alterations of Cereal Polymers

In the baking industry, the hydration properties of wheat flour are of special interest in determining the dough processability and affecting the quality of bread [46]. As shown in Figure 6A, isolated thermal stress (TS) did not modify significantly the water retention capacity (WRC) of heat-treated gluten, starch or flour. WRC of MS and TMS wheat flours was raised with an increase in the rotation speed of an ultra-centrifugal grinder (up to 18,000 rpm) from 58.61 ± 0.82% dm to 65.94 ± 1.82% dm and 66.62 ± 0.38% dm, respectively. Elevated hydration properties of flours after grinding are explainable by the reduction in particle size and consequently the enhancement of the surface of flour particles. Furthermore, alterations in chemical bonds of the surface of flour particles can occur, leading to a rise in hydrophilic bonds [5,47]. Since MS and TMS showed same effects on WRC, alterations in hydration properties of starch in excess water are induced solely by a mechanical modification of flours.

These insights bring advantages for the construction of modern mills and the control of grinding processes, since flour functionalities and qualities can be set during grinding, depending on the purposed usage. If hydration properties are in the foreground, heat development during grinding can be neglected and consequently costs for cooling units can be economized. However, if the gelatinization properties are relevant, for instance in flours for bread production, heat development in grinders should be controlled during the grinding procedure to achieve a standardization of the flour quality.

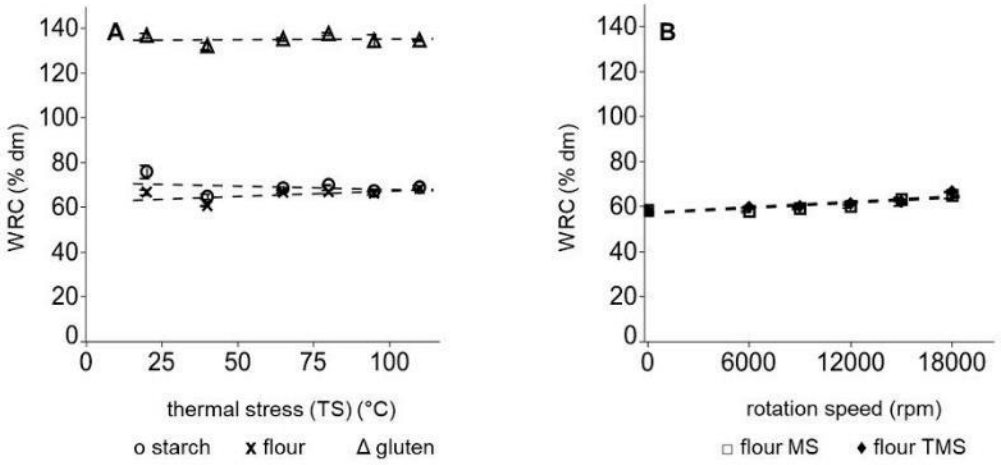

Figure 6. Mean water retention capacity after (**A**) exclusively thermal treatment of flour x, starch O and gluten Δ and (**B**) after mechanical treatment with thermal stress (TMS) ♦ or without thermal stress (MS) ≤ of wheat flours. Error bars represent standard deviations; $n = 3$

However, results from the applied WRC method (AACC 56–11.02), which determine the hydration properties of flours in excess water, cannot be directly transferred to a matrix with limited water content, as for doughs [48]. To investigate hydration properties under limited water content and to elucidate alterations in dough characteristic of physically modified flours, a Z-kneading system was additionally used. The results are summarized in Table 2.

Table 2. Mean (± SD) dough water absorption and dough development time of thermally treated wheat flour determined by a Z-kneading system. SD = standard deviation; $n = 3$.

Thermal Treatment (°C)	Water Absorption (%)	Dough Development Time (min)
+20	61.0 ± 0.3^{B}	3.4 ± 0.8^{A}
+40	$61.6 \pm 0.2^{A,B}$	3.4 ± 0.4^{A}
+65	$61.2 \pm 0.0^{A,B}$	3.4 ± 0.2^{A}
+80	$61.3 \pm 0.5^{A,B}$	3.7 ± 0.4^{A}
+95	$61.4 \pm 0.3^{A,B}$	3.8 ± 1.3^{A}
+110	62.0 ± 0.3^{A}	4.0 ± 0.4^{A}

Different letters demonstrate significant differences in columns.

Water absorption of thermally stressed flours increased slightly from 61.0 ± 0.3% (+20 °C) to 62.0 ± 0.3 (+110 °C) suggesting a modification of wheat polymers. Significant alterations occurred solely for a temperature of 110 °C, hence above the denaturation temperature of proteins. This denaturation can result in a higher water binding capacity of proteins and consequently firmer dough, if the water amount is not adapted. Since WRC is performed in excess water, slight changes in hydration properties might not be visible. However, even small alterations in hydration properties may significantly alter the functional properties of doughs. The dough development times for TS flours stayed constant for temperature up to 110 °C.

To summarize, in limited water systems (DoughLab) and as well in excess water (DSC gelatinization onset), the impact of thermal treatment on polymer functionality was visible. The onset temperature and furthermore the water absorption was elevated. Thus, thermal stresses of flours during grinding or transportation of flours influence the processing of wheat dough and the

manufacturing of wheat products. Consequently, temperatures during the processing of grains, the transportation and storage of flours should be controlled to a enable a constant flour quality.

However, wheat functionality and the ability to enclose high amounts of gas is mainly attributed to the unique properties of the gluten network. To clarify if findings are generally valid for further cereals, TS (thermal stress), MS (mechanical stress) and TMS (thermomechanical stress) was applied to rice, rye and a second wheat variety (Table 3).

Table 3. Impact of thermal stress (TS) at 110 °C, thermal-mechanical stress (TMS) in non-temperature-controlled grinder (18,000 rpm) and mechanical stress (MS) in temperature-controlled grinder (18,000 rpm) on mean (± SD) water retention capacity (WRC) and starch modification degree (SMD) of rice, rye and an additional wheat flour. SD = standard deviation; $n = 3$.

Sample	WRC (% dm)	SMD (% dm)
Ref rice	95.97 ± 0.68 ^{D}	8.71 ± 0.25 ^{C}
TS rice	104.58 ± 0.70 ^{C}	8.44 ± 0.12 ^{C}
TMS rice	110.56 ± 1.18 ^{B}	14.60 ± 0.12 ^{B}
MS rice	114.57 ± 1.41 ^{A}	15.96 ± 0.28 ^{A}
Ref rye	124.74 ± 0.30 ^{D}	4.67 ± 0.09 ^{C}
TS rye	135.60 ± 0.97 ^{B}	4.64 ± 0.15 ^{C}
TMS rye	138.85 ± 0.97 ^{A}	6.06 ± 0.13 ^{A}
MS rye	132.95 ± 0.76 ^{C}	5.73 ± 0.13 ^{B}
Ref wheat 2	49.02 ± 0.30 ^{B}	6.91 ± 0.21 ^{C}
TS wheat 2	51.55 ± 3.41 ^{A,B}	6.67 ± 0.28 ^{C}
TMS wheat 2	54.28 ± 1.55 ^{A}	7.79 ± 0.39 ^{B}
MS wheat 2	55.26 ± 0.47 ^{A}	8.81 ± 0.14 ^{A}

Different letters demonstrate significant differences in columns.

3.5. Transferability to Other Cereal Flours

In accordance to previous findings, TS did not affect WRC or SMD of a second wheat flour (wheat 2). Thus, results from previous chapters are transferable to other wheat varieties.

However, thermal stress of rice and rye flour caused a rise in WRC from 95.97 ± 0.68% dm to 104.58 ± 0.70% dm and from 124.74 ± 0.30% dm to 135.60 ± 0.97% dm, respectively. Interestingly, TS of rice and rye flour caused higher WRC, but did not elevate the starch modification degree. Since accessibility of starch is not altered by TS, a higher amount of water is retained by non-starch components of rice and rye flours. Further research should focus in detail on the alterations of proteins and arabinoxylans from rice and rye caused by thermal stress.

Mechanical treatments (18,000 rpm) with or without thermal stress caused significant alterations of the WRC and SMD within all flours. For rye flours, the combination of thermal and mechanical stress (TMS) caused significantly higher WRC and SMD than pure mechanical stress MS (compare TMS rye and MS rye). The rise of WRC of TMS rye could be explained by the enhanced swelling of heat treated arabinoxylans [49], however, to prove this hypothesis, pure arabinoxylans should be exposed to a dry heat treatment. Thus, flours with additional and influential polymers, as arabinoxylans, should be further analyzed in particular to understand the polymer interactions in detail. In summary, starch modification can be achieved by mechanical treatments in all analyzed cereals, however, in cereals with further functional biopolymers than starch and gluten, modification of all biopolymers should be considered.

4. Conclusions

Physical treatments (for instance during grinding) can be used to gain more detailed knowledge of structure-function relations in complex cereal matrices, as wheat flour, and to produce flours with altered functional properties for the use in clean label products. This study showed that functional alteration of wheat starch is exclusively based on mechanical stress during grinding, however, thermal stress can cause a modification of the gelatinization onset of wheat flour. Thus, proteins and/or other components of flours have to exhibit changes in structure or conformation leading to a rise in gelatinization onset and flour hydration. This indicates that wheat proteins are thermally modified even in dry matrices, which necessitates a detailed investigation of the functional alteration of gluten caused by thermo-mechanical processes. Depending on the field of application, thermal forces should therefore be monitored and, moreover, controlled during the grinding process and any further processing of grains.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4360/11/3/448/s1. Table S1. Mean (± SD) gelatinization enthalpy ΔH of thermally treated wheat flour and wheat starch. SD = standard deviation; $n = 3$.

Author Contributions: Conceptualization, S.P.; Investigation, S.P.; Methodology, S.P.; Project administration, S.P.; Supervision, M.J. and T.B.; Writing—original draft, S.P.; Writing—review & editing, M.J. and T.B.

Funding: This research was funded by Forschungskreis der Ernährungsindustrie (FEI) grant number 18679N.

Acknowledgments: This IGF Project of the FEI is supported via AiF (18679 N) within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lee, M.-R.; Swanson, B.G.; Baik, B.-K. Influence of amylose content on properties of wheat starch and breadmaking quality of starch and gluten blends. *Cereal Chem.* **2007**, *78*, 701–706. [CrossRef]
2. Barak, S.; Mudgil, D.; Khatkar, B.S. Effect of flour particle size and damaged starch on the quality of cookies. *J. Food Sci. Technol.* **2014**, *51*, 1342–1348. [CrossRef] [PubMed]
3. Ali, R.; Khan, M.S.; Sayeed, S.A.; Ahmed, R.; Saeed, G.; Mobin, L. Relationship of damaged starch with some physicochemical parameters in assessment of wheat flour quality. *Pak. J. Bot.* **2014**, *46*, 2217–2225.
4. León, A.E.; Barrera, G.N.; Pérez, G.T.; Ribotta, P.D.; Rosell, C.M. Effect of damaged starch levels on flour-thermal behaviour and bread staling. *Eur. Food Res. Technol.* **2006**, *224*, 187–192. [CrossRef]
5. Jakobi, S.; Jekle, M.; Becker, T. Direct link between specific structural levels of starch and hydration properties. *Carbohydr. Polym.* **2018**, *181*, 159–166. [CrossRef] [PubMed]
6. Martínez, M.; Oliete, B.; Gómez, M. Effect of the addition of extruded wheat flours on dough rheology and bread quality. *J. Cereal Sci.* **2013**, *57*, 424–429. [CrossRef]
7. Hackenberg, S.; Verheyen, C.; Jekle, M.; Becker, T. Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. *Eur. Food Res. Technol.* **2017**, *243*, 287–296. [CrossRef]
8. Schlesinger, J. Results of ball-milling buhler experimentally milled hard winter wheat flour. *Cereal Chem.* **1964**, *41*, 465–473.
9. Mao, Y.; Flores, R.A. Mechanical starch damage effects on wheat flour tortilla texture. *Cereal Chem. J.* **2001**, *78*, 286–293. [CrossRef]
10. Hormdok, R.; Noomhorm, A. Hydrothermal treatments of rice starch for improvement of rice noodle quality. *LWT Food Sci. Technol.* **2007**, *40*, 1723–1731. [CrossRef]
11. Khunae, P.; Tran, T.; Sirivongpaisal, P. Effect of heat-moisture treatment on structural and thermal properties of rice starches differing in amylose content. *Starch Stärke* **2007**, *59*, 593–599. [CrossRef]
12. Anderson, A.K.; Guraya, H.S. Effects of microwave heat-moisture treatment on properties of waxy and non-waxy rice starches. *Food Chem.* **2006**, *97*, 318–323. [CrossRef]
13. Gunaratne, A.; Hoover, R. Effect of heat–moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydr. Polym.* **2002**, *49*, 425–437. [CrossRef]

14. Herceg, I.L.; Režek Jambrak, A.; Šubarić, D.; Brnčić, M.; Rimac Brnčić, S.; Badanjak, M.; Tripalo, B.; Ježek, D.; Novotni, D.; Herceg, Z. Texture and pasting properties of ultrasonically treated corn starch. *Czech J. Food Sci.* **2010**, *28*, 83–93. [CrossRef]
15. Luo, Z.; Fu, X.; He, X.; Luo, F.; Gao, Q.; Yu, S. Effect of ultrasonic treatment on the physicochemical properties of maize starches differing in amylose content. *Starch Stärke* **2008**, *60*, 646–653. [CrossRef]
16. Jakobi, S.; Jekle, M.; Becker, T. High-pressure treatment of non-hydrated flour affects structural characteristics and hydration. *Foods* **2018**, *7*, 78. [CrossRef]
17. Tran, T.T.B.; Shelat, K.J.; Tang, D.; Li, E.; Gilbert, R.G.; Hasjim, J. Milling of rice grains. The degradation on three structural levels of starch in rice flour can be independently controlled during grinding. *J. Agric. Food Chem.* **2011**, *59*, 3964–3973. [CrossRef]
18. Giorni, P.; Dall'Asta, C.; Gregori, R.; Cirlini, M.; Galaverna, G.; Battilani, P. Starch and thermal treatment, important factors in changing detectable fumonisins in maize post-harvest. *J. Cereal Sci.* **2015**, *61*, 78–85. [CrossRef]
19. Neill, G.; Al-Muhtaseb, A.H.; Magee, T.R.A. Optimisation of time/temperature treatment, for heat treated soft wheat flour. *J. Food Eng.* **2012**, *113*, 422–426. [CrossRef]
20. Qin, Y.; Liu, C.; Jiang, S.; Cao, J.; Xiong, L.; Sun, Q. Functional properties of glutinous rice flour by dry-heat treatment. *PLoS ONE* **2016**, *11*, e0160371. [CrossRef]
21. Sun, Q.; Gong, M.; Li, Y.; Xiong, L. Effect of dry heat treatment on the physicochemical properties and structure of proso millet flour and starch. *Carbohydr. Polym.* **2014**, *110*, 128–134. [CrossRef] [PubMed]
22. Zia-ud-Din; Xiong, H.; Fei, P. Physical and chemical modification of starches: A review. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2691–2705. [CrossRef] [PubMed]
23. Hareland, G.A. Evaluation of flour particle size distribution by laser diffraction, sieve analysis and near-infrared reflectance spectroscopy. *J. Cereal Sci.* **1994**, *20*, 183–190. [CrossRef]
24. James, B.; Whistler, R. *Starch Chemistry and Technology*, 3rd ed.; James, B., Whistler, R., Eds.; Elsevier: Amsterdam, The Netherlands, 2009; ISBN 9780080926551.
25. Kumar, R.; Kumar, A.; Sharma, N.K.; Kaur, N.; Chunduri, V.; Chawla, M.; Sharma, S.; Singh, K.; Garg, M. Soft and hard textured wheat differ in starch properties as indicated by trimodal distribution, morphology, thermal and crystalline properties. *PLoS ONE* **2016**, *11*, e0147622. [CrossRef] [PubMed]
26. Ahmed, J.; Mulla, M.Z.; Arfat, Y.A. Particle size, rheological and structural properties of whole wheat flour doughs as treated by high pressure. *Int. J. Food Prop.* **2017**, *20*, 1829–1842. [CrossRef]
27. Weegels, P.L.; de Groot, A.M.G.; Verhoek, J.A.; Hamer, R.J. Effects on gluten of heating at different moisture contents. II changes in physico-chemical properties and secondary structure. *J. Cereal Sci.* **1994**, *19*, 39–47. [CrossRef]
28. Schofield, J.D.; Bottomley, R.C.; Timms, M.F.; Booth, M.R. The effect of heat on wheat gluten and the involvement of sulphydryl-disulphide interchange reactions. *J. Cereal Sci.* **1983**, *1*, 241–253. [CrossRef]
29. Hossen, M.S.; Sotome, I.; Nanayama, K.; Sasaki, T.; Okadome, H. Functional properties of submicron-scale rice flour produced by wet media grinding. *Cereal Chem. J.* **2016**, *93*, 53–57. [CrossRef]
30. Sharma, P.; Chakkaravarthi, A.; Singh, V.; Subramanian, R. Grinding characteristics and batter quality of rice in different wet grinding systems. *J. Food Eng.* **2008**, *88*, 499–506. [CrossRef]
31. Sharif Hossen, M.; Sotome, I.; Takenaka, M.; Isobe, S.; Nakajima, M.; Okadome, H. Starch damage and pasting properties of rice flours produced by dry jet grinding. *Cereal Chem.* **2011**, *88*, 6–11. [CrossRef]
32. Angelidis, G.; Protonotariou, S.; Mandala, I.; Rosell, C.M. Jet milling effect on wheat flour characteristics and starch hydrolysis. *J. Food Sci. Technol.* **2016**, *53*, 784–791. [CrossRef] [PubMed]
33. Mu, T.-H.; Zhang, M.; Raad, L.; Sun, H.-N.; Wang, C. Effect of α-amylase degradation on physicochemical properties of pre-high hydrostatic pressure-treated potato starch. *PLoS ONE* **2015**, *10*, e0143620. [CrossRef]
34. Blazek, J.; Gilbert, E.P. Effect of enzymatic hydrolysis on native starch granule structure. *Biomacromolecules* **2010**, *11*, 3275–3289. [CrossRef] [PubMed]
35. Altay, F.; Gunasekaran, S. Influence of drying temperature, water content, and heating rate on gelatinization of corn starches. *J. Agric. Food Chem.* **2006**, *54*, 4235–4245. [CrossRef] [PubMed]
36. Ayaslan, A.; Seib, P.; Chung, O. Properties of starch and vital gluten isolated from wheat flour by three different wet-milling methods. *J. Appl. Biol. Sci.* **2010**, *4*, 51–56.
37. Zhang, Y.; Guo, Q.; Feng, N.; Wang, J.; Wang, S.; He, Z. Characterization of A- and B-type starch granules in Chinese wheat cultivars. *J. Integr. Agric.* **2016**, *15*, 2203–2214. [CrossRef]

38. Arns, B.; Bartz, J.; Radunz, M.; do Evangelho, J.A.; Pinto, V.Z.; da Rosa Zavareze, E.; Dias, A.R.G. Impact of heat-moisture treatment on rice starch, applied directly in grain paddy rice or in isolated starch. *LWT Food Sci. Technol.* **2015**, *60*, 708–713. [CrossRef]
39. Ratnayake, W.S.; Jackson, D.S. Gelatinization and solubility of corn starch during heating in excess water: New insights. *J. Agric. Food Chem.* **2006**, *54*, 3712–3716. [CrossRef]
40. Muhrbeck, P.; Svensson, E. Annealing properties of potato starches with different degrees of phosphorylation. *Carbohydr. Polym.* **1996**, *31*, 263–267. [CrossRef]
41. Maaurf, A.; Che Man, Y.; Asbi, B.; Junainah, A.; Kennedy, J. Gelatinisation of sago starch in the presence of sucrose and sodium chloride as assessed by differential scanning calorimetry. *Carbohydr. Polym.* **2001**, *45*, 335–345. [CrossRef]
42. Chen, Y.; Yang, Q.; Xu, X.; Qi, L.; Dong, Z.; Luo, Z.; Lu, X.; Peng, X. Structural changes of waxy and normal maize starches modified by heat moisture treatment and their relationship with starch digestibility. *Carbohydr. Polym.* **2017**, *177*, 232–240. [CrossRef] [PubMed]
43. Liu, C.; Hong, J.; Zheng, X. Effect of heat-moisture treatment on morphological, structural and functional characteristics of ball-milled wheat starches. *Starch Stärke* **2017**, *69*, 1500141. [CrossRef]
44. Baldwin, P.M. Starch granule-associated proteins and polypeptides: A Review. *Starch Stärke* **2001**, *53*, 475. [CrossRef]
45. Svihus, B.; Uhlen, A.K.; Harstad, O.M. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Anim. Feed Sci. Technol.* **2005**, *122*, 303–320. [CrossRef]
46. Rosell, C.M.; Collar, C. Effect of temperature and consistency on wheat dough performance. *Int. J. Food Sci. Technol.* **2009**, *44*, 493–502. [CrossRef]
47. Saad, M.; Gaiani, C.; Scher, J.; Cuq, B.; Ehrhardt, J.J.; Desobry, S. Impact of re-grinding on hydration properties and surface composition of wheat flour. *J. Cereal Sci.* **2009**, *49*, 134–140. [CrossRef]
48. Jacobs, P.J.; Hemdane, S.; Dornez, E.; Delcour, J.A.; Courtin, C.M. Study of hydration properties of wheat bran as a function of particle size. *Food Chem.* **2015**, *179*, 296–304. [CrossRef]
49. Keppler, S.; Bakalis, S.; Leadley, C.E.; Sahi, S.S.; Fryer, P.J. Evaluation of dry heat treatment of soft wheat flour for the production of high ratio cakes. *Food Res. Int.* **2018**, *107*, 360–370. [CrossRef]

2.5 High-pressure treatment of non-hydrated flour affects structural characteristic and hydration

foods

MDPI

Article

High-Pressure Treatment of Non-Hydrated Flour Affects Structural Characteristics and Hydration

Sabina Jakobi, Mario Jekle * and Thomas Becker

Research Group Cereal Technology and Process Engineering, Institute of Brewing and Beverage Technology, Technical University of Munich, 85354 Freising, Germany; sabina.jakobi@tum.de (S.J.); tb@tum.de (T.B.)

* Correspondence: mjekle@tum.de; Tel.: +49-8161-71-3669; Fax: +49-8161-3883

Received: 27 April 2018; Accepted: 14 May 2018; Published: 16 May 2018

check for updates

Abstract: In recent years, high-pressure treatment (HPT) has become an established process concerning the preservation of food. However, studies dealing with the structural, and consequently functional modification of non-hydrated starchy matrices (moisture content $\leq$ 15%) by HPT are missing. To close this knowledge gap, pressure (0–600 MPa, 10 min) and pressurization time depending (0–20 min, 450 MPa) alterations of wheat flour were investigated. Pressure rise from 0 to 600 MPa or pressurization time rise from 0 to 20 min resulted in a decline of amylopectin content from 68.3 $\pm$ 2.0% to 59.7 $\pm$ 1.5% (linearly, R^2 = 0.83) and 59.6 $\pm$ 0.7% (sigmoidal), respectively. Thereby, detectable total amount of starch decreased from 77.7 $\pm$ 0.8% linearly to 67.6 $\pm$ 1.7%, and sigmoidal, to 69.4 $\pm$ 0.4%, respectively. Increase in pressure caused a linear decrease in gelatinization enthalpy of 33.2 $\pm$ 5.6%, and linear increase in hydration properties by 11.0 $\pm$ 0.6%. The study revealed structural and technological relevant alterations of starch-based food matrices with low moisture content by HPT, which must be taken into consideration during processing and preservation of food.

Keywords: starch modification; wheat flour; physical modification; starch alteration

1. Introduction

In recent years, consumer expectations on safety, sensory, and nutritive characteristics of processed food have increased, resulting in the evolution of novel processing technologies as high-pressure treatment (HPT). Thereby in the beverage industry, HPT diminishes the microbial load of products without significantly affecting quality parameters [1,2]. However, studies performed on cereal biopolymers revealed HPT induced alterations of matrices, resulting in the usage of HPT for the functionalization and beneficial modification of product quality [3–6]. In gluten-free products, HPT of corn or rice starches resulted in a reduced staling rate of breadcrumbs due to structural alterations of starch, and thus, extended the shelf-life of the products [7].

Although in some food matrices, HPT should not cause alteration of covalent bonds and applied pressure should have minimal effects on processed food [8], research dealing with starchy matrices investigated great changes of polymers resulting in modified enzymatic reactions. Pressure-treated potato starch or barley/wheat flour slurries exhibited a higher enzymatic digestion, due to the increased accessibility of starch, boosting the content of reducing sugars [9,10]. Besides changes in matrix composition, HPT alterations of starch are monitored visually by the transformation of starch granules from a spherical/elliptical to an irregular and ruptured shape [9], as well as the loss of birefringence [11,12]. Granular destruction of starch, in turn, increased swelling and initial viscosity when pressures above 500 MPa are applied [12,13].

To predict the extent of high pressure-induced starch modification, the knowledge of influencing factors is indispensable. Kudta and Tomasik observed a pressure and time dependent structural modification of starch [14]. Thereby, HPT of starch in aqueous solution resulted in significant

changes of starch, however, (pea) starch suspended in ethanol exhibited no structural and functional changes [13]. Even the addition of salt, as ferric chlorides, to aqueous media affected the modification process [15]. Furthermore, intrinsic factors, such as type of starch (A, B, C) [16], starch composition as amylose/amylopectin ratio [17,18], and the origin of starch [17,19] influence the effectiveness of pressure treatments.

To date, investigation on the impact of HPT on starch were performed mainly in starch–water slurries [20]. However, during several cereal processing steps, such as grinding, starch is exposed to high pressure without the addition of extra water. Since HPT resulted in a partial replacement of internal starch hydrogen bonds and gelatinization of starch, it is assumed that former exploration cannot be transferred easily to high-pressure treatments on starch or flour in powdered state (≤14%). Insights into the structural alteration of high pressure on starch in cereal matrices, such as flour, are essential to understand starch-modifying processes during grinding and milling. With the aid of highly compressible vials containing the wheat flour sample, HPT could be successfully applied to non-hydrated wheat flour. Thus, the impact of varied pressure (up to 600 MPa) and pressurization time (up to 20 min) on flour characteristics was determined. This study presents the effects of HPT on the structural and functional modification of wheat flour, and on this basis, enables the investigation of HPT on low-moisture products.

2. Materials and Methods

2.1. Material

High pressure was applied to commercial wheat flour (Type 550, variety "Akteur") provided by Rosenmühle GmBH (Ergolding, Germany). The moisture content of the flour was determined according to the approved method of the AACC 44-40 (2000). Moisture content was constant at 14.64 ± 0.27% before and after treatment. The protein content (10.55 ± 0.03% flours DM (dry matter)) was evaluated using Kjeldahl method AACC 46-12.01 with a conversion factor of 5.7. Ash content was 0.67 ± 0.02 g/100 g analyzed in accordance to AACC 08-12.

2.2. High-Pressure Treatment

HPT experiments were performed on a self-constructed high-pressure device at the Chair of Technical Microbiology at Technical University of Munich. Wheat flour was filled in elastic cryotubes, slightly compressed and hermetically sealed. The surrounding housing was filled with polyethylene–glycol–water to achieve a satisfying pressure transfer to the sample tubes. In two experimental test series (Table 1), the impact of pressurization time (at constant pressure) and of pressure (at constant pressurization time) was analyzed. Depending on the applied pressure, pressure build-up varied between 0.75 and 3.00 min (Table 1). The pressurization time started when the applied pressure was reached. All pressure treatments were performed at least in triplicates.

Table 1. High-pressure treatment (HPT) parameters applied to wheat flour sample and pressure built-up time.

Pressure (MPa)	Pressurization Time (min)	Pressure Build-Up (min)
0	10	0
150	10	0.75
300	10	1.50
450	10	2.25
600	10	3.00
450	1	2.25
450	2	2.25
450	5	2.25
450	10	2.25
450	20	2.25

2.3. Molecular Structure of Starch

Determination of glucose, maltose, maltotriose, as well as fructose and saccharose, was performed using HPAEC-ED (high-performance anion-exchange chromatography with electrochemical detector from Dionex Softron GmbH (Germering, Germany). Flours were dissolved in a methanol–water (1:1) solution (1 g/flour/8 mL solvent) to inhibit enzymatic reactions. Afterwards, samples were filtered through 0.45 µm syringe filters and stored at −18 ± 1 °C before the analysis. Relative amylose content was determined with an amylose/amylopectin assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Total starch content was analyzed according to the AACC Methode 76-13.01 using Total Starch Enzyme Kit from Megazyme International Ireland Ltd. (Wicklow, Ireland). To purify starch and remove interfering dextrin of the sample, flour samples were purified in concentrated ethanol solution (80%).

2.4. Crystalline Properties

Impact of HPT on crystalline structures of starch was studied using dynamic scanning calorimetry (DSC) (Pyris Diamond, Perkin Elmer, Waltham, MA, USA) equipped with a cooling system (Intracooler 2P). Flour samples were mixed with distilled water (flour/water = 1:3), filled in aluminum pans (20–35 mg) and hermetically sealed. After 2 min equilibration time at −40 °C, samples were heated from −40 °C to +95 °C, and held again for 2 min. The gelatinization enthalpy (calculated from the peak endotherm) describes the amount of energy which is necessary to melt crystalline parts of starch during heating.

2.5. Granular Structure

Particle size distribution prior and after HPT was analyzed by static light scattering using Mastersizer 3000 equipped with AeroS unit for dry measurements (Malvern Instruments Ltd., Worcestershire, UK). "Mie Theory" was applied to calculate the particle size distribution using a refractive index of 1.59, the "general purpose mode", and an obscuration ranging between 2 and 8% during measurements. All measurements were performed in triplicates.

2.6. Starch Modification Degree

Starch modification degree (formerly known as starch damage) was determined according to the approved method (AACC method 76-31.01) using the Starch Damage Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The starch modification degree (SMD) specifies the amount of starch which can be hydrolyzed at 40 °C by fungal α-amylase to low molecular weight dextrin, and is calculated using Formula (1):

$$SMD = \Delta E \times F \times 60 \times (1/1000) \times (100/W) \times (162/180), \quad (1)$$

with SMD = starch modification degree (%), ΔE = absorbance (reaction) read against the reagent blank, F = (150 µg of glucose)/(absorbance of 150 µg of glucose), W = sample weight (mg).

2.7. Hydration Properties

The solvent (water) retention capacity (WRC) of flours states the amount of water which can be bound by the sample after centrifugation at low rotational speed (AACC 56-11.02). The approved method had to be modified due to the low sample amount. The ratio flour/solvent remained constant, however, flour weight was reduced to 1.00 ± 0.05 g, and distilled water to 5.00 ± 0.05 g.

2.8. Statistical Evaluation

The statistical analysis was performed using JMP® Pro (Version 12.2.0, JMP Software, SAS Institute Inc., Cary, NC, USA). The effects of HPT on starch structures and functionality were determined using Tukey's pairwise comparisons with a confidence level of 95.0% with ANOVA.

3. Results and Discussion

3.1. Molecular Alterations of Starch

Starch mainly consists of the highly branched polymer amylopectin and the linear glucan amylose, which determine the functional properties of starch. The polymer constitution of starch in flour samples gives information of the origin of starch and provides fundamental insights into the extent of stress acting on starch during physical treatments. As shown in Table 2, with the increase in pressure from 0 to 600 MPa, a significant rise of relative amylose content from 31.7 ± 1.7% of total starch to 40.3 ± 1.2% of total starch (linearly, R^2 = 0.834), and decrease of total starch from 77.7 ± 0.8% to 67.6 ± 1.7% (linearly, R^2 = 0.892) occurred. The simultaneous increase of percentage amylose content and decrease of total starch can be reduced to the destruction of the starch polymer amylopectin. It is well known from literature, that the degradation of the highly-branched polymer amylopectin (~10^8 Da in comparison to amylose ~10^6 Da) is favored when physical forces are applied to pure starch or flours [21,22], explainable by the higher molecular weight and rigidity of amylopectin. The predominant degradation of amylopectin to low molecular weight dextrin or the debranching of this polymer resulted in a percentage rise of amylose in total starch. However, the extent of the degradation of total starch content was surprising. Applied pressure of 600 MPa caused a reduction of the starch content of 13.0%, despite a low moisture content in the present flour samples (14.64 ± 0.27%). Extrusion processes of starchy matrices are known to reduce, extremely, the hydrodynamic radius of starches caused by the splitting of polymer chains, as well [23]. However, this study was performed in matrices with glycerol/water plasticizer of 30 or 40%, facilitating cleavage of polymer chains.

Table 2. Molecular modification of starch in wheat flour visualized by the maltose content, amylose content, and total starch content in dependency of pressurization time and pressure, $\bar{x} \pm SD$, $n = 3$, DM: dry matter.

Variation of Pressure (for 10 min Pressurization Time)				Variation of Pressurization Time (at 450 MPa)			
Pressure (MPa)	Maltose (mg/g Flour)	Amylose (% of Total Starch)	Total Starch (% DM)	Pressurization Time (min)	Maltose (mg/g Flour)	Amylose (% of Total Starch)	Total Starch (% DM)
0	0.8 ± 0.1^{A}	31.7 ± 1.7^{C}	77.7 ± 0.8^{A}	0	0.8 ± 0.1^{A}	31.7 ± 1.7^{C}	77.7 ± 0.8^{A}
150	$0.8 \pm 0.1^{A,B}$	$33.2 \pm 1.2^{B,C}$	$76.8 \pm 0.8^{A,B}$	1	0.7 ± 0.1^{A}	$34.2 \pm 1.0^{B,C}$	$76.2 \pm 0.9^{A,B}$
300	0.6 ± 0.1^{B}	$35.2 \pm 0.5^{B,C}$	$74.0 \pm 0.5^{B,C}$	2	0.6 ± 0.1^{A}	35.7 ± 0.7^{B}	$74.7 \pm 0.5^{B,C}$
450	0.6 ± 0.0^{B}	$37.0 \pm 1.3^{A,B}$	71.7 ± 0.7^{C}	5	0.7 ± 0.2^{A}	36.2 ± 0.6^{B}	$73.7 \pm 0.3^{C,D}$
600	0.6 ± 0.1^{B}	40.3 ± 1.2^{A}	67.6 ± 1.7^{D}	10	0.6 ± 0.0^{A}	$37.0 \pm 1.3^{A,B}$	71.7 ± 0.7^{D}
				20	0.6 ± 0.1^{A}	40.4 ± 0.7^{A}	69.4 ± 0.4^{E}

Different letters in columns mark statistically significant differences between means ($p \leq 0.05$).

Although the determination of total starch content using a Megazyme assay kit presents a fast and reproductive method, it is questionable if an underestimation of the dextrin content after severe physical treatment of starchy matrices occurs. In the first step of this assay, interfering substances are removed by washing the sample in ethanol. The cleavage of starch leads to the formation of low molecular weight fragments showing a higher solubility than starch. Those dextrins are removed prior to the enzymatic digestion, and thus, not detected.

A slight (significant) decrease of maltose content was further investigated, when pressure increased from 0 to 600 MPa (0.8 ± 0.1 to 0.6 ± 0.1 mg/g flour). To date, it remains unclear if the reduction of maltose content is due to a mechanically induced splitting of the disaccharide. An enzymatic hydrolyzation of starch was excluded, since enzymatic degradation of maltose would

cause an increase in glucose content, which could not be determined so far (data not shown), and furthermore, temperature did not rise during the pressure treatment above (30 ± 1 °C). Although decrease in maltose content is negligible for practical application, it provides insights into the mechanism of high pressure on the molecular modification of starch.

Surprisingly, decrease in maltose content could not be determined with an increase in pressurization time from 0 to 20 min at a pressure level of 450 MPa. Furthermore, the analysis of the impact of pressurization time on starch structures elucidates the time-dependent destruction of starch. Due to the natural logarithmic destructive behavior of starch with increase in pressurization time, during the first 5 min of the treatment, the total starch content decreased about 5.1%; within the following 15 min (min 5–20), starch content only decreased about 5.8%.

Due to the huge impact of HPT, even in low moisture matrices on starch, pressure treatments of food systems should not be neglected. Since a favored destruction of amylopectin occurred during HPT, measurements of starch crystallinity are necessary to predict the modified behavior of HPT starches during non-isothermal processes.

3.2. Alterations in Gelatinization Properties and Starch Modification Degree of Flours

Alterations in starch gelatinization properties on nanoscopic scale can be visualized using differential scanning calorimetry. In starch–water suspensions, a destruction of starch crystallinity was already determined by differential scanning calorimetry (DSC) and X-ray method [24]. These results coincide with findings in these studies, despite the significant lower moisture content in the present study. With increase in pressure, a linear decrease in the gelatinization enthalpy of HPT samples from 5.0 ± 0.1 J/g to 3.4 ± 0.4 J/g was noticed (R^2 = 0.851) (Figure 1). Thus, the pressure treatment of 600 MPa resulted in a reduction of total starch and crystallinity of 13.0 and 32.6%, respectively. Consequently, reduction of the total starch content could entail the multiple decline of crystallinity. Furthermore, the severe reduction of gelatinization enthalpy could be referred to a partial gelatinization of starch. In high-moisture matrices, 15–88% of wheat starch was melted, when pressure between 300 and 500 MPa was applied [25]. Thus, a partial gelatinization of starch in HPT non-hydrated samples is possible, causing an accelerated decline in gelatinization enthalpy. The stepwise increase in pressurization time up to 20 min caused a reduction of gelatinization enthalpy to 3.8 ± 0.3 J/g, following a natural logarithmic curve shape (comparable to total starch content): within the first minute of treatment (plus pressure built-up time), gelatinization enthalpy decreased to 4.3 ± 0.2 J/g. Afterward, a decelerated decline in gelatinization enthalpy was noticed, which is consistent with findings of Bauer and Knorr [26]. In general, severe alterations of starch crystallinity were preceded by destruction of granular constitution of starch, and thus facilitated enzymatic digestion.

The starch modification degree (SMD) increased with pressure level (up to 600 MPa) by 37% (linear dependency, R^2 = 0.97). A dependency of SMD from the applied pressure was also revealed by Ahmed et al. on matrices containing 20–50% wheat flour [27]. The rise in pressurization time (up to 20 min) resulted in an increase of SMD of 38%. At the applied pressure of 450 MPa, main alterations in SMD occurred during the first minute of treatment. Thereby, SMD was raised from 4.40 ± 0.13% to 5.66 ± 0.15%, and stayed constant during the following 9 min, demonstrating the need for a detailed control of HPT-induced alterations of starchy matrices, especially during short HPT.

Thereby, the starch modification degree of flours is a key value for the enzymatic degradation of flour particles by amylases during fermentation processes. To draw conclusions regarding the enzymatic hydrolysis and as well to monitor physically-induced changes of flours, the particle size distribution of flours is often determined. Furthermore, changes on microscopic scale can be identified using static light scattering.

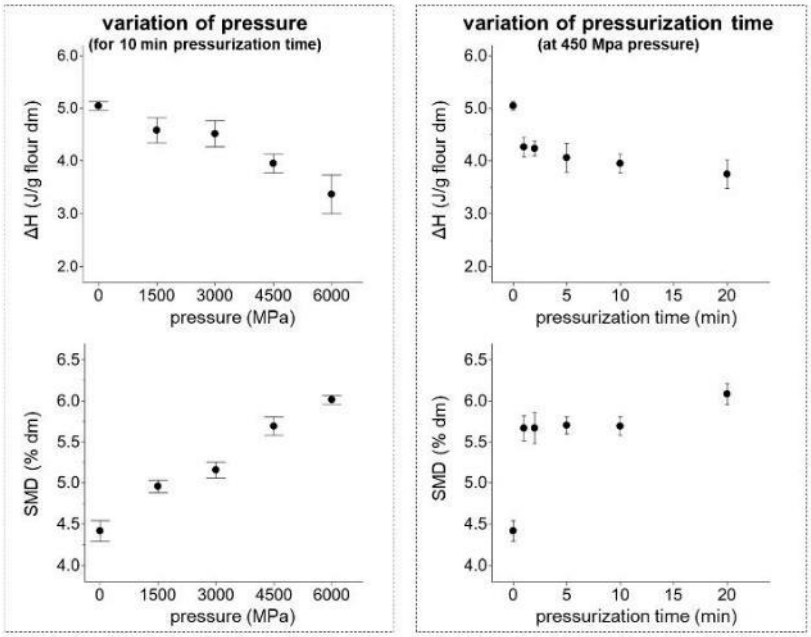

Figure 1. Impact of pressure and pressurization time on the gelatinization enthalpy (ΔH) and starch modification degree (SMD) of wheat flour, $\bar{x} \pm$ SD, $n = 3$.

3.3. Granular Modification of Wheat Flour Particles

Wheat flours, modified by grinding procedures, showed in previous studies a good correlation of SMD and the particle size distribution, so that authors assumed that rise in SMD was caused by the surface enlargement due to the particle size reduction [22]. However, for HPT flours, no reduction of bigger flour particles was visible, as demonstrated by the $D_{3,90}$ of the pressure and pressurization time test series (Table 3). Low pressurization time of 1 min caused a significant reduction of the medium particle size $D_{3,50}$ from 90.4 ± 0.8 to 74.0 ± 4.7 µm without any alterations for longer treatments. A comparable behavior was detected for the pressure level, whereby for pressure treatments above 150 MPa, no significant changes of the medium particle size $D_{3,50}$ were noticed anymore. In literature, contradictory results and dependencies concerning particle size alterations of HPT starches were found: while application of high pressure caused a disruption of bigger particles in high moisture wheat slurry (>50%) [27], an increase of particle size of starch by five- to six-fold was noticed in almond milk or wheat slurries containing sugars [28,29]. However, in the present study, no alterations of flour particles were visible for low moisture matrixes as non-hydrated wheat flour (14%), which is in accordance to the findings of Douzals on wheat starch suspensions (5% DM) [30].

Since particle size distribution (PSD) values are in contrast with the development of SMD under pressure treatments in non-hydrated matrices, where a continuous increase in SMD over the whole pressure range was measured, PSD is not, in general, an adequate analytical method for the prediction of the accessibility of particles for enzymatic digestion. Consequently, surface alterations of wheat flour particles occur, which is not related to the particle size reduction. Changes of starch–gluten agglomerates are known to occur by mechanical treatments, for example grinding, even in low moisture matrices, due to the removal of starch-covering proteins by friction forces. However, since during HPT, 3-dimensional and low friction forces are applied on starch–protein agglomerates, no removal of starch-covering proteins was expected, increasing the accessibility of starch. Therefore, possible explanations for the rise in SMD are microcracks and the deformation of starch granules, which enlarge

the surface of starch, and consequently, enzymatic accessibility, without altering noticeably the particle size distribution.

Table 3. Granular alterations of wheat flour particles determined by the particle size distribution (volume) in dependency of pressurization time and pressure, $\bar{x} \pm$ SD, $n = 3$.

Variation of Pressure (10 min Pressurization Time)				Variation of Pressurization Time (450 MPa)			
Pressure (MPa)	$D_{3,10}$ (µm)	$D_{3,50}$ (µm)	$D_{3,90}$ (µm)	Pressurization Time (min)	$D_{3,10}$ (µm)	$D_{3,50}$ (µm)	$D_{3,90}$ (µm)
0	24.2 ± 0.4^{A}	90.4 ± 0.8^{A}	185.2 ± 5.9^{A}	0	24.2 ± 0.4^{A}	90.4 ± 0.8^{A}	185.2 ± 5.9^{A}
150	19.8 ± 0.4^{A}	81.5 ± 1.7^{B}	184.5 ± 7.3^{B}	1	17.6 ± 1.1^{C}	74.0 ± 4.7^{B}	$182.8 \pm 4.5^{A,B,C}$
300	19.9 ± 0.6^{A}	82.2 ± 1.5^{B}	184.4 ± 2.3^{B}	2	17.4 ± 1.3^{C}	73.5 ± 5.1^{B}	$179.0 \pm 3.3^{B,C}$
450	20.2 ± 2.1^{A}	81.0 ± 6.5^{B}	184.3 ± 6.1^{B}	5	18.0 ± 1.4^{C}	75.9 ± 5.2^{B}	177.9 ± 3.0^{C}
600	21.1 ± 2.0^{A}	83.4 ± 7.1^{B}	191.7 ± 9.9^{B}	10	20.2 ± 2.1^{B}	77.1 ± 4.9^{B}	$184.3 \pm 6.1^{A,B}$
				20	17.0 ± 0.9^{C}	71.7 ± 3.8^{B}	$179.6 \pm 3.7^{A,B,C}$

Different letters in columns mark statistically significant differences between means ($p \leq 0.05$).

To summarize, high pressure-treated flours show comparable molecular (total starch content), crystalline (gelatinization enthalpy) and surface-related (enzymatic accessibility) alterations, which cannot be correlated to the modification of particle size. Thus, using static light scattering, HPT-caused alterations of wheat flours cannot be determined. For the application of pressure-treated starchy matrices in food industry, beside structural alterations, the functional properties of HPT flours are of special interest, too.

3.4. Facilitated Hydration of HPT Flours

The water retention capacity (WRC) of flours describes the amount of water which can be bound by flours after centrifugation. For an increase in WRC, a high accessibility of flours to water is prerequisite. Existing studies in high-moisture matrices have already shown a dependency of pressure and the swelling index of wheat starch [31], and a higher retention capacity of medium moisture matrices (~40%) treated by pressure treatment at 600 MPa [32]. Those findings are also mainly transferable to non-hydrated, starch-based flours. Both test series (pressure level and pressurization time) led to a significant increase in WRC, according to the curve shapes of SMD (compared in Figure 2). The increase in pressure up to 600 MPa resulted in a linear rise of WRC ($R^2 = 0.942$) from $65.68 \pm 0.43\%$ to $73.77 \pm 0.92\%$. This is in agreement with findings from authors dealing with alterations of flour hydration introduced by high-pressure treatments in starch suspensions. However, less effects of high-pressure on starch or flour are expected, as in comparison to HPT of starch suspension, since the hydration highly depends on the availability of free water [33]. A pressurization time of 2 min at 450 MPa caused an increase of WRC to $70.12 \pm 0.70\%$, however, afterwards, only a slight increase of WRC was noticed ($72.91 \pm 0.67\%$ for 20 min HPT). The relation of starch modification degree, pressure, and increased hydration properties of starch in dispersions was also noticed by Ahmed et al. [27]. Hence, the hypothesis can be confirmed, that high-pressure treatment of low moisture starchy matrices significantly modifies the flour particle accessibility, as demonstrated by SMD and WRC measurements, without evoking severe alterations of particle size.

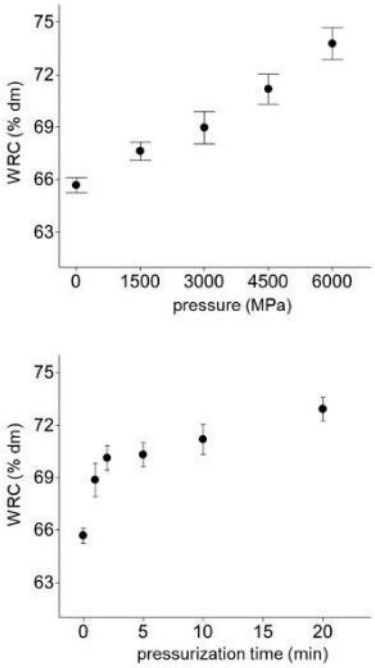

Figure 2. Impact of pressure and pressurization time on hydration properties (WRC = water retention capacity) of wheat flour, $\bar{x} \pm$ SD, $n = 3$.

4. Conclusions

High-pressure treatment of non-hydrated starch–protein particles causes structural modifications of starch, and consequently, functionality of the matrix. Thus, evaluation of high-pressure treatments of starchy food should not exclusively focus on the improvement of the microbiological stability of food products, but also consider functional alterations of the matrix by HPT. These findings are of special interest for the practical, industrial application. The reduction of microbiological stability, in combination with a better enzymatic accessibility of starch, facilitates long-term fermentation processes under controlled conditions. Since high-pressure treatments of starchy matrices, even under low moisture conditions, evoke modifications of starch, grinding processes with (proportional) compressive forces—as found in roller grinding processes—should be examined and adjusted precisely, to prevent undesired alterations. This knowledge can lead to a reconsideration of high-pressure treatments of food systems, and to a targeted selection of monitoring parameters during HPT.

Author Contributions: Conceptualization, methodology, data curation, writing—S.J.; supervision—M.J. and T.B.

Acknowledgments: This IGF Project of the FEI is supported via AiF (18679 N) within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament, and financed by the German Research Foundation (DFG) and the Technical University of Munich within the funding programme Open Access Publishing. Furthermore, the authors want to thank the Chair of Technical Microbiology at Technical University of Munich for the possibility to perform the experiments on the high-pressure plant of the Chair.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bull, M.K.; Zerdin, K.; Howe, E.; Goicoechea, D.; Paramanandhan, P.; Stockman, R.; Sellahewa, J.; Szabo, E.A.; Johnson, R.L.; Stewart, C.M. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innov. Food Sci. Emerg. Technol.* **2004**, *5*, 135–149. [CrossRef]

2. Timmermans, R.A.H.; Mastwijk, H.C.; Knol, J.J.; Quataert, M.C.J.; Vervoort, L.; Van der Plancken, I.; Hendrickx, M.E.; Matser, A.M. Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. *Innov. Food Sci. Emerg. Technol.* **2011**, *12*, 235–243. [CrossRef]
3. Balasubramaniam, V.M.; Barbosa-Cánovas, G.V.; Lelieveld, H.L.M. *High Pressure Processing of Food*; Food Engineering Series; Springer: New York, NY, USA, 2016; ISBN 978-1-4939-3233-7.
4. Dunford, N.T. *Food and Industrial Bioproducts and Bioprocessing*; Wiley-Blackwell: Hoboken, NJ, USA, 2012; ISBN 9780813821054.
5. Kim, H.-S.; Kim, B.-Y.; Baik, M.-Y. Application of Ultra High Pressure (UHP) in Starch Chemistry. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 123–141. [CrossRef] [PubMed]
6. Liu, P.-L.; Hu, X.-S.; Shen, Q. Effect of high hydrostatic pressure on starches: A review. *Starch-Stärke* **2010**, *62*, 615–628. [CrossRef]
7. Cappa, C.; Barbosa-Cánovas, G.V.; Lucisano, M.; Mariotti, M. Effect of high pressure processing on the baking aptitude of corn starch and rice flour. *LWT Food Sci. Technol.* **2016**, *73*, 20–27. [CrossRef]
8. Muntean, M.-V.; Marian, O.; Barbieru, V.; Cătunescu, G.M.; Ranta, O.; Drocas, I.; Terhes, S. High Pressure Processing in Food Industry—Characteristics and Applications. *Agric. Agric. Sci. Procedia* **2016**, *10*, 377–383. [CrossRef]
9. Mu, T.-H.; Zhang, M.; Raad, L.; Sun, H.-N.; Wang, C. Effect of α-Amylase Degradation on Physicochemical Properties of Pre-High Hydrostatic Pressure-Treated Potato Starch. *PLoS ONE* **2015**, *10*. [CrossRef] [PubMed]
10. Gomes, M.R.A.; Clark, R.; Ledward, D.A. Effects of high pressure on amylases and starch in wheat and barley flours. *Food Chem.* **1998**, *63*, 363–372. [CrossRef]
11. Błaszczak, W.; Valverde, S.; Fornal, J. Effect of high pressure on the structure of potato starch. *Carbohydr. Polym.* **2005**, *59*, 377–383. [CrossRef]
12. Oh, H.E.; Hemar, Y.; Anema, S.G.; Wong, M.; Neil Pinder, D. Effect of high-pressure treatment on normal rice and waxy rice starch-in-water suspensions. *Carbohydr. Polym.* **2008**, *73*, 332–343. [CrossRef]
13. Leite, T.S.; de Jesus, A.L.T.; Schmiele, M.; Tribst, A.A.L.; Cristianini, M. High pressure processing (HPP) of pea starch: Effect on the gelatinization properties. *LWT Food Sci. Technol.* **2017**, *76*, 361–369. [CrossRef]
14. Kudta, E.; Tomasik, P. The Modification of Starch by High Pressure. Part I: Air- and Oven-dried Potato Starch. *Starch-Stärke* **1992**, *44*, 167–173. [CrossRef]
15. Kudla, E.; Tomasik, P. The Modification of Starch by High Pressure. Part II: Compression of Starch with Additives. *Starch-Stärke* **1992**, *44*, 253–259. [CrossRef]
16. Stute, R.; Heilbronn, R.W.; Klingler, R.W.; Boguslawski, S.; Eshtiaghi, M.N.; Knorr, D. Effects of High Pressures Treatment on Starches. *Starch-Starke* **1996**, *48*, 399–408. [CrossRef]
17. Oh, H.E.; Pinder, D.N.; Hemar, Y.; Anema, S.G.; Wong, M. Effect of high-pressure treatment on various starch-in-water suspensions. *Food Hydrocoll.* **2008**, *22*, 150–155. [CrossRef]
18. Simonin, H.; Marzouki, S.; Guyon, C.; Orlowska, M.; Le-Bail, A.; de Lamballerie, M. Pasting properties of high-pressure-treated starch suspensions. *High Press. Res.* **2009**, *29*, 726–731. [CrossRef]
19. Liu, Y.Q.; Liu, Y.N.; Li, H.; Shen, R.L.; Li, X.H.; Yang, L.Z. The Study on Gelatinization Pressure of Starch by Ultra-High Pressure Processing. *Adv. Mater. Res.* **2011**, *295–297*, 131–134. [CrossRef]
20. Kawai, K.; Fukami, K.; Yamamoto, K. Effect of temperature on gelatinization and retrogradation in high hydrostatic pressure treatment of potato starch–water mixtures. *Carbohydr. Polym.* **2012**, *87*, 314–321. [CrossRef]
21. Dhital, S.; Shrestha, A.K.; Flanagan, B.M.; Hasjim, J.; Gidley, M.J. Cryo-milling of starch granules leads to differential effects on molecular size and conformation. *Carbohydr. Polym.* **2011**, *84*, 1133–1140. [CrossRef]
22. Jakobi, S.; Jekle, M.; Becker, T. Direct link between specific structural levels of starch and hydration properties. *Carbohydr. Polym.* **2018**, *181*, 159–166. [CrossRef] [PubMed]
23. Li, M.; Hasjim, J.; Xie, F.; Halley, P.J.; Gilbert, R.G. Shear degradation of molecular, crystalline, and granular structures of starch during extrusion. *Starch-Stärke* **2014**, *66*, 595–605. [CrossRef]
24. Liu, M.; Wu, N.-N.; Yu, G.-P.; Zhai, X.-T.; Chen, X.; Zhang, M.; Tian, X.-H.; Liu, Y.-X.; Wang, L.-P.; Tan, B. Physicochemical properties, structural properties, and in vitro digestibility of pea starch treated with high hydrostatic pressure. *Starch-Stärke* **2018**, *70*. [CrossRef]
25. Douzals, J.P.; Marechal, P.A.; Coquille, J.C.; Gervais, P. Microscopic Study of Starch Gelatinization under High Hydrostatic Pressure. *J. Agric. Food Chem.* **1996**, *44*, 1403–1408. [CrossRef]

26. Bauer, B.A.; Knorr, D. The impact of pressure, temperature and treatment time on starches: Pressure-induced starch gelatinisation as pressure time temperature indicator for high hydrostatic pressure processing. *J. Food Eng.* **2005**, *68*, 329–334. [CrossRef]
27. Ahmed, J.; Mulla, M.Z.; Arfat, Y.A. Particle size, rheological and structural properties of whole wheat flour doughs as treated by high pressure. *Int. J. Food Prop.* **2017**, *20*, 1829–1842. [CrossRef]
28. Dhakal, S.; Giusti, M.M.; Balasubramaniam, V. Effect of high pressure processing on dispersive and aggregative properties of almond milk. *J. Sci. Food Agric.* **2016**, *96*, 3821–3830. [CrossRef] [PubMed]
29. Reineke, K.; Weich, H.; Knorr, D. The Influence of Sugars on Pressure Induced Starch Gelatinization. *Procedia Food Sci.* **2011**, *1*, 2040–2046. [CrossRef]
30. Douzals, J.P.; Perrier Cornet, J.M.; Gervais, P.; Coquille, J.C. High-Pressure Gelatinization of Wheat Starch and Properties of Pressure-Induced Gels. *J. Agric. Food Chem.* **1998**, *46*, 4824–4829. [CrossRef]
31. Douzals, J.P.; Perrier-Cornet, J.M.; Gervais, P.; Coquille, J.C. Hydration and Pressure-Temperature Phase Diagrams of Wheat Starch. In *Advances in High Pressure Bioscience and Biotechnology*; Springer: Berlin/Heidelberg, Germany, 1999; pp. 333–336, ISBN 978-3-642-60196-5.
32. Cappa, C.; Lucisano, M.; Barbosa-Cánovas, G.V.; Mariotti, M. Physical and structural changes induced by high pressure on corn starch, rice flour and waxy rice flour. *Food Res. Int.* **2016**, *85*, 95–103. [CrossRef] [PubMed]
33. Rumpold, B.A. Impact of High Hydrostatic Pressure on Wheat, Tapioca, and Potato Starches. Ph.D. Thesis, Technical University of Berlin, Berlin, Germany, August 2005.

2.6 Characterizing the impact of starch and gluten-induced alterations on gelatinization behavior of physically modified model dough

Food Chemistry 301 (2019) 125276

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Characterizing the impact of starch and gluten-induced alterations on gelatinization behavior of physically modified model dough

Sabina Paulik[a], Wen Wen Yu[b], Bernadine Flanagan[b], Robert G. Gilbert[b,c], Mario Jekle[a,*], Thomas Becker[a]

[a] Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology and Process Engineering, 85354 Freising, Germany
[b] The University of Queensland, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD 4072, Australia
[c] Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou 225009, Jiangsu Province, China

ARTICLE INFO

Keywords:
Milling
Physical modification
Wheat starch
Structure-function relation
Hydration
NMR
SEC
Rheometry

ABSTRACT

Gelatinization properties of physically modified starch-gluten matrices are often exclusively traced back to starch constitution without considering the state of gluten. Thus, gelatinization of model dough, combining reference (rS)/modified starch (mS) with reference (rG)/modified gluten (mG), was investigated using nuclear magnetic resonance and differential scanning calorimetry to relate structural alterations of biopolymers to their hydration properties. No differences were found in gelatinization onsets of model dough consisting of rS and mS combined with mG (starch: gluten = 50:50 (m/m)), although gelatinization enthalpy of mS mG (1.7 ± 0.4 J/g dm) was significantly lowered in comparison to rS mG (2.2 ± 0.2 J/g dm). Relaxation time T_2 was significantly reduced for mG in comparison to rG, demonstrating a tighter water binding of mG. This suggests that reduced gelatinization enthalpy of modified starch–gluten matrices is caused by a destruction of crystal parts of modified starch and by a tighter water binding of modified gluten.

1. Introduction

Functional properties of wheat biopolymers enable the inclusion of high amounts of gas during proofing of wheat dough and subsequent baking (Gan, Ellis, & Schofield, 1995; Sroan, Bean, & MacRitchie, 2009), resulting in desired low density crumbs. This is used in the baking industry, whereby the usage of physically modified wheat flour is avoided, due to altered hydration and foam stabilizing properties of flours, which lead to a diminished product quality (Hackenberg, Verheyen, Jekle, & Becker, 2017). Physical modification during grinding or subsequent processes can alter functional changes of all cereal biopolymers; however, in recent years, focus of interest has been on elucidating the modification of the quantitatively dominating polymer 'starch' and its functional alterations (Arora, 2007; Susanto, Fitrianingtyas, Rokhati, Hakiim, & Sistihapsari, 2015), necessitating the usage of several analyzing methods (El-Porai, Salama, Sharaf, Hegazy, & Gadallah, 2013), including differential scanning calorimetry (DSC), as well as amperometric (AACC 76-33) and enzymatic (AACC 76-31) starch damage determination (Angelidis, Protonotariou, Mandala, & Rosell, 2016; Li, Hasjim, Xie, Halley, & Gilbert, 2014). However, complex interactions of cereal biopolymers and minor components make it difficult to trace functional changes of dough and breads back to a modified structure of starch and/ or gluten polymers (Li, Dhital, & Hasjim, 2014). Since low-carb products, often enriched with gluten gain more importance in baking industry, the influence of possible gluten modification on analysis methods and more importantly on product quality should not be neglected. The presence of (modified) gluten complicates precise analysis and a targeted modification of flours. Especially protein-starch-interactions can affect starch-related analysis through DSC or starch damage determination (Hu et al., 2018), since (1) an enhanced protein hydration can reduce the amount of available water and thus starch swelling, impeding starch gelatinization and/ or (2) cracks in the protein-based cover layer or removal of surface proteins of starch facilitate penetration of water and promote enzymatic digestion (Zou, Sissons, Gidley, Gilbert, & Warren, 2015; Zou, Sissons, Warren, Gidley, & Gilbert, 2016), resulting in a reduced gelatinization onset and enhanced SMD.

On the other hand, changes in gluten network formation can be induced by structural alterations of native gluten proteins themselves or by modified starch granules, which affect gluten network formation (Hackenberg, Jekle, & Becker, 2018; Ma et al., 2016). To date, it is not known if the gluten structure is altered by grinding processes or if

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

https://doi.org/10.1016/j.foodchem.2019.125276
Received 22 February 2019; Received in revised form 23 July 2019; Accepted 26 July 2019
Available online 29 July 2019

Table 1
Characterization of starch and gluten by means of mean (± SD) moisture content, mean (± SD) hydrodynamic radius of amylose (SEC, 10–100 nm) and amylopectin (SEC, 100–1000 nm), mean (± SD) starch modification degree and mean (± SD) water solvent retention capacity prior and after modification.

	Moisture (%)	Amylose R_h (nm)	Amylopectin R_h (nm)	Particle size $D_{3,50}$ (µm)	Starch modification degree (%)	Water Solvent retention capacity (%)
rS*	13.0 ± 0.0^{A**}	27.4 ± 0.2^{A**}	315.9 ± 5.8^{A**}	22.3 ± 0.7^{C**}	4.8 ± 0.1^{B**}	82.5 ± 0.4^{D**}
mS*	12.0 ± 0.0^{B**}	27.0 ± 0.1^{A**}	311.3 ± 1.5^{A**}	19.4 ± 0.2^{D**}	6.2 ± 0.2^{A**}	101.9 ± 0.5^{C**}
rG*	10.4 ± 0.0^{C**}			81.2 ± 5.5^{B**}		159.4 ± 0.4^{B**}
mG*	7.6 ± 0.0^{D**}	–		186.0 ± 48.4^{A**}		170.6 ± 0.9^{A**}

SD = standard deviation; n = 3.
* rS = reference starch, mS = modified starch, rG = reference gluten; mG = modified gluten.
** Different letters demonstrate significant differences between starch or gluten samples in columns (p-values < 0.05), identified by one-way ANOVA Tukey Test.

modified starch influences the hydration of gluten and thus its functionality during dough processing.

Among these approaches, rheological measurements deliver valuable information of the behavior of modified starch in cereal-based matrices during isothermal and temperature-scan conditions. In rheometric measurements, Jekle, Mühlberger, and Becker (2016) examined the functional manipulation of starch by gluten during heating. This knowledge of starch-gluten interactions in limited water matrices revealed the need for detailed investigations of starch and protein modification, as well as their interaction. To the best of our knowledge, there is no unambiguous identification of how starch and/ or protein modifications affect thermal transition properties of wheat-based dough, since common methods and experimental setups are mainly dependent on starch-gluten interactions.

In the present study, the impact of physically modified starch and/ or gluten on the hydration and gelatinization properties of starch-gluten model dough was determined by using DSC, rheometer and NMR measurements. The separate modification of polymers and usage of model dough are supposed to achieve a detailed understanding of the water distribution in starch-gluten matrices during processing and the decoding of the impact of physical stress on starch as well as gluten functionality.

2. Experimental

2.1. Physical treatments of raw materials and characterization

Wheat starch and gluten were provided by Kroener-Staerke GmbH (Ibbenbueren, Germany). Reference starch (rS) and reference gluten (rG) were converted to modified starch (mS) or modified gluten (mG) using a non-temperature controlled impact grinder (ZM 200, Retsch, Haan, Germany). Samples were ground at rotational speed of 18,000 rpm using a 250 µm mesh size sieve. To minimize heat-induced alterations of starch and gluten, the mill was allowed to cool down after 200 ± 10 g sample were ground. After modification, samples were hermetically sealed and stored at 4 °C ± 2 °C in the dark until analysis. All modification procedures were performed in triplicates (n = 3).

2.2. Characterization of reference and modified samples

Analysis of reference starch (rS) and reference gluten (rG) were performed in triplicates. Each modified starch (mS) and modified gluten (mG) sample was measured at least once, so that at least three measured value were achieved per modification setting. The moisture content of all samples was determined by thermogravimetric analysis (Kern & Sohn, Balingen, Germany) using 6–8 g sample per measurement. Changes in particle size were evaluated using Mastersizer 3000 (static light scattering, Aero S Unit, Malvern Instruments Ltd, Worcestershire, UK). The dry dispersion unit enabled the analysis of reference gluten (rG), reference starch (rS), modified gluten (mG) and modified starch (mS) without hydration of the samples during the measurement. The particle size was calculated from the light diffraction using Mie theory with a refractive index of 1.54 and the general-purpose mode. $D_{50,3}$ was used to describe changes in particle size distribution ($D_{50,3}$: 50% of a sample's volume is comprised of smaller particles). The starch modification degree (%) of reference and modified starch (rS and mS, respectively) was measured enzymatically (AACC 76-31, starch damage, Megazyme International Ltd., Ireland), determining the percentage of starch hydrolysable by amylases within a defined time range. Hydration properties of samples were determined in accordance with AACC 56-11.02 (water solvent retention capacity). This method was performed with water and describes the weight of water retained by 5 g starch or gluten samples after 10 min of centrifugation at 1000 g based on dry mass. The hydrodynamic radii of amylose and amylopectin was determined with the method, set-up and procedures given in (Vilaplana & Gilbert, 2010). Results are summarized in Table 1.

2.3. Measurement of pasting properties

Prior rheological measurements, model dough were prepared in a Z-kneading system according to AACC method 54-70.01 (DoughLab; Perten Instruments, Germany). Distilled water addition was adapted for each model dough to achieve a final moisture of dough of 46.25%, which was determined as the optimum water addition for the reference model dough rS rG and is comparable to the water addition during the wheat bread preparation. Model dough consisted of reference starch – reference gluten (rS rG), reference starch – modified gluten (rS mG), modified starch – reference gluten (mS rG) and modified starch – modified gluten (mS mG).

Gluten amount in starch-gluten model dough (based on dry mass) was 0, 11, 14, 17, 50, 100%. Dough mixing time was kept constant to reduce effects of various mixing times on sample hydration. All doughs were mixed for 3.0 min, which was the optimum dough development time for reference model dough rS rG, previously determined using AACC method 54–70.01. Water addition and mixing time were kept constant to draw conclusions regarding the effects of sample modification on the hydration, gelatinization and pasting properties of model dough. Afterwards, pasting properties (rise in viscosity) of starch or starch-gluten dough were determined using a rheometer (AR-G2 rheometer, TA instruments, New Castle, USA), equipped with parallel, cross-hatched plates (4 cm diameter). The plate gap was kept constant (2 mm). The model dough was placed between plates and the gap was set. After removing excess dough and edge, the surface was coated with paraffin oil. A temperature sweep was performed to investigate the functional properties of model dough during heating until 96 °C simulating the baking process. The dough was rested for 30 s at 20 °C followed by a heat treatment (oscillatory test) to 96 °C (10 K/min) and resting period at 96 °C for 2 min. Oscillatory measurements were performed in the linear-viscoelastic region (deformation 0.01%, prior determined by a deformation sweep) to ensure a non-destructive measurement of functional properties of the model dough. The onset of pasting was set as the inflection point of tan δ using Matlab software (The Mathworks, Inc., Natick, USA).

Storage module (G′), which is an indicator for elastic material behavior, and loss module (G″), which is an indicator for viscous

components of the material, and tan δ – the ratio of viscous to the elastic proportion – was calculated using Eq. (1):

$$\tan\delta = \frac{G''}{G'} \quad (1)$$

If a material is ideally elastic, G″ = 0 and consequently tan δ = 0. If a material becomes more viscous, loss modulus rises and storage modulus decreases resulting in an increase of tan δ.

2.4. Determination of gelatinization properties

Gelatinization properties (destruction of crystalline parts) of starch or starch-gluten samples were determined using a differential scanning calorimeter (DSC) (Pyris Diamond, Perkin Elmer, Waltham, USA) equipped with an Intracooler 2P cooling system. Calibration was performed with indium and *n*-decane for temperatures ranging from +20 to +95 °C. Samples were mixed with deionized water (starch-gluten: water ratio = 1:3), placed in DSC pans (20–35 mg) and sealed hermetically. After 2 min of equilibration at 25 °C, samples were heated to 95 °C with a temperature ramp of 10 K/min in a nitrogen atmosphere. The onset (T_o), peak (T_p), and conclusion temperatures (T_c), and the enthalpy of gelatinization (ΔH) were evaluated from the curves using Pyris software.

2.5. Analysis of the water mobility of model dough

Water mobility in model dough (46.25%) was measured using NMR (topspin 3.2, Bruker Corporation, Billerica, USA). Spin-spin relaxation time T_2 corresponds to the transverse nuclear spin magnetization and provides information about the water mobility in dough in solid state NMR. In this study, A Carr-Purcell-Meiboom-Gill sequence (CPMG) was used, which allows to measure spin-spin T_2 relaxation times of nuclei. After reverse transformation, CPMG spectra displayed a typical curve. The higher T_2 is, the more mobile water is in the dough. Since of the interest was the water binding of starch and gluten of the most mobile water fraction, the T_2 of peak E was analyzed for each model dough using Matlab software (The Mathworks, Inc., Natick, USA).

2.6. Statistical analysis

Statistical analysis was performed using JMP Pro (Version 12.2.0, JMP Software, SAS Institute Inc., Cary, NC, USA). Data was checked for outliners through the Distribution function. No outliners could be identified. Significant differences were determined by Tukey's pairwise comparison with a confidence level of 95% with ANOVA. Prior applying Tukey's test, datasets were checked, if they meet the criteria of normality and homoscedasticity.

3. Results and discussion

3.1. Characterization of starch and gluten

Structural and functional modifications of starch and gluten arising from the grinding procedure are summarized in Table 1. Grinding of the reference starch (rS) caused a significant reduction in moisture content from 13.0 ± 0.0% (rS) to 12.0 ± 0.0% (modified starch, mS). A significant decrease in particle size $D_{3,50}$ from 22.3 ± 0.7 µm to 19.4 ± 0.2 µm was caused by grinding, without achieving any changes in molecular starch structure: hydrodynamic radius of amylose (27.4 ± 0.2 nm and 27.0 ± 0.1 nm) and amylopectin (315.9 ± 5.8 nm and 311.3 ± 1.5 nm) did not change significantly compared to modified starch (mS). The enzymatic accessibility (see starch modification degree) significantly increased from 4.8 ± 0.1% to 6.2 ± 0.2% and water retention capacity significantly increased from 82.5 ± 0.4% to 101.9 ± 0.5% of rS and mS, respectively. This could be traced back to the shift of the particle size distribution to smaller particles (de la Hera, Gomez, & Rosell, 2013) and most likely to changes in starch surface properties; which cause an increase in starch surface through grinding procedures. Especially surface associated proteins affect the functional properties and accessibility of starch, as reviewed by Baldwin (2001). Thus, grinding of starch caused less changes in molecular structure, but significant alterations of the hydration properties of starch.

Grinding of reference gluten led to a significant reduction of moisture content from 10.4 ± 0.0% (rG) to 7.6 ± 0.0% (mG) and a significant, very pronounced increase in particle size $D_{3,50}$ from 81.2 ± 5.5 µm (rG) to 186.0 ± 48.4 µm (mG). These findings agree with earlier studies of gluten samples forming new disulfide bonds and consequently agglomerates, when exposed to high temperatures (Weegels, de Groot, Verhoek, & Hamer, 1994). In general, the particle size of cereal-based samples correlates significantly negatively with the solvent retention capacity (SRC) of samples (Jakobi, Jekle, & Becker, 2018a). In this study, water solvent retention capacity of gluten significantly increased from 159.4 ± 0.4% to 170.6 ± 0.9% due to grinding procedure, despite the rise in particle size. Thus, particle size for gluten samples is not suitable to forecast hydration behavior of gluten polymers.

This primal characterization of wheat polymers demonstrated structural alterations of gluten and starch, as visualized by the rise in particle size of modified gluten by 129% and a particle reduction of modified starch by 13% in comparison to the reference samples. Although, the molecular analysis of starch showed no significant changes in molecular constitution (amylose and amylopectin R_h), a severe increase in enzymatic accessibility (29%) and SRC (24%) of modified starch was displayed. Functional alterations of modified gluten polymers were also displayed by a slight increase in SRC (7%). Consequently, an impact of modified gluten on the gelatinization, pasting and water mobility of starch-gluten model dough is expected on the basis of the structural and functional alterations caused by grinding.

3.2. Pasting and gelatinization onset of starch-gluten model dough

Rheological tests are often used to determine mechanical properties of cereal-based and synthetic matrices (Brandner, Becker, & Jekle, 2018). During heating of starch-gluten dough, polymers undergo structural changes leading to an altered visco-elastic behavior. With rise in temperature, G'' and consequently tan δ increase, possibly due to the swelling of starch granules and an elevation of viscosity. At temperatures about 60 °C, gelatinization of starch granules and the formation of new cross-links of proteins (Attenburrow, Barnes, Davies, & Ingman, 1990) take place, leading to a rise in G'. Thus, tan δ starts to decrease and a typical curve progression is obtained, as illustrated in supplementary data (Fig. 3). The pasting onset was defined as the inflection point of tan δ based on the method of Jekle et al. (2016) (Jekle et al., 2016). At the inflection point (where the second derivative of tan δ is 0) the slope of tan δ decreases, since gelatinization favors elastic material behavior.

The determination of pasting onset showed that with an increase in gluten ratio in starch-gluten model dough (0 → 50%), pasting onset of model dough rose (Table 2). At constant water addition, model dough containing 50% gluten had a pasting onset around 73.5 ± 0.1 °C, while pure reference starch (rS) and modified starch (mS) showed a pasting onset of 57.6 ± 0.1 °C and 58.4 ± 0.3 °C, respectively. Pasting onset was elevated, since gluten polymers compete with starch for water and can additionally form a barrier around starch granules, hindering the diffusion of water to starch granules. Thus, pasting onset is affected without any noticeable effects on the pasting peak temperature, which is in agreement with the literature (Chen, Deng, Wu, Tian, & Xie, 2010; Jekle et al., 2016).

At a low gluten ratio in model dough, which corresponds to the starch – gluten ratio in wheat flour, no influence of gluten modification

Table 2
Mean (± SD) pasting onset of each starch-gluten model dough measured by means of rheometer. Pasting onset determined as the inflection point of tan δ.

Ratio of gluten in model dough (% dm)	Pasting onset of starch-gluten model dough (°C)			
	rS rG*	rS mG*	mS rG*	mS mG*
50	73.6 ± 0.5^{A**}	73.5 ± 1.6^{A**}	73.3 ± 1.9^{A**}	73.5 ± 1.6^{A**}
17	68.2 ± 0.4^{AB**}	68.1 ± 0.3^{AB**}	67.7 ± 0.4^{A**}	68.3 ± 0.3^{AB**}
14	65.6 ± 0.2^{B**}	65.7 ± 0.5^{B**}	66.5 ± 0.2^{A**}	66.6 ± 0.2^{A**}
11	61.4 ± 0.2^{B**}	61.5 ± 0.1^{B**}	62.4 ± 0.2^{A**}	63.6 ± 1.1^{AB**}
0	57.6 ± 0.1^{B**}		58.4 ± 0.3^{A**}	

SD = standard deviation; n = 3.
* rS = reference starch, mS = modified starch, rG = reference gluten; mG = modified gluten.
** Different letters demonstrate significant differences in rows (p-values < 0.05), identified by one-way ANOVA Tukey Test.

on pasting onset was seen. Nevertheless, results are not directly transferable to wheat flour: in wheat flour, starch granules are encapsulated by wheat proteins and embedded in a gluten matrix, but in this study, starch is probably not incorporated in a protein structure. The diminished embedment of starch into gluten matrix is likely due to the presence of two particulate polymer systems (starch granules and gluten particles). Jenkins et al. (1987) showed, that the subsequent addition of gluten to a starch matrix did not lower the digestibility of wheat starch (measured through the rise in blood glucose level). On the other hand, a regular wheat flour - based matrix showed a significantly reduced digestibility in comparison to a gluten free starchy matrices or matrices, containing subsequently added gluten, which the authors referred to changes in naturally occurring starch – protein interactions. Although other studies showed, that subsequently added gluten affects the functional properties of starch (Petrofsky & Hoseney, 1995) by barrier effects (Jekle et al., 2016), the effects of gluten and consequently modified gluten functionality on starch pasting can be less pronounced.

For higher gluten ratios in model dough, grinding of pure wheat proteins affected the pasting onset of starch, due to a protein modification. On the other side, an embedding of starch granules could not be guaranteed in this study, thus, starch–gluten interactions might not be captured in full. However, model dough provides the best way to assign alterations in dough behavior to a starch or gluten modification.

Model dough rS rG (reference starch – reference gluten (86:14), 65.6 ± 0.2 °C) did not differ in pasting onset T_o significantly from model dough rS rG (reference starch – modified gluten (86:14), 65.7 ± 0.5 °C). No differences in pasting onset of model dough (starch: gluten = 86:14) were detected when modified gluten (mG) and reference gluten (rG) were mixed with modified starch (mS) (66.6 ± 0.2 °C and 66.5 ± 0.2 °C, respectively).

Starch modification alone marginally affected the pasting onset (see 0% gluten in model dough). Interestingly, modification of starch led to a retarded rise in viscosity, which is in constrast to studies of Ali et al. (2014) and Ma et al. (2016), who found a negative correlation between the starch modification degree and the pasting onset in a micro visco-amylo-graph or the pasting temperature in a rapid visco analyser. In the study of Ali et al., flour suspensions were heated and cooled down under controlled conditions in a rotating bowl of the micro visco-amylo-graph, containing excess water (15 g flour mixed with 100 mL distilled water). Thus, due to differences in the water content and geometry of the measuring devices results are not fully comparable to results achieved in this study.

The gelatinization onset (DSC), which describes the beginning of the destruction of crystalline parts of starch, did not vary significantly between pure reference (rS) and modified starch (mS) (50.1 ± 0.1 °C and 49.9 ± 0.1 °C, respectively) (see Table 3). This finding are in agreement with findings of Hasjim, Li and Dhital, who analyzed the gelatinization onset of ground rice grains (Hasjim, Li, & Dhital, 2013). Further research might be carried out on the role of water in the determination of pasting or gelatinization onsets of modified flours, and analysis methods should be critically scrutinized, since differences between rS and mS were not noticeable for DSC measurements, which were performed in excess water (starch – gluten: distilled water = 1:3), while pasting onsets or rS and mS, measured under limited water, differed significantly.

Furthermore, DSC measurements of starch – gluten dough displayed an earlier gelatinization onset (DSC) in comparison to the pasting onset (inflection point of tan δ). Changes rheological measurements and investigations of crystalline structures have also reported by other studies (Nakazawa, Noguchi, Takahashi, & Takada, 1984) possibly due to differences in evaluation methods and moisture content in the measurements.

For all starch – gluten doughs, a positive correlation between gelatinization onset and gluten ratio in model dough was found, which could be due to shielding effects for starch by proteins and, moreover, the reduction in free water for starch gelatinization, since water is also bound by proteins (Kaushik, Kumar, Sihag, & Ray, 2015). For rS rG suspensions, gelatinization onset was elevated from 50.1 ± 0.1 °C (0% gluten) to 52.3 ± 0.8 °C (50% gluten). Rise in pasting onset (Table 2) was even more pronounced, when gluten concentration increased from 0% (57.6 ± 0.1 °C) to 50% (73.6 ± 0.5 °C). Comparable to rheological measurements, modification of gluten had no impact on gelatinization onset of either reference or modified starch in model dough with the same starch: gluten ratio. For 14% gluten in model systems, rS rG and rS mG (51.1 ± 0.1 °C and 51.0 ± 0.1 °C, respectively) as well as mS rG and mS mG (50.7 ± 0.1 °C and 50.7 ± 0.1 °C, respectively) showed no significant differences. On the other hand, starch modification had an influence on gelatinization onset, comparable to the pasting properties. Gelatinization onset of rS rG (51.1 ± 0.1 °C) was higher than for mS rG (50.7 ± 0.1 °C).

To summarize, grinding of pure gluten – as a kind of physical modification – had no impact on pasting onset (increase in viscosity) or gelatinization onset (beginning of destruction of crystallinity) of starch-gluten model dough. However, starch modification significantly affected the pasting and gelatinization onset of starch - gluten matrices. Thus it seems that alterations of the gelatinization enthalpy, which is a key value for the evaluation of dough-like systems, also occurred due to the physical modification of starch or gluten.

3.3. Gelatinization enthalpy of starch-gluten model dough

Gelatinization enthalpy is affected by the structure of starch and the presence of additives, such as salts, hydrocolloids and proteins (Beck, Jekle, & Becker, 2011; Correa & Ferrero, 2015; Jakobi, Jekle, & Becker, 2018b). In one report, no correlations between the gelatinization onset and the melting of crystallite structures were found (Beck et al., 2011). The gelatinization enthalpy (ΔH) mainly depends on the content of available water (Wootton & Bamunuarachchi, 1979), which hampers the comparison between studies. In this investigation, gelatinization enthalpy decreased from 4.1 ± 0.2 J g^{-1} dm to 1.9 ± 0.4 J g^{-1} dm reference model dough (rS rG), as gluten content was changed from 14% to 50% (see Fig. 1). Probable reasons for the reduced ΔH are (1)

Table 3
Mean (± SD) gelatinization onset of starch – gluten model dough measured by means of DSC.

Ratio of gluten in model dough (% dm)	Gelatinization onset of starch-gluten model dough (°C)			
	rS rG*	rS mG*	mS rG*	mS mG*
50	52.3 ± 0.8^{A**}	51.73 ± 2.13^{A**}	54.3 ± 0.8^{A**}	53.6 ± 0.3^{A**}
17	51.2 ± 0.2^{A**}	51.1 ± 0.0^{A**}	51.3 ± 0.2^{A**}	51.0 ± 0.1^{A**}
14	51.1 ± 0.1^{A**}	51.0 ± 0.1^{AB**}	50.7 ± 0.1^{B**}	50.7 ± 0.1^{B**}
11	50.6 ± 0.1^{B**}	51.4 ± 0.3^{A**}	50.7 ± 0.2^{B**}	50.5 ± 0.1^{B**}
0	50.1 ± 0.1^{A**}		49.9 ± 0.1^{A**}	
	50.1 ± 0.1^{A}		49.9 ± 0.1^{A}	

SD = standard deviation; n = 3.
* rS = reference starch, mS = modified starch, rG = reference gluten; mG = modified gluten.
** Different letters demonstrate significant differences in rows (p-values < 0.05), identified by one-way ANOVA Tukey Test.

the rise in protein content of model dough led to a reduction in dry mass of starch and (2) the water binding of protein fraction resulted in a reduced amount of available water for starch. Model doughs made of reference starch (rS) mixed with reference gluten (rG) and modified gluten (mG) had the same ΔH, when same ratio of starch: gluten was used (4.1 ± 0.2 J g^{-1} dm and 4.5 ± 0.1 J g^{-1} dm, respectively). Despite a reduction of starch particle size and an elevated starch modification degree (see Section 3.1), no difference of gelatinization enthalpy in model dough rS rG and mS rG were noticeable, possibly due to primary granular modification of starch. This is also confirmed by size-exclusion chromatographic measurements (SEC) (Section 3.1), where the average amylopectin hydrodynamic radius shows that amylopectin is not destroyed by the grinding procedure.

However, reference starch and modified starch mixed with modified gluten (50:50) revealed significant different gelatinization enthalpies (2.2 ± 0.2 J g^{-1} dm (rS mG 50:50) and 1.7 ± 0.4 J g^{-1} dm (mS mG 50:50), respectively), as well as model dough containing 14% gluten as to whether or not the altered water binding properties of modified gluten, which were also noticed by the increase in SRC (see Section 3.1), affected the gelatinization enthalpy of model dough. To explore this, the water distribution in model dough was analyzed.

3.4. Water mobility – NMR

^{1}H nuclear magnetic resonance (NMR) is an established method to investigate the water mobility in dough (Bosmans et al., 2012; d'Avignon et al., 1991; Engelsen, Jensen, Pedersen, Nørgaard, & Munck, 2001). The relaxation time of protons depends on the interaction between polymer and water. The higher the polymer - water interaction of a sample is, the lower the relaxation rate of protons is. Inversely, with a rise in T_2 of a sample, the mobility of water increases. The relaxation rate decreases (water mobility is reduced) when moisture content is decreased, biopolymers are substituted (for example bran by wheat flour) or the temperature of samples is elevated (Engelsen et al., 2001; Hemdane et al., 2017). Since the moisture content of model dough as well as rehydrated substances (pure starch or gluten) was 46.25%, alterations of relaxation time are due to modifed polymer – water interactions. Consequently, shifts in T_2 to lower times can be ascribed to tighter water binding.

As illustrated in Fig. 2, modification of starch (compare rS 100 and mS 100) led to looser water binding and higher water mobility (100% reference starch rS: 14.8 ± 0.2 ms; 100% modified starch mS: 15.8 ± 0.2 ms). It is known that modification of starch results in an increased amount of bound water in matrices with excess water (Jakobi et al., 2018b, 2018a). However, in matrices with limited water content, as in the present study (46.25%), most of the water is already interacting with starch and/or gluten. Thus, modification of starch could have led to a higher extent of water binding in this study, but on the contrary, it resulted in the weakening of bonds between starch polymers and water. The strength of water binding, as necessary for the formation of hydrogels, is thereby mainly determined by the molecular weight of hydrocolloids, as starch (Boulos, Greenfield, & Wills, 2000; Wang & Cui, 2005). As the hydrodynamic radius of amylose after grinding decreases (see characterization of starch Section 3.1), the water binding to modified starch is reduced as well.

Modification of gluten resulted in a reduction of relaxation time T_2 of reference gluten (rG 100) from 10.2 ± 0.2 ms to 9.1 ± 0.1 ms of modified gluten (mG 100). Lower relaxation times of dough with

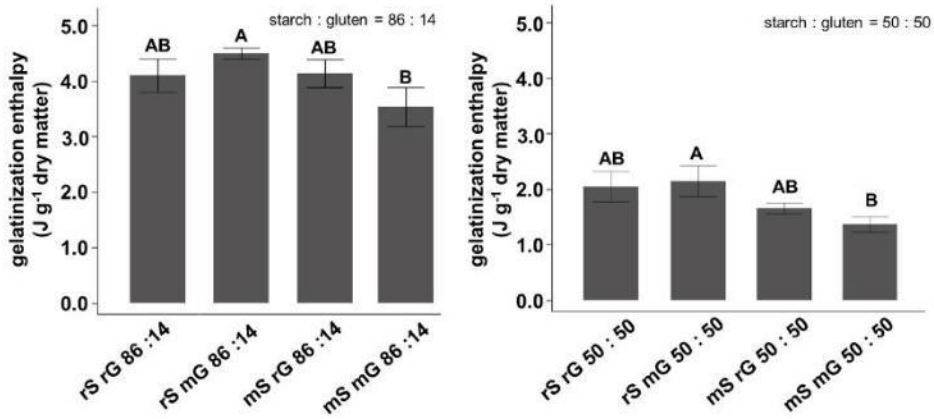

Fig. 1. Mean gelatinization enthalpy of starch-gluten model dough measured by DSC. Starch: gluten ratio in model dough: 86:14 (left or) 50:50 (right). rS = reference starch, mS = modified starch, rG = reference gluten; mG = modified gluten. Error bars represent standard deviations; n = 3. Different letters demonstrate significant differences in model dough (p-values < 0.05), identified by one-way ANOVA Tukey Test.

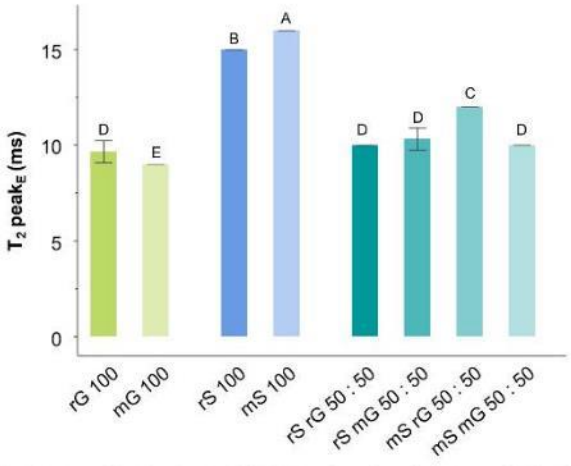

Fig. 2. Mean relaxation time T_2 of CPMG peak E of starch-gluten model dough, as well as reference gluten (rG 100), modified gluten (mG 100), reference starch (rS 100) and modified starch (mS 100) measured by NMR. Starch - gluten ratio in model dough: 50:50. rS = reference starch, mS = modified starch, rG = reference gluten; mG = modified gluten. Error bars represent standard deviations; n = 3. Different letters demonstrate significant differences in samples (p-values < 0.05), identified by one-way ANOVA Tukey Test.

constant moisture constant showed a stronger polymer – water interactions, in this case gluten – water interactions, as revealed by measurement of relaxation times of the water distribution between the cereal polymers starch and gluten.

Roman-Gutierrez, Guilbert, and Cuq (2002) analyzed the water distribution between wheat flour components (starch:gluten = ~86:14) by water adsorption isotherms. They suggested that in non-heated flours, starch has bound 88%; gluten 10% and pentosans 2% of the available water. However, heat-treatments and other physical treatments could result in an altered water – polymer distribution. To analysis wether a functional gluten modification occurs during grinding and to investigate its effect on the hydration of starch – gluten matrices, NMR study of model dough was performed, analyzing T_2 of model dough with a starch: gluten ratio of 50:50. No difference in T_2 of model dough rS rG 50:50, rS mG 50:50 and mS mG 50:50 were seen. However, model dough of modified starch: reference gluten mS rG 50:50 showed a significantly higher T_2 (12.0 ± 0.1 ms) than model dough made of reference starch: reference gluten (10.1 ± 0.3 ms). The increase is attributable to two factors: (1) rG exhibited a higher relaxation time than mG and (2) mS a higher relaxation time than rS. Thus, combined effects and a higher relaxation time of model dough mS rG occurred, when mS rG are mixed.

Furthermore, measurement of T_2 of single polymers and model dough provides an explanation for the significantly reduced gelatinization enthalpy of mS mG 50:50 in comparison to other model doughs (see Section 3.3):

As mentioned before, modification of gluten resulted in lower T_2 in model dough with limited water content, indicating a tighter binding of water by modified gluten (mG 100 (9.1 ± 0.1 ms) in comparison to reference gluten rG 100 (10.2 ± 0.2 ms)). On the other hand, modification of starch resulted in a higher T_2, indicating a looser binding of water of modified starch mS 100 (15.8 ± 0.2 ms) in comparison to reference starch rS 100 (14.8 ± 0.2 ms). These opposing effects (higher water binding of modified gluten and lower water binding of modified starch) led to a local water shift from modified starch polymers to modified gluten polymers in the model dough mS mG 50:50. Due to the water becoming less associated with modified starch polymers and more with modified gluten polymers, a reduced amount of water is localized at modified starch polymers, possibly resulting in a reduced gelatinization enthalpy of starch.

Consequently, beside the destruction of crystal structure of starch through the grinding procedure (Jakobi et al., 2018a), the relocation of mobile water to the gluten fraction could provide an explanation for the reduced gelatinization enthalpy of physically modified model dough.

4. Conclusion

The gelatinization enthalpy in starch – gluten matrices with limited water content, as wheat dough, was strongly related to the structural constitution of starch. However, less was known about the impact of grinding on gluten properties and its influence on the gelatinization properties of starch in starch – gluten matrices. This study demonstrated that starch modification caused a rise in starch modification degree of 29% and solvent retention capacity of 23.5%; however, water binding was looser in comparison to reference starch. Protein modification led to a higher solvent retention capacity and a tighter water binding in starch - gluten model dough in comparison to reference gluten. Thus, gluten functionality is also altered during the grinding process, in addition to the well-known modification of starch. The tighter water binding of modified gluten, accompanied by the looser water binding of modified starch might contribute to a shift in water from starch to gluten polymers, causing a reduced gelatinization extent of starch. Whilst functional gluten modification due to moderate grinding procedures has been difficult to detect in starch – gluten matrices so far, this investigation provides considerable information about the interplay of starch – gluten – water in simplified matrices with limited water content. The type and extent of structural gluten modification changes are areas for future work.

Declaration of Competing Interest

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

Acknowledgements

The authors would like to thank the 'Weihenstephaner Institut für Getreideforschung' (WIG) and the Queensland Alliance for Agriculture and Food Innovation for enabling this research, as well as Kroener-Staerke GmbH (Ibbenbueren, Germany) for the supply of starch and gluten samples.

This IGF Project of the FEI (18679N) was supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125276.

References

Ali, R., Khan, M. S., Sayeed, S. A., Ahmed, R., Saeed, G., & Mobin, L. (2014). Relationship of damaged starch with some physicochemical parameters in assessment of wheat flour quality. *Pakistan Journal of Botany, 46*(6), 2217–2225.

Angelidis, G., Protonotariou, S., Mandala, I., & Rosell, C. M. (2016). Jet milling effect on wheat flour characteristics and starch hydrolysis. *Journal of Food Science and Technology, 53*(1), 784–791. https://doi.org/10.1007/s13197-015-1990-1.

Arora, S. (2007). The effect of enzymes and starch damage on wheat flour tortilla quality. Retrieved from http://oaktrust.library.tamu.edu/handle/1969.1/4864.

Attenburrow, G., Barnes, D. J., Davies, A. P., & Ingman, S. J. (1990). Rheological properties of wheat gluten. *Journal of Cereal Science, 12*(1), 1–14. https://doi.org/10.1016/S0733-5210(09)80152-5.

Baldwin, P. M. (2001). Starch granule-associated proteins and polypeptides: A review. *Starch – Stärke, 53*(10), 475. https://doi.org/10.1002/1521-379X(200110)53:10<475::AID-STAR475>3.0.CO;2-E.

Beck, M., Jekle, M., & Becker, T. (2011). Starch re-crystallization kinetics as a function of various cations. *Starch – Stärke, 63*(12), 792–800. https://doi.org/10.1002/star.201100071.

Bosmans, G. M., Lagrain, B., Deleu, L. J., Fierens, E., Hills, B. P., & Delcour, J. A. (2012). Assignments of proton populations in dough and bread using NMR relaxometry of starch, gluten, and flour model systems. *Journal of Agricultural and Food Chemistry, 60*(21), 5461–5470. https://doi.org/10.1021/jf3008508.

Boulos, N. N., Greenfield, H., & Wills, R. B. H. (2000). Water holding capacity of selected soluble and insoluble dietary fibre. *International Journal of Food Properties, 3*(2), 217–231. https://doi.org/10.1080/10942910009524629.

Brandner, S., Becker, T., & Jekle, M. (2018). Wheat dough imitating artificial dough system based on hydrocolloids and glass beads. *Journal of Food Engineering, 223*, 144–151. https://doi.org/10.1016/J.JFOODENG.2017.12.014.

Chen, J., Deng, Z., Wu, P., Tian, J., & Xie, Q. (2010). Effect of gluten on pasting properties of wheat starch. *Agricultural Sciences in China, 9*(12), 1836–1844. https://doi.org/10.1016/S1671-2927(09)60283-2.

Correa, M. J., & Ferrero, C. (2015). Thermal behaviour of wheat starch and flour at different water levels: Effect of pectins, modified celluloses and NaCl. *Starch – Stärke, 67*(3–4), 338–347. https://doi.org/10.1002/star.201400116.

d'Avignon, D. A., Hung, C. C., Pagel, M. T., Hart, B., Bretthorst, G. L., & Ackerman, J. J. (1991). 1H and 2H NMR studies of water in work-free wheat flour doughs. *Advances in Experimental Medicine and Biology, 302*, 485–508 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1746346.

de la Hera, E., Gomez, M., & Rosell, C. M. (2013). Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties. *Carbohydrate Polymers, 98*(1), 421–427. https://doi.org/10.1016/j.carbpol.2013.06.002.

El-Porai, E. S., Salama, A. E., Sharaf, A. M., Hegazy, A. I., & Gadallah, M. G. E. (2013). Effect of different milling processes on Egyptian wheat flour properties and pan bread quality. *Annals of Agricultural Sciences, 58*(1), 51–59. https://doi.org/10.1016/j.aoas.2013.01.008.

Engelsen, S. B., Jensen, M. K., Pedersen, H. T., Nørgaard, L., & Munck, L. (2001). NMR-baking and multivariate prediction of instrumental texture parameters in bread. *Journal of Cereal Science, 33*(1), 59–69. https://doi.org/10.1006/JCRS.2000.0343.

Gan, Z., Ellis, P. R., & Schofield, J. D. (1995). Gas cell stabilisation and gas retention in wheat bread dough. *Journal of Cereal Science, 21*(3), 215–230. https://doi.org/10.1006/JCRS.1995.0025.

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

Hackenberg, S., Verheyen, C., Jekle, M., & Becker, T. (2017). Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. *European Food Research and Technology, 243*(2), https://doi.org/10.1007/s00217-016-2743-8.

Hasjim, J., Li, E., & Dhital, S. (2013). Milling of rice grains: Effects of starch/flour structures on gelatinization and pasting properties. *Carbohydrate Polymers, 92*(1), 682–690. https://doi.org/10.1016/j.carbpol.2012.09.023.

Hemdane, S., Jacobs, P. J., Bosmans, G. M., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2017). Study of biopolymer mobility and water dynamics in wheat bran using time-domain 1H NMR relaxometry. *Food Chemistry, 236*, 68–75. https://doi.org/10.1016/J.FOODCHEM.2017.01.020.

Hu, P., Fan, X., Lin, L., Wang, J., Zhang, L., & Wei, C. (2018). Effects of surface proteins and lipids on molecular structure, thermal properties, and enzymatic hydrolysis of rice starch. *Food Science and Technology, 38*(1), 84–90. https://doi.org/10.1590/1678-457x.35016.

Jakobi, S., Jekle, M., & Becker, T. (2018a). Direct link between specific structural levels of starch and hydration properties. *Carbohydrate Polymers, 181*(1), 159–166. https://doi.org/10.1016/J.CARBPOL.2017.10.062.

Jakobi, S., Jekle, M., & Becker, T. (2018b). High-pressure treatment of non-hydrated flour affects structural characteristics and hydration. *Foods, 7*(5), 78. https://doi.org/10.3390/foods7050078.

Jekle, M., Mühlberger, K., & Becker, T. (2016). Starch-gluten interactions during gelatinization and its functionality in dough like model systems. *Food Hydrocolloids, 54*, 196–201. https://doi.org/10.1016/j.fuel.2005.08.014.

Jenkins, D. J., Thorne, M. J., Wolever, T. M., Jenkins, A. L., Rao, A. V., & Thompson, L. U. (1987). The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. *The American Journal of Clinical Nutrition, 45*(5), 946–951.

Kaushik, R., Kumar, N., Sihag, M. K., & Ray, A. (2015). Isolation, characterization of wheat gluten and its regeneration properties. *Journal of Food Science and Technology, 52*(9), 5930–5937. https://doi.org/10.1007/s13197-014-1690-2.

Li, E., Dhital, S., & Hasjim, J. (2014). Effects of grain milling on starch structures and flour/starch properties. *Starch – Stärke, 66*(1–2), 15–27. https://doi.org/10.1002/star.201200224.

Li, M., Hasjim, J., Xie, F., Halley, P. J., & Gilbert, R. G. (2014). Shear degradation of molecular, crystalline, and granular structures of starch during extrusion. *Starch – Stärke, 66*(7–8), 595–605. https://doi.org/10.1002/star.201300201.

Ma, S., Li, L., Wang, X., Zheng, X., Bian, K., & Bao, Q. (2016). Effect of mechanically damaged starch from wheat flour on the quality of frozen dough and steamed bread. *Food Chemistry, 202*, 120–124. https://doi.org/10.1016/j.foodchem.2016.01.075.

Nakazawa, F., Noguchi, S., Takahashi, J., & Takada, M. (1984). Gelatinization and retrogradation of rice starch studied by differential scanning calorimetry. *Agricultural and Biological Chemistry, 48*(1), 201–203. https://doi.org/10.1080/00021369.1984.10866115.

Petrofsky, K. E., & Hoseney, R. C. (1995). Rheological properties of dough made with starch and gluten from several cereal sources'. *Cereal Chemistry (USA), 72*(1), 53–58 Retrieved from https://pdfs.semanticscholar.org/c67c/ede1d74d490f8877103d113f86861ba90690.pdf.

Roman-Gutierrez, A. D., Guilbert, S., & Cuq, B. (2002). Distribution of water between wheat flour components: A dynamic water vapour adsorption study. *Journal of Cereal Science, 36*(3), 347–355. https://doi.org/10.1006/JCRS.2002.0470.

Sroan, B. S., Bean, S. R., & MacRitchie, F. (2009). Mechanism of gas cell stabilization in bread making. I. The primary gluten–starch matrix. *Journal of Cereal Science, 49*(1), 32–40. https://doi.org/10.1016/j.jcs.2008.07.003.

Susanto, H., Fitrianingtyas, M., Rokhati, N., Hakiim, A., & Sistihapsari, F. (2015). Physical and chemical modification of sorghum flour for the potential substitute of wheat flour. *Advanced Materials Research, 1123*, 368–372. https://doi.org/10.4028/www.scientific.net/AMR.1123.368.

Vilaplana, F., & Gilbert, R. G. (2010). Two-dimensional size/branch length distributions of a branched polymer. *Macromolecules, 43*(17), 7321–7329. https://doi.org/10.1021/ma101349t.

Wang, Q., & Cui, S. W. (2005). Understanding the physical properties of food polysaccharides. In S. Cui (Ed.). *Food carbohydrates chemistry, physical properties, and applications*CRC Presshttps://doi.org/10.1201/9780203485286.

Weegels, P. L., de Groot, A. M. G., Verhoek, J. A., & Hamer, R. J. (1994). Effects on gluten of heating at different moisture contents. II. Changes in physico-chemical properties and secondary structure. *Journal of Cereal Science, 19*(1), 39–47. https://doi.org/10.1006/JCRS.1994.1006.

Wootton, M., & Bamunuarachchi, A. (1979). Application of differential scanning calorimetry to starch gelatinization. I. Commercial native and modified starches. *Starch – Stärke, 31*(6), 201–204. https://doi.org/10.1002/star.19790310606.

Zou, W., Sissons, M., Gidley, M. J., Gilbert, R. G., & Warren, F. J. (2015). Combined techniques for characterising pasta structure reveals how the gluten network slows enzymic digestion rate. *Food Chemistry, 188*, 559–568. https://doi.org/10.1016/J.FOODCHEM.2015.05.032.

Zou, W., Sissons, M., Warren, F. J., Gidley, M. J., & Gilbert, R. G. (2016). Compact structure and proteins of pasta retard in vitro digestive evolution of branched starch molecular structure. *Carbohydrate Polymers, 152*, 441–449. https://doi.org/10.1016/j.carbpol.2016.06.016.

3 Discussion, conclusion and outlook

Thesis outcome

A rising demand for clean label products offers an opportunity for operators of mills to expand their traditional product range of large quantity batches with partially undefined flour property towards a production of highly functional and standardized flours. Enabled by the knowledge of the impact of different physical forces on the structural and functional behavior of flour components, mills can be specifically adjusted to the desired matters. The usage of new grinding technologies expands the opportunities and sphere of action of small and middle-sized grinding companies in particular by the production and offer of individualized flours and starches of small-scale batches.

Based on the initial hypothesis, mechanisms of flour/ starch modification were elucidated in this thesis, resulting in the disclosure of the following results: The compilation of previous research revealed a dependency of the density of highly inflated baked products, especially on the hydration and pasting properties of flours, and moreover, the interaction of starch and gluten. Increased starch accessibility under room temperature was responsible for a facilitated hydration of modified flours. Rise in hydration of flours were evoked by the mechanical forces occurring during grinding, excluding the impact of thermally induced flour modification regarding the water retention capacity. Thus, the physical modification during grinding can be simplified traced back to a mechanically induced modification of wheat flours. A detailed investigation of the water distribution, as well as gelatinization and pasting behavior of reference/ modified starch combined with reference/ modified gluten polymers revealed a water shift from starch to gluten polymers through grinding. Thereby, altered gelatinization behavior of wheat flour polymers was not merely due to a modification of starch, but also due to a strengthen water binding of gluten polymers. In summary, modification of flours during grinding rest on a physical modification of both main wheat polymers - starch and gluten.

Scientific uncertainties of causal structure-function relations

A poor distinction of causal relations of different parameters is often found in food science, resulting in a misleading interpretation of results and incorrect recommendations for consumers and food industry. Consequently, scientific studies should not only focus on the production of scientific results correlating structural and

functional characteristics of polymers, but moreover, on the elucidation of causal, structure-function relations of food matrices (Jekle, 2018). Due to varying processing conditions and raw material fluctuation, however, it is complicated to determine coherent relations between material changes and their impact on the texture of wheat based products. The complex arrangement of starch polymers into starch granules and their interplay with amylolytic enzymes, proteins, lipids and minor components, which are present in wheat flour, complicate additionally the identification of required properties of starch to produce low-density (highly inflated) baked goods. Consequently, parameters of production processes and alterations of recipe components are non-mechanistically related to the product texture.

A study of Srirejeki et al. (2018) describes the impact of water addition during the preparation of composite flours dough (made of wheat flour and physically modified cassava starch) on the characteristic of dough and bread. A correlation of the water addition/ mixing time and the specific loaf volume was found (Srirejeki, Manuhara, Amanto, Atmaka, & Laksono, 2018). For different wheat flours, a correlation of the specific loaf volume and specific dough properties, determined by Alveograph measurements, was also shown (Addo, Coahran, & Pomeranz, 1990). However, the cause of these correlations remained unclear. An influence of chemically modified cassava starch in composite wheat flour blends on the specific loaf volume was further mentioned by Mo, Go, & An (2015). The effects of different chemical modification techniques on the specific loaf volume were assumed to be caused by alterations of the hydrophilic and hydrophobic character of modified starches (Mo, Go, & An, 2015), but a valid proof of evidence was missing. There is scientific uncertainty, if those results provide an added value regarding the selection of raw materials and adaption of processes, since mechanistic principles were disregarded. Thereby, the knowledge of causal relations is a prerequisite to develop new technologies and to control conventional manufacturing processes of baked goods, as evidenced by the application of physically modified wheat flours in baking industry.

Jet-milled wheat flours display a higher water retention capacity, resulting in a higher water absorption of dough during kneading (Lazaridou et al., 2018), which should theoretically be advantageous for the stabilization of a higher amount of gas and, consequently, the production of baked products with a high specific loaf volume.

However, baking trials showed a reduced specific loaf volume and thereof a poor gas stabilization throughout the production process (Figure 3.1). To benefit from the enhanced water absorption, which partially leads to a higher bread yield, but to minimize negative effects on the loaf volume, it is recommended to substitute only small amounts of native wheat flours by physically modified flours (Martínez, Oliete, & Gómez, 2013; Miller, Maningat, & Hoseney, 2008). To the best of our knowledge, however, no study provide an adequate explanation, why a partial substitution of native wheat flour provided more promising outcomes, than the usage of fully, but marginal modified wheat flour (compare Figure 3.1). As a result, the physical modification of wheat flours is often seen to be undesired due to negative effects on the texture and specific volume of baking products (Araki et al., 2009). To fill the knowledge gap regarding physically modified flours and consequently to control the flour functionality, the identification of relevant structural and functional changes of flour polymers, especially of starch and gluten, are indispensable.

Since structural alterations of starch, which reduce the specific loaf volume of cereal based products were unknown so far, a precise definition and thus modification instruction was missing. To overcome this lack of information and to satisfy consumer requirements of low-density products, starch structures and dough properties, which are responsible to achieve highly inflated products, had to be determined. This enabled the reduction of performed, descriptive analysis of wheat flours, since previously applied analysis of starch were mainly irrelevant to forecast the effects of physically modified wheat flours on gas forming kinetics and gas holding capacity of wheat based materials. However, the need to understand the effects of the physical modification is not limited to wheat flours. In the production of gluten-free baked goods, the biggest challenge faces the manufacturing of products containing a juicy crumb in combination with a high specific loaf volume (Horstmann, Lynch, & Arendt, 2017; Krupa, Rosell, Sadowska, & Soral-Śmietana, 2010). A high viscosity of dough during mixing and baking, reached by the usage of physically modified starches, could help to meet these needs. For this purpose, the knowledge of flour characteristic is essential to select and purchase target-oriented gluten-free flours (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016). As it remains unclear, which parameters of flour characteristic contribute positively to the production of low-density, gluten-free products, the development of precise flour specifications failed, so far. A targeted selection of gluten-

free flours was solely enabled by trial and error small-scale baking trials, which caused substantial costs for bakeries, since limitations of analysis methods are rarely possible. Consequently, new strategies were required to establish cause-effect links.

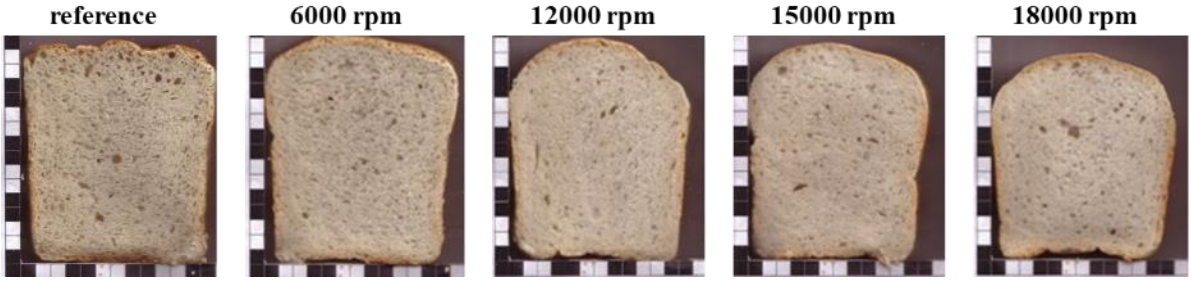

Figure 3.1: Bread produced with reference or modified wheat flour. Rotation speed of impact mill was increased from 6000 rpm to 18000 rpm to achieve different starch modification degrees. Starch modification degree increased from 5.52 g/100 g (reference flour) to 7.01 g/ 100 g (18000 rpm) using 250 µm mesh sieve (FEI report 2016; project 18679N).

Through the development and usage of a reverse approach, relevant modified starch structures and furthermore major dough functionalities for the production of low-density wheat breads were determined. This was achieved by identifying the dough functionalities at first, which mainly affect the specific loaf volume of baked goods. It was shown that the viscosity of dough during processing, as kneading, and during baking, as well as the pasting onset are mainly responsible for the gas holding capacity of wheat foams, as dough. Those properties depend on the hydration of wheat polymers. Hereinafter, starch structures and the role of gluten was considered in their influence on those major dough functionalities (viscosity and pasting onset). In this case, the amylose content, starch content and disruption of granular starch structures were determined as major influencing factors. Subsequently, starch structures, modified by physical methods, especially grinding, were reviewed. Finally, the comparison of influential starch structures and starch structures modified by grinding processes revealed, which structural alterations of starch were relevant to control in the baking process (Paulik, Jekle, & Becker, 2019a). This reverse approach (from the product characteristic to the starch constitution) enabled to elucidate systematically relevant physical modifications in dough characteristics, and constitutes a method to determine decisive starch structures and underlying relations (compare Figure 3.2).

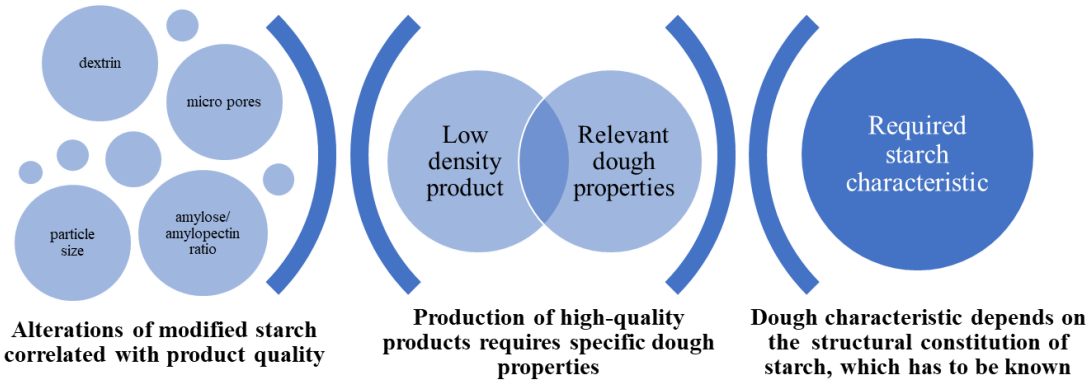

Figure 3.2: Systematic, reverse approach to determine underlying mechanism of modified starch and the resulting impact on the specific loaf volume of baked goods. Product texture/ density and required dough properties take center stage (1 and 2) enclosed by starch structures of native or physically modified flours (3a and 3b). Thus, reverse approach differs from the traditional approach, where modified starch structures take center place and are correlated randomly with product texture/ density.

Thereby, non-relevant correlations of starch structures and product texture were removed. Focusing on relevant starch structures allows the reduction of analysis methods and consequently costs to a minimum, as shown for wheat based baking products (compare Figure 3.3).

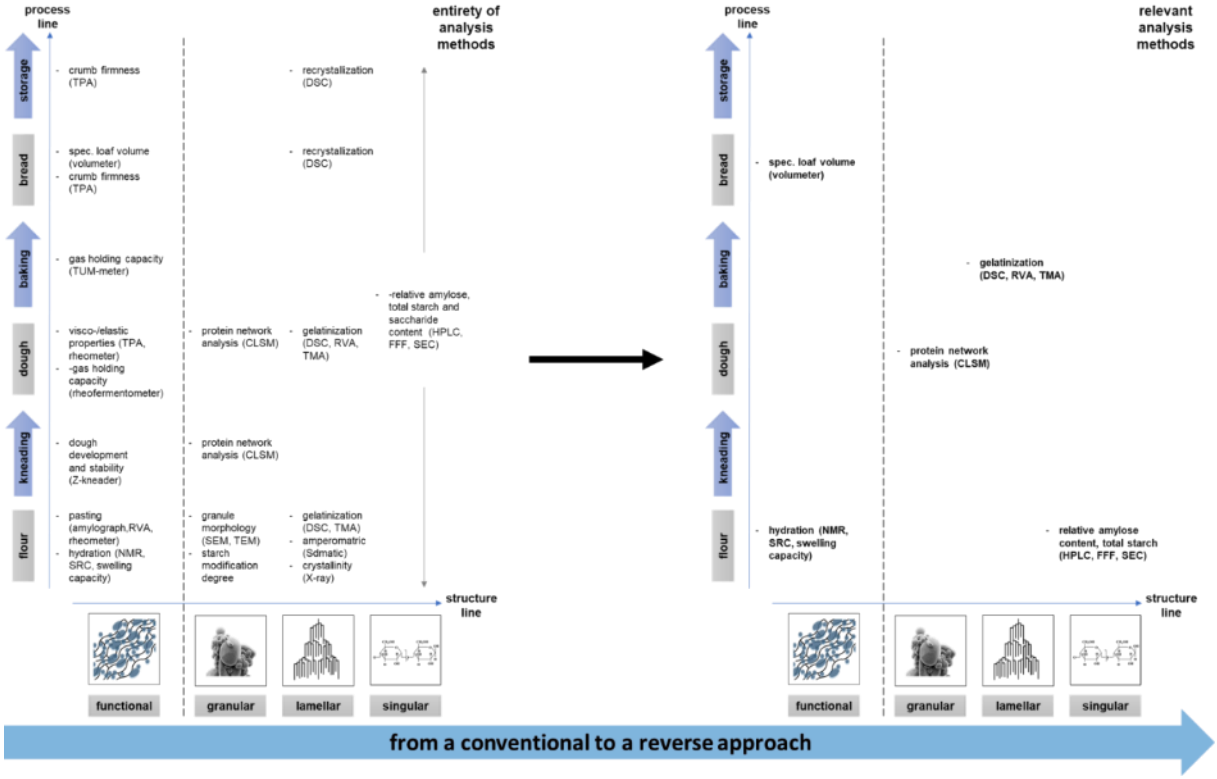

Figure 3.3: Number of performed analysis methods can be reduced by focusing on relevant starch structures in the reverse approach. RVA = rapid visco-analyzer; CLSM = confocal laser scanning microscopy; TEM = Transmission Electron Microscopy; DSC = differential scanning microscopy; TMA = thermomechanical analysis; X-ray = X-radiation; SEM = scanning electron microscopy; FFF= field-flow-fractionation; HPLC = high performance liquid chromatography; SEC = size exclusion chromatography; NMR = nuclear magnetic resonance

Furthermore, it became obvious through the application of the reverse approach, that modified wheat starch (granules) cause a modification of gluten functionality during processing. This was referred to the enhanced swelling and hydration of starch granules and consequently reduced amount of water for gluten biopolymers. However, this approach did not provide information about a possible, structural modification of gluten polymers itself. In addition, effects of possibly modified gluten polymers on starch functionality, as pasting and gelatinization, were neglected in this approach, although former studies already revealed a functional manipulation of starch by gluten polymers (used in varying ratios) in model dough during thermal transition of starch (Jekle et al., 2016).

To prove systematically a modification of gluten polymers during grinding, Differential Scanning Calorimetry (DSC) and Rapid Visco Analyser (RVA) measurements on separately modified (by grinding) gluten and/ or starch were performed, revealing a reduction of the gelatinization enthalpy of modified starch, when using ground (modified) gluten (Paulik, Wen Yu, et al., 2019). NMR trials disclosed a tighter water binding of physically modified gluten in comparison to reference gluten, causing a more intense competition of starch and gluten among water and consequently a reduced amount of freely available water for starch polymers during pasting. Thus, the reduction in gelatinization enthalpy of physically modified flours can be induced by a degradation of starch crystallinity and, moreover, by a tighter water binding of modified gluten (Paulik, Wen Yu, et al., 2019).

Although DSC measurements of the thermal transition of physically modified starch are known to capture exclusively alterations in starch constitution after physical treatments, this methodology demonstrated that a simultaneously occurring modification of wheat proteins and further polymers can affect the results of DSC measurements. Thus, the modification of other wheat polymers, as gluten, must not

be neglected due to their impact on gelatinization properties of starch. Hence, a proper selection of suitable analysis methods is necessary to capture the interplay of starch, gluten and water. Conventional strategies should be questioned regarding their suitability to mechanistically elucidate polymer alterations in complex cereal matrices, as wheat flour. To complete previous investigations, gluten polymers should encapsulate starch granules, as present in wheat flours, to achieve a realistic behavior of starch and gluten during grinding. This requires, however, the development of a new method to create starch-gluten particles, without altering the original structure of polymers.

The shift of water from modified starch polymers to modified gluten polymers (Paulik, Wen Yu, et al., 2019) could additionally affect the water distribution and recrystallization of starch during storage of baked goods. Thus, a changed firming behavior is expected. The effects of modified starch and modified gluten on bread firming could furthermore be enhanced by the presence of enzymes or short-chain dextrin (see Figure 3.4 (Paulik & Jekle, 2019)), which are known to form complex interactions with wheat components and, thus, retard crumb firming (Błaszczak, Sadowska, Rosell, & Fornal, 2004; Durán, León, Barber, & Benedito de Barber, 2001; Rojas, Rosell, & Benedito de Barber, 2001). Beside an increased crumb void fraction caused by an enhanced gas formation rate, amylases and dextrin affect the gelatinization process (compare Figure 3.4). Finally, additives function in the crumb matrix by showing effects on recrystallization of amylopectin and probably water redistribution between cereal polymers (Paulik & Jekle, 2019).

Although detailed studies on the enzymatic hydrolysis of modified wheat flour are still missing, different effects of enzymes in physically modified, wheat based matrices can be hypothesized:

1. An enzymatic hydrolyzation of modified starch is hampered. Shift in water distribution from modified starch to modified gluten polymers leads to a reduction in available water content surrounding starch polymers, weakening the enzymatic degradation of starch.
2. An enzymatic hydrolyzation of modified starch is facilitated. Shift in water distribution from modified starch to modified gluten polymers leads to a reduction in available water content surrounding starch polymers, in which

amylases are concentratedly present. Thus, interactions of starch polymers and amylase are increased. This effect could be intensified, since starch surface and consequently enzymatic starch accessibility is increased, due to the particle size reduction of flours caused by mechanical flour treatments.

These effects highly depend on the water addition/ dough yield during dough preparation (Hackenberg, Leitner, Jekle, & Becker, 2018), necessitating detailed studies with varying water content in wheat flour dough.

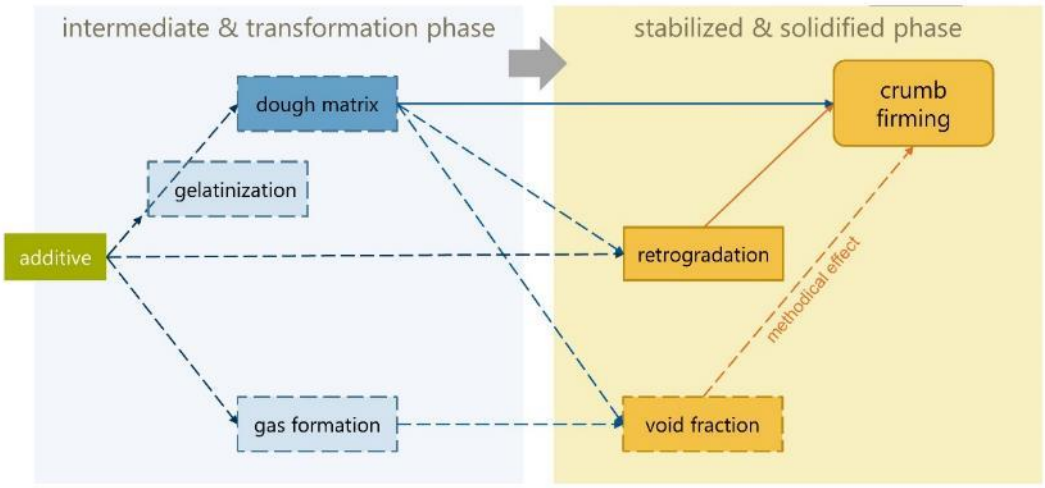

Figure 3.4: Potential mechanisms of (anti-staling) additives as amylases and short-chain dextrin during the dough phase (intermediate & transformation phase) and crumb phase (stabilized & solidified phase) regarding the anti-staling effects (Paulik & Jekle, 2019).

Especially when it comes to determine protein functionality and structure after physical treatment, non-fundamental, superficial methods are often used in cereal industry and research, for instance Z-kneaders, (DoughLab, Farinograph), Alveograph, Glutopeak or Extensograph. Those analysis allow to evaluate and adapt baking processes and often deliver highly transferable results to baking trials (Bouachra, Begemann, Aarab, & Hüsken, 2017; Dang & Bason, 2014; Rakita, Dokić, Dapčević Hadnađev, Hadnađev, & Torbica, 2018; Różyło & Laskowski, 2011; Simurina et al., 2016), but provide only little insights into the structural alterations of gluten polymers. Thus, fundamental methods, as Confocal Laser Scanning Microscopy (CLSM), where selective gluten proteins are stained, as shown in Figure 3.5 (S. Jakobi, Vogel, Jekle, Köhler, & Becker, 2017), are used to analyze and characterize protein network formation (Lucas, Stauner, Jekle, & Becker, 2018). Investigations mainly revealed an elevated increase in porosity of protein strands (CLSM measurement) with increase in starch modification

degree (measured with SDmatic, amperometric method), caused by grinding procedures of wheat flours (Hackenberg, Jekle, et al., 2018). However, modified gluten formation in wheat flours can either be evoked by a modification of gluten polymers themselves or by a modification of starch, which results in a rise in water retention of starch polymers and consequently a modified hydration of gluten (S. Jakobi et al., 2017). Thus, a modified gluten network formation cannot be directly traced back to an altered gluten structure.

On the other hand, structural analysis as the Osborne fractionation provide insights into the solubility behavior of gluten polymers, but less information about further functional properties of gluten.

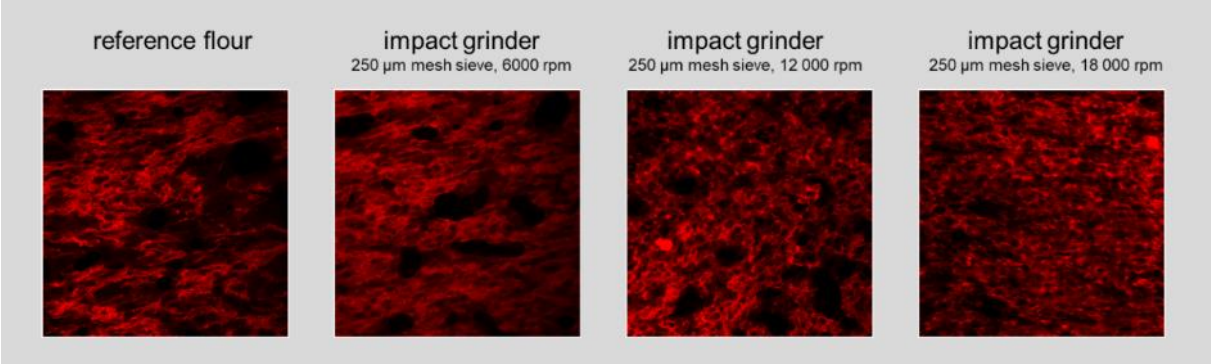

Figure 3.5: Gluten network formation in dough prepared of native and physically modified wheat flours in a impact mill at 6000, 12000, 18000 rpm. Gluten network was visualized using confocal laser scanning microscope (CLSM). Gluten polymers were stained using dye rhodamin B, which stains selectively proteins. Dye was solved in bulk water. Addition of bulk water was kept constant and was prior the experiments adapted to the reference to achieve 500 FU in Farinograph (modified from (S. Jakobi et al., 2017)).

In order to differentiate between a gluten or starch functionality and to exclude a manipulation of polymers among each other, starch or gluten should be extracted from the cereal matrix ‘wheat flour’. Therefore, a procedure has to be developed, which does not alter the functionality of starch or gluten during extraction. The mutual influence of starch and gluten become visible even in case of model dough, when modified gluten interacted, impeding a separate consideration of modifications. Only when both polymers were modified, visible alterations occurred. This was further indicated by the study of Paulik, Jekle and Becker (2019), which dealt with the influence of different physical forces on cereal biopolymers (Paulik, Jekle, & Becker, 2019b):

1. Dry mechanical (temperature-controlled impact mill), thermo-mechanical (impact mill with temperature development) or thermal treatment (up to 110 °C) of wheat flour as well as isolated wheat starch and isolated gluten revealed no alterations of gluten regarding the water retention capacity for all three treatment. However, changes in gelatinization enthalpy of starch-gluten model dough occurred, when modified gluten was used. Thus, WRC method was unable to capture modification in hydration properties of gluten polymers.
2. Mechanical and thermo-mechanical treatment caused a comparable rise in WRC of wheat flour and isolated starch, consequently modification of starch (isolated or in wheat flour) was evoked by mechanical forces during grinding.
3. The investigation showed an increase in gelatinization onset, intersection of the tangent and base line at the left side of the gelatinization peak, of wheat flours, however not for wheat starch, when samples were subjected to dry heat stress.

As shown in Figure 3.6, starch granules are embedded in wheat flour in a matrix consisting of proteins and other non-starch components. Consequently, alterations in the interplay of starch and gluten or other flour polymers occurred probably by shielding starch granules from water, causing a delayed gelatinization of starch in wheat flour. Due to matrix changes during grinding of wheat based materials, investigation on isolated materials would neglect modified interactions of starch with other cereal biopolymers.

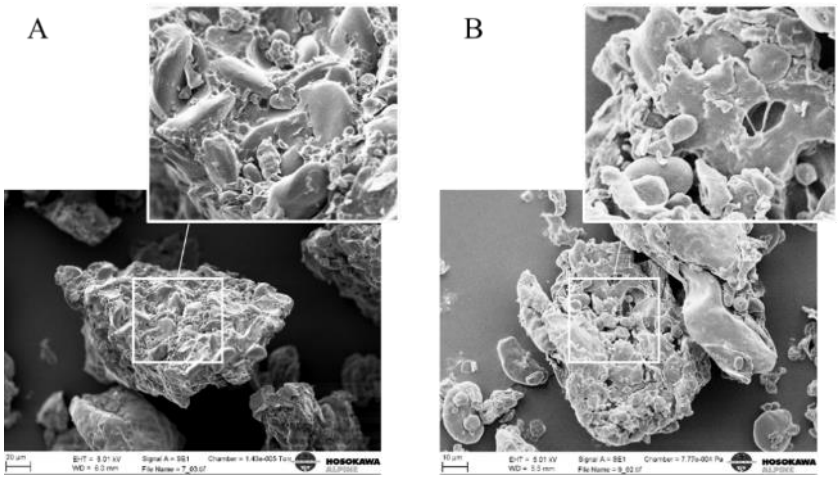

Figure 3.6: Scanning electron microscope (SEM) images of reference wheat flour 'Akteur' (A) and physically modified wheat flour 'Akteur' (B). Modification was performed at 18000 rpm using 80 µm mesh sieve in impact mill. Reference wheat flour A showed starch granules embedded in a rigid protein matrix, whereby grinding of wheat flour lead to a formation of a amylose network or gluten network-like

structure and the breaking out of starch granules. Images made by Hosokawa Alpine AG (modified from (S. Jakobi et al., 2017)).

Furthermore, WRC method of modified samples provides only information about the accessibility of wheat flour/ starch particles. When standard wheat flour was modified using an impact and a cryogenic mill, a linear dependency of WRC and the enzymatically determined starch modification degree was determined for both modification methods. Consequently, water retention capacity of wheat flours depends on the accessibility of starch particles (S. Jakobi et al., 2018). Alterations of gluten polymers regarding water retention capacity of flours, however, were neglected.

Therefore, sensitive methods should preferably be applied to capture alterations in water distribution and recrystallization. The suitability of methods recording global changes of polymer water interactions, for instance the water retention capacity, water binding capacity or water swelling capacity, should be reconsidered in elucidating altered polymer functionalities in dough and crumbs of complex cereal based matrices. As an alternative, NMR or X-ray methods should standardly be integrated in the analysis of food systems, which are already used in polymer science by default (Di Tullio, Capitani, & Proietti, 2018; Garcea, Wang, & Withers, 2018; Lv, Tian, Zhang, & Xiang, 2018; Roe, 2000; Sapiga & Sergeev, 2019; Tran, Lin, Chaurasia, & Lin, 2019; Wu et al., 2019; Zisti et al., 2019), but only sporadically applied in food science (Ariyantoro et al., 2018; Nielsen, Canibe, & Larsen, 2018). The sample preparation and the interpretation of NMR and X-ray results necessitate a lot of experience in systems with partially large raw material fluctuations, why the methods are only sporadically used for the study of food materials. Nonetheless, to understand the effects of mechanical treatments on water-gluten interactions, gluten polymers should be elucidated in detail.

In this thesis, analytical approaches for a systemic investigation of physically modified starches in cereal based foams were developed and applied to elucidate the hydration properties of physically modified wheat starch. Thus, it was possible to identify the mechanism of modified starch affecting the density of wheat based products: effects the higher water retention of physically modified starch in wheat flours lead to a rise in wheat dough viscosity and an early pasting of wheat dough during heating, increasing the density of the product. The increased hydration of physically modified starch was

based on the rise in starch accessibility, however, does not correlate to the particle size reduction of wheat flour particles. Consequently, the particle size of flours produced on different mills is not an appropriate tool to predict the hydration properties of wheat flours. The enhanced starch-water interaction at room temperature was caused solely by a mechanical modification of the starch. However, grinding in mills, where mechanical and thermal forces acting simultaneously on grain (products), caused additionally a modification of gluten, which became visible during heating of modified wheat flour slurries. A systemic model study revealed a tighter water binding of modified gluten, causing a reduced gelatinization enthalpy of physically modified starch-gluten matrices. Relations between a physical modification and resulting hydration characteristics of cereal biopolymers were revealed forming a solid basis for a further mechanistic elucidation of complex interactions in wheat flours and starch based matrices.

4 References

Abebe, W., Collar, C., & Ronda, F. (2015). Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours. *Carbohydrate Polymers*, *115*, 260–268. https://doi.org/10.1016/J.CARBPOL.2014.08.080

Addo, K. ., Coahran, D. ., & Pomeranz, Y. (1990). A New Parameter Related to Loaf Volume Based on the First Derivative of the Alveograph Curve. *Cereal Chemistry*, *67*(1), 64–69.

Aguilera, J. M., & Stanley, D. W. (1999). *Microstructural principles of food processing and engineering*. Aspen Publishers.

Altemimi, A. B. (2018). Extraction and Optimization of Potato Starch and Its Application as a Stabilizer in Yogurt Manufacturing. *Foods*, *7*(2), 14. https://doi.org/10.3390/foods7020014

Araki, E., Ikeda, T. M., Ashida, K., Takata, K., Yanaka, M., & Iida, S. (2009). Effects of Rice Flour Properties on Specific Loaf Volume of One-loaf Bread Made from Rice Flour with Wheat Vital Gluten. *Food Science and Technology Research*, *15*(4), 439–448. https://doi.org/10.3136/fstr.15.439

Ariyantoro, A. R., Katsuno, N., & Nishizu, T. (2018). Effects of Dual Modification with Succinylation and Annealing on Physicochemical, Thermal and Morphological Properties of Corn Starch. *Foods*, *7*(9), 133. https://doi.org/10.3390/foods7090133

Arvisenet, G. ., Bail, P. L., Voilley, A. ., & Cayot, N. . (2002). Influence of Physicochemical Interactions between Amylose and Aroma Compounds on the Retention of Aroma in Food-like Matrices. *Jorunal of Agricultural and Food Chemistry*, *50*(24), 7088–7093. https://doi.org/10.1021/JF0203601

Banerjee, S., & Bhattacharya, S. (2012). Food Gels: Gelling Process and New Applications. *Critical Reviews in Food Science and Nutrition*, *52*(4), 334–346. https://doi.org/10.1080/10408398.2010.500234

Bao, J., & Bergman, C. J. (2004). The functionality of rice starch. In *Starch in Food* (pp. 258–294). Elsevier. https://doi.org/10.1533/9781855739093.2.258

Bayarri, S., Izquierdo, L., Durán, L., & Costell, E. (2006). Effect of addition of sucrose and aspartame on the compression resistance of hydrocolloids gels. *International Journal of Food Science and Technology*, *41*(8), 980–986. https://doi.org/10.1111/j.1365-2621.2005.01156.x

BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydrate Polymers*, *86*(2), 386–423. https://doi.org/10.1016/J.CARBPOL.2011.05.064

Bertoft, E. (2017). Understanding Starch Structure: Recent Progress. *Agronomy*, *7*(3), 56. https://doi.org/10.3390/agronomy7030056

Błaszczak, W., Sadowska, J., Rosell, C. M., & Fornal, J. (2004). Structural changes in the wheat dough and bread with the addition of alpha-amylases. *European Food Research and Technology*, *219*(4), 348–354. https://doi.org/10.1007/s00217-004-0972-8

Blazek, J., & Copeland, L. (2008). Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydrate Polymers*, *71*(3), 380–387. https://doi.org/10.1016/J.CARBPOL.2007.06.010

Bouachra, S., Begemann, J., Aarab, L., & Hüsken, A. (2017). Prediction of bread wheat baking quality using an optimized GlutoPeak ® -Test method. *Journal of Cereal Science*, *76*, 8–16. https://doi.org/10.1016/j.jcs.2017.05.006

Brunnschweiler, J., Luethi, D., Handschin, S., Farah, Z., Escher, F., & Conde-Petit, B. (2005). Isolation, Physicochemical Characterization and Application of Yam (Dioscorea spp.) Starch as Thickening and Gelling Agent. *STARCH - ST*[illegible]*RKE*, *57*(3–4), 107–117. https://doi.org/10.1002/star.200400327

Chen, J., Deng, Z., Wu, P., Tian, J., & Xie, Q. (2010). Effect of Gluten on Pasting Properties of Wheat Starch. *Agricultural Sciences in China*, *9*(12), 1836–1844. https://doi.org/10.1016/S1671-2927(09)60283-2

Cho, S.-H., Lee, B.-H., & Eun, J.-B. (2019). Physicochemical properties of dry- and semi-wet-milled rice flours after fermentation by Lactobacillus amylovorus. *Journal of Cereal Science*, *85*, 15–19. https://doi.org/10.1016/J.JCS.2018.11.003

Cornell, H. (2004). The functionality of wheat starch. In *Starch in Food: Structure, Function and Applications* (pp. 211–240). CRC Press. Retrieved from https://books.google.com/books?id=6IO3eV7KfDIC&pgis=1

Dang, J. M. C., & Bason, M. L. (2014). AACCI Approved Methods Technical Committee Report: Collaborative Study on a Method for Determining the Mixing Properties of Dough Using High-Energy Mixing. *Http://Dx.Doi.Org/10.1094/CFW-58-4-0199*. https://doi.org/10.1094/CFW-58-4-0199

Davis, H., Skrzypek, W., & Khan, A. (1994). Iodine binding by amylopectin and stability of the amylopectin–iodine complex. *Journal of Polymer Science Part A: Polymer*

Chemistry, *32*(12), 2267–2274. https://doi.org/10.1002/pola.1994.080321208

Dengate, H. N., Baruch, D. W., & Meredith, P. (1978). The Density of Wheat Starch Granules: A Tracer Dilution Procedure for Determining the Density of an Immiscible Dispersed Phase. *Starch - Stärke*, *30*(3), 80–84. https://doi.org/10.1002/star.19780300304

Dhaka, V., & Khatkar, B. S. (2015). Effects of Gliadin/Glutenin and HMW-GS/LMW-GS Ratio on Dough Rheological Properties and Bread-Making Potential of Wheat Varieties. *Journal of Food Quality*, *38*(2), 71–82. https://doi.org/10.1111/jfq.12122

Dhital, S., Shrestha, A. K., Flanagan, B. M., Hasjim, J., & Gidley, M. J. (2011). Cryo-milling of starch granules leads to differential effects on molecular size and conformation. *Carbohydrate Polymers*, *84*(3), 1133–1140. https://doi.org/10.1016/J.CARBPOL.2011.01.002

Di Tullio, V., Capitani, D., & Proietti, N. (2018). Unilateral NMR to study water diffusion and absorption in stone-hydrogel systems. *Microporous and Mesoporous Materials*, *269*, 180–185. https://doi.org/10.1016/J.MICROMESO.2017.07.011

Donald, A., Kato, K. L., Perry, P., & Waigh, T. (2001). Scattering Studies of the Internal Structure of Starch Granules. *Starch*, *53*(10), 504–512. https://doi.org/10.1002/1521

dos santos, D. M. J., & da Silva, J. A. L. (2002). Influence of pentosans on the rheological and thermal properties of starch isolated from two different wheat varieties. In *Gums and Stabilisers for the Food Industry 11* (pp. 137–144). https://doi.org/10.1039/9781847551016-00137

Durán, E., León, A., Barber, B., & Benedito de Barber, C. (2001). Effect of low molecular weight dextrins on gelatinization and retrogradation of starch. *European Food Research and Technology*, *212*(2), 203–207. https://doi.org/10.1007/s002170000205

Durrani, C. M., & Donald, A. M. (1995). Physical characterisation of amylopectin gels. *Polymer Gels and Networks*, *3*(1), 1–27. https://doi.org/10.1016/0966-7822(94)00005-R

European Parliament, ; (2008). Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32008R1333#d1e32-31-1

Ferrero, C. (2017). Hydrocolloids in wheat breadmaking: A concise review. *Food*

Hydrocolloids, *68*, 15–22. https://doi.org/10.1016/J.FOODHYD.2016.11.044

Fiszman, S. M., Costell, E., & Durán, L. (1986). Effects of the addition of sucrose and cellulose on the compression behaviour of κ-carrageenan gels. *Food Hydrocolloids*, *1*(2), 113–120. https://doi.org/10.1016/S0268-005X(86)80013-3

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydrate Polymers*, *32*(3–4), 177–191. https://doi.org/10.1016/S0144-8617(97)00008-8

Garcea, S. C., Wang, Y., & Withers, P. J. (2018). X-ray computed tomography of polymer composites. *Composites Science and Technology*, *156*, 305–319. https://doi.org/10.1016/J.COMPSCITECH.2017.10.023

Goering, K. J., Fritts, D. H., & Eslick, R. F. (1973). A Study of Starch Granule Size and Distribution in 29 Barley Varieties. *Starch - Stärke*, *25*(9), 297–302. https://doi.org/10.1002/star.19730250904

González, L. C., Loubes, M. A., & Tolaba, M. P. (2018). Incidence of milling energy on dry-milling attributes of rice starch modified by planetary ball milling. *Food Hydrocolloids*, *82*, 155–163. https://doi.org/10.1016/J.FOODHYD.2018.03.051

Gunaratne, A., & Corke, H. (2016). Starch, Analysis of Quality. *Reference Module in Food Science*. https://doi.org/10.1016/B978-0-08-100596-5.00092-5

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

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

He, S., Qin, Y., Walid, E., Li, L., Cui, J., & Ma, Y. (2014). Effect of ball-milling on the physicochemical properties of maize starch. *Biotechnology Reports*, *3*, 54–59. https://doi.org/10.1016/J.BTRE.2014.06.004

Hong, J., Zeng, X.-A., Brennan, C. S., Brennan, M., & Han, Z. (2016). Recent Advances in Techniques for Starch Esters and the Applications: A Review. *Foods (Basel, Switzerland)*, *5*(3). https://doi.org/10.3390/foods5030050

Horstmann, S. W., Belz, M. C. E., Heitmann, M., Zannini, E., & Arendt, E. K. (2016). Fundamental Study on the Impact of Gluten-Free Starches on the Quality of Gluten-Free Model Breads. *Foods (Basel, Switzerland)*, *5*(2).

https://doi.org/10.3390/foods5020030

Horstmann, S. W., Lynch, K. M., & Arendt, E. K. (2017). Starch Characteristics Linked to Gluten-Free Products. *Foods (Basel, Switzerland)*, *6*(4). https://doi.org/10.3390/foods6040029

Huang, Z.-Q., Xie, X., Chen, Y., Lu, J., & Tong, Z.-F. (2008). Ball-milling treatment effect on physicochemical properties and features for cassava and maize starches. *Comptes Rendus Chimie*, *11*(1–2), 73–79. https://doi.org/10.1016/j.crci.2007.04.008

Imberty, A., & Perez, S. (1988). A revisit to the three-dimensional structure of B-type starch. *Biopolymers*, *27*(8), 1205–1221. https://doi.org/10.1002/bip.360270803

Jackson, D. S. (2003). STARCH | Structure, Properties, and Determination. *Encyclopedia of Food Sciences and Nutrition*, 5561–5567. https://doi.org/10.1016/B0-12-227055-X/01141-X

Jakobi, S., Jekle, M., & Becker, T. (2018). Direct link between specific structural levels of starch and hydration properties. *Carbohydrate Polymers*, *181*(1), 159–166. https://doi.org/10.1016/J.CARBPOL.2017.10.062

Jakobi, S., Jekle, M., & Becker, T. (2018). High-Pressure Treatment of Non-Hydrated Flour Affects Structural Characteristics and Hydration. *Foods*, *7*(5), 78. https://doi.org/10.3390/foods7050078

Jakobi, S., Vogel, C., Jekle, M., Köhler, P., & Becker, T. (2017). *FEI Intermediate report 18679*.

James, B., & Whistler, R. (2009). *Starch - Chemistry and Technology*. (B. James & R. Whistler, Eds.) (3rd ed.).

Jane, J. (2009). Structural Features of Starch Granules II. *Starch*, 193–236. https://doi.org/10.1016/B978-0-12-746275-2.00006-9

Jekle, M. (2018). Zum Stand der Wissenschaft in der heutigen Zeit. *Cereal Technology*, *4*, 288–291.

Jekle, M., Mühlberger, K., & Becker, T. (2016). Starch-gluten interactions during gelatinization and its functionality in dough like model systems. *Food Hydrocolloids*, *54*, 196–201. https://doi.org/10.1016

Jobling, S. (2004). Improving starch for food and industrial applications. *Current Opinion in Plant Biology*, *7*(2), 210–218. https://doi.org/10.1016/J.PBI.2003.12.001

Kaptso, K. G., Njintang, Y. N., Nguemtchouin, M. M. G., Scher, J., Hounhouigan, J., &

Mbofung, C. M. (2015). Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: bambara groundnut (Vigna subterranea). *Journal of Food Science and Technology*, *52*(8), 4915–24. https://doi.org/10.1007/s13197-014-1580-7

Kasaai, M. R. (2007). Calculation of Mark–Houwink–Sakurada (MHS) equation viscometric constants for chitosan in any solvent–temperature system using experimental reported viscometric constants data. *Carbohydrate Polymers*, *68*(3), 477–488. https://doi.org/10.1016/j.carbpol.2006.11.006

Kaufman, R. C., Wilson, J. D., Bean, S. R., Herald, T. J., & Shi, Y.-C. (2015). Development of a 96-well plate iodine binding assay for amylose content determination. *Carbohydrate Polymers*, *115*, 444–447. https://doi.org/10.1016/j.carbpol.2014.09.015

Khunae, P., Tran, T., & Sirivongpaisal, P. (2007). Effect of Heat-Moisture Treatment on Structural and Thermal Properties of Rice Starches Differing in Amylose Content. *Starch - Stärke*, *59*(12), 593–599. https://doi.org/10.1002/star.200700618

Kijima, N., Katumi, N., Takasago, T., Ikeda, T. M., Shimoyamada, M., & Nishikawa, M. (2015). Characterization of Rice Flour Milled with Water and Effects of Soaking Conditions. *Food Science and Technology Research*, *21*(6), 771–778. https://doi.org/10.3136/fstr.21.771

Knutson, C. A. (1990). Annealing of maize starches at elevated temperatures. *Cereal Chemistry*, *67*(4), 376.

Kohajdová, Z., & Karovičová, J. (2009). Application of hydrocolloids as baking improvers. *Chemical Papers*, *63*(1), 26–38. https://doi.org/10.2478/s11696-008-0085-0

Kong, F., & Singh, R. P. (2011). Chemical deterioration and physical instability of foods and beverages. *Food and Beverage Stability and Shelf Life*, 29–62. https://doi.org/10.1533/9780857092540.1.29

Krueger, B. R. ., Walker, C. E. ., Knutson, C. A., & Inglett, G. E. (1987). Differential scanning calorimetry of raw and annealed starch isolated from normal and mutant maize genotypes. *Cereal Chemistry*, *64*(3), 187. Retrieved from https://pubag.nal.usda.gov/catalog/23932

Krupa, U., Rosell, C. M. ., Sadowska, J., & Soral-Śmietana, M. (2010). Bean starch as ingredient for gluten-free bread. *Journal of Food Processing and Preservation*, *34*,

501–518. https://doi.org/10.1111/j.1745-4549.2009.00366.x

Kumar, C. S., Malleshi, N. G., & Bhattacharya, S. (2008). A Comparison of Selected Quality Attributes of Flours: Effects of Dry and Wet Grinding Methods. *International Journal of Food Properties*, *11*(4), 845–857. https://doi.org/10.1080/10942910701657686

Kumar, R., & Khatkar, B. S. (2017). Thermal, pasting and morphological properties of starch granules of wheat (Triticum aestivum L.) varieties. *Journal of Food Science and Technology*, *54*(8), 2403–2410. https://doi.org/10.1007/s13197-017-2681-x

Laaman, T. (2011). *Hydrocolloids in food processing*. Wiley-Blackwell.

Lazaridou, A. ., Vouris, D. G. ., Zoumpoulakis, P., & Biliaderis, C. G. . (2018). Physicochemical properties of jet milled wheat flours and doughs. *Food Hydrocolloids*, *80*, 111–121. https://doi.org/10.1016/J.FOODHYD.2018.01.044

Lee, Y.-T., Shim, M.-J., Goh, H.-K., Mok, C., & Puligundla, P. (2019). Effect of jet milling on the physicochemical properties, pasting properties, and in vitro starch digestibility of germinated brown rice flour. *Food Chemistry*, *282*, 164–168. https://doi.org/10.1016/J.FOODCHEM.2018.07.179

Li, H., Wen, Y., Wang, J., & Sun, B. (2018). Relations between chain-length distribution, molecular size, and amylose content of rice starches. *International Journal of Biological Macromolecules*, *120*, 2017–2025. https://doi.org/10.1016/J.IJBIOMAC.2018.09.204

Liu, C.; Liu, Lin; Li, Limin; Hao, Chunming; Zheng, X., & Bian, Ke; Zhang, Jie; Wang, X. (2015). Effects of different milling processes on whole wheat flour quality and performance in steamed bread making. *LWT - Food Science and Technology*, *62*(1), 310–318. https://doi.org/10.1016/J.LWT.2014.08.030

Liu, T. Y., Ma, Y., Yu, S. F., Shi, J., & Xue, S. (2011). The effect of ball milling treatment on structure and porosity of maize starch granule. *Innovative Food Science & Emerging Technologies*, *12*(4), 586–593. https://doi.org/10.1016/J.IFSET.2011.06.009

Liu, X. ., Xiao, X. ., Liu, P. ., Yu, L., Li, M., Zhou, S., & Xie, F. (2017). Shear degradation of corn starches with different amylose contents. *Food Hydrocolloids*, *66*, 199–205. https://doi.org/10.1016/J.FOODHYD.2016.11.023

Lucas, I., Stauner, B., Jekle, M., & Becker, T. (2018). Staining methods for dough systems – Impact on microstructure and functionality. *LWT*, *88*, 139–145. https://doi.org/10.1016/J.LWT.2017.10.010

Lv, C., Tian, H., Zhang, X., & Xiang, A. (2018). LF-NMR analysis of the water mobility, state and distribution in sorbitol plasticized polyvinyl alcohol films. *Polymer Testing*, *70*, 67–72. https://doi.org/10.1016/J.POLYMERTESTING.2018.06.024

Malumba, P., Massaux, C., Deroanne, C., Masimango, T., & Béra, F. (2009). Influence of drying temperature on functional properties of wet-milled starch granules. *Carbohydrate Polymers*, *75*(2), 299–306. https://doi.org/10.1016/J.CARBPOL.2008.07.027

Mandala, I. G. (2012). Viscoelastic Properties of Starch and Non-Starch Thickeners in Simple Mixtures or Model Food. In J. de Vicente (Ed.), *Viscoelasticity - From Theory to Biological Applications*. InTech. https://doi.org/10.5772/50221

Marcotte, M., Taherian Hoshahili, A. R., & Ramaswamy, H. S. (2001). Rheological properties of selected hydrocolloids as a function of concentration and temperature. *Food Research International*, *34*(8), 695–703. https://doi.org/10.1016/S0963-9969(01)00091-6

Martínez-Bustos, F., López-Soto, M., San Martín-Martínez, E., Zazueta-Morales, J. J., & Velez-Medina, J. J. (2007). Effects of high energy milling on some functional properties of jicama starch (Pachyrrhizus erosus L. Urban) and cassava starch (Manihot esculenta Crantz). *Journal of Food Engineering*, *78*(4), 1212–1220. https://doi.org/10.1016/J.JFOODENG.2005.10.043

Martínez, M., Oliete, B., & Gómez, M. (2013). Effect of the addition of extruded wheat flours on dough rheology and bread quality. *Journal of Cereal Science*, *57*(3), 424–429. https://doi.org/10.1016/j.jcs.2013.01.007

Megazymea Assay Procedure. (2018). *Starch damage AACC Method 76-31.01, K-SDAM 06/18*.

Milani, J., & Maleki, G. (2012). Hydrocolloids in Food Industry. In *Food Industrial Processes - Methods and Equipment*. InTech. https://doi.org/10.5772/32358

Miller, R. A., Maningat, C. C., & Hoseney, R. C. (2008). Modified Wheat Starches Increase Bread Yield. *Cereal Chemistry Journal*, *85*(6), 713–715. https://doi.org/10.1094/CCHEM-85-6-0713

Mills, E. N. C., Wilde, P. J., Salt, L. J., & Skeggs, P. (2003). Bubble Formation and Stabilization in Bread Dough. *Food and Bioproducts Processing*, *81*(3), 189–193. https://doi.org/10.1205/096030803322437956

Miskelly, D., & Suter, D. (2017). Assessing and Managing Wheat-Flour Quality Before, During and After Milling. In *Cereal Grains* (pp. 607–634). Woodhead Publishing.

https://doi.org/10.1016/B978-0-08-100719-8.00022-X

Mo, I., Go, O., & An, A. (2015). Production and Evaluation of Bread Made from Modified Cassava Starch and Wheat Flour Blends. *Agrotechnology*, *04*(01), 1–6. https://doi.org/10.4172/2168-9881.1000133

Mukerjea, R., & Robyt, J. F. (2010). Isolation, structure, and characterization of the putative soluble amyloses from potato, wheat, and rice starches. *Carbohydrate Research*, *345*(3), 449–451. https://doi.org/10.1016/j.carres.2009.11.021

Muñoz, L. A., Pedreschi, F., Leiva, A., & Aguilera, J. M. (2015). Loss of birefringence and swelling behavior in native starch granules: Microstructural and thermal properties. *Journal of Food Engineering*, *152*, 65–71. https://doi.org/10.1016/J.JFOODENG.2014.11.017

Ngamnikom, P., & Songsermpong, S. (2011). The effects of freeze, dry, and wet grinding processes on rice flour properties and their energy consumption. *Journal of Food Engineering*, *104*(4), 632–638. https://doi.org/10.1016/j.jfoodeng.2011.02.001

Nielsen, T. S., Canibe, N., & Larsen, F. H. (2018). Butyrylation of Maize and Potato Starches and Characterization of the Products by Nuclear Magnetic Resonance and In Vitro Fermentation. *Foods*, *7*(5), 79. https://doi.org/10.3390/foods7050079

Nishita, K. D.; Bean, M. M. (1982). Grinding Methods: Thier Impact on Rice Flour Properties. *Cereal Chemistry*, *59*(1), 46–49.

Niu, M., Zhang, B., Jia, C., & Zhao, S. (2017). Multi-scale structures and pasting characteristics of starch in whole-wheat flour treated by superfine grinding. *International Journal of Biological Macromolecules*, *104*, 837–845. https://doi.org/10.1016/J.IJBIOMAC.2017.06.125

Paulik, S., & Jekle, M. (2019). Novel approach to investigate the mechanical properties of crumb matrix during storage – Re-engineering of gas-free crumb pellets. *Food Chemistry*, *288*, 333–340. https://doi.org/10.1016/j.foodchem.2019.03.014

Paulik, S., Jekle, M., & Becker, T. (2019a). A review: Reverse approach to analyze the impact of starch modification on the inflation and gas holding properties of wheat-based matrices. *Trends in Food Science & Technology*, *91*, 231–239. https://doi.org/10.1016/J.TIFS.2019.07.031

Paulik, S., Jekle, M., & Becker, T. (2019b). Mechanically and Thermally Induced Degradation and Modification of Cereal Biopolymers during Grinding. *Polymers*, *11*(3), 448. https://doi.org/10.3390/polym11030448

Paulik, S., Jekle, M., Becker, T., Paulik, S., Jekle, M., & Becker, T. (2019). Mechanically and Thermally Induced Degradation and Modification of Cereal Biopolymers during Grinding. *Polymers*, *11*(3), 448. https://doi.org/10.3390/polym11030448

Paulik, S., Wen Yu, W., Flanagan, B., Gilbert, R. G., Jekle, M., & Becker, T. (2019). Characterizing the impact of starch and gluten-induced alterations on gelatinization behavior of physically modified model dough. *Food Chemistry*, 125276. https://doi.org/10.1016/J.FOODCHEM.2019.125276

Pauly, A., Pareyt, B., De Brier, N., Fierens, E., & Delcour, J. A. (2012). Starch isolation method impacts soft wheat (Triticum aestivum L. cv. Claire) starch puroindoline and lipid levels as well as its functional properties. *Journal of Cereal Science*, *56*(2), 464–469. https://doi.org/10.1016/J.JCS.2012.06.003

Peat, S. ., Whelan, W. J. ., & Thomas, G. J. . (1952). Evidence of multiple branching in waxy maize starch. *Journal of the Chemical Society (Resumed)*, 4536–4538. Retrieved from https://miami.pure.elsevier.com/en/publications/evidence-of-multiple-branching-in-waxy-maize-starch

Pérez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch - Stärke*, *62*(8), 389–420. https://doi.org/10.1002/star.201000013

Phillips, G. O., & Williams, P. A. (2009). *Handbook of Hydrocolloids.* Woodhead Pub.

Posner, E. S., & Hibbs, A. N. (Arthur N. (2005). *Wheat flour milling*. American Association of Cereal Chemists.

Rakita, S., Dokić, L., Dapčević Hadnađev, T., Hadnađev, M., & Torbica, A. (2018). Predicting rheological behavior and baking quality of wheat flour using a GlutoPeak test. *Journal of Texture Studies*, *49*(3), 339–347. https://doi.org/10.1111/jtxs.12308

Razavi, S. M. A. (2019). *Emerging natural hydrocolloids : rheology and functions*. Retrieved from https://www.wiley.com/en-us/Emerging+Natural+Hydrocolloids%3A+Rheology+and+Functions-p-9781119418665

Roe, R. J. (Ryong-J. (2000). *Methods of X-ray and neutron scattering in polymer science*. Oxford University Press. Retrieved from https://global.oup.com/academic/product/methods-of-x-ray-and-neutron-scattering-in-polymer-science-9780195113211?cc=us&lang=en&

Rojas, J. A., Rosell, C. M., & Benedito de Barber, C. (2001). Role of maltodextrins in the staling of starch gels. *European Food Research and Technology*, *212*(3), 364–368. https://doi.org/10.1007/s002170000218

Roman, L., Gomez, M., Hamaker, B. R., & Martinez, M. M. (2019). Banana starch and molecular shear fragmentation dramatically increase structurally driven slowly digestible starch in fully gelatinized bread crumb. *Food Chemistry*, *274*, 664–671. https://doi.org/10.1016/J.FOODCHEM.2018.09.023

Różyło, R., & Laskowski, J. (2011). Predicting Bread Quality (Bread Loaf Volume and Crumb Texture). *Polish Journal of Food and Nutrition Sciences*, *61*(1), 61–67. https://doi.org/10.2478/v10222-011-0006-8

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: a critical review. *Journal of Food Science and Technology*, *47*(6), 587–97. https://doi.org/10.1007/s13197-010-0162-6

Sanderson, J. S., Daniels, R. D., Donald, A. M., Blennow, A., & Engelsen, S. B. (2006). Exploratory SAXS and HPAEC-PAD studies of starches from diverse plant genotypes. *Carbohydrate Polymers*, *64*(3), 433–443. https://doi.org/10.1016/j.carbpol.2005.12.026

Sapiga, A. V., & Sergeev, N. A. (2019). NMR study of microscopic mechanism of water molecules diffusion in natrolite. *Solid State Nuclear Magnetic Resonance*, *97*, 1–6. https://doi.org/10.1016/J.SSNMR.2018.10.001

Schirmer, M., Höchstötter, A., Jekle, M., Arendt, E., & Becker, T. (2013). Physicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio. *Food Hydrocolloids*, *32*(1), 52–63. https://doi.org/10.1016/J.FOODHYD.2012.11.032

Seisun, D., Phillips, G. O., & Seisun, D. (2002). Overview of the hydrocolloid market (pp. 3–10). https://doi.org/10.1039/9781847551016-00003

Shen, Y., Zhang, N., Xu, Y., Huang, J., Yuan, M., Wu, D., & Shu, X. (2019). Physicochemical properties of hydroxypropylated and cross-linked rice starches differential in amylose content. *International Journal of Biological Macromolecules*, *128*, 775–781. https://doi.org/10.1016/J.IJBIOMAC.2019.01.194

Shewry, P. R., Halford, N. G., Belton, P. S., & Tatham, A. S. (2002). The structure and properties of gluten: an elastic protein from wheat grain. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*,

357(1418), 133–42. https://doi.org/10.1098/rstb.2001.1024

Shyichuk, A. V. (1997). Condition of gel-formation under crosslinking with simultaneous scission of macromolecules. *European Polymer Journal*, *33*(8), 1267–1269. https://doi.org/10.1016/S0014-3057(96)00273-X

Simurina, O., Filipcev, B., Belic, Z., Jambrec, D., Krulj, J., Brkljaca, J., & Pestoric, M. (2016). The influence of plant protein on the properties of dough and the quality of wholemeal spelt bread. *Croatian Journal of Food Science and Technology*, *8*(2), 107–111. https://doi.org/10.17508/CJFST.2016.8.2.10

Singh, A. V. ; Nath, L. K. ; Singh, A. (2010). Pharmaceutical, food and non-food applications of modified starches: a critical review. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *9*(7), 1214–1221. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20103366090

Srichuwong, S., Curti, D., Austin, S., King, R., Lamothe, L., & Gloria-Hernandez, H. (2017). Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. *Food Chemistry*, *233*, 1–10. https://doi.org/10.1016/J.FOODCHEM.2017.04.019

Srirejeki, S., Manuhara, G. J., Amanto, B. S., Atmaka, W., & Laksono, P. W. (2018). The effect of water volume and mixing time on physical properties of bread made from modified cassava starch-wheat composite flour. *IOP Conference Series: Materials Science and Engineering*, *333*, 012072. https://doi.org/10.1088/1757-899X/333/1/012072

Tan, I. (2014). “Structure-Property” Relationships of Genetically Modified Starch. *Starch Polymers*, 31–75. https://doi.org/10.1016/B978-0-444-53730-0.00020-8

Tang, H., Mitsunaga, T., & Kawamura, Y. (2006). Molecular arrangement in blocklets and starch granule architecture. *Carbohydrate Polymers*, *63*(4), 555–560. https://doi.org/10.1016/j.carbpol.2005.10.016

Tegge, G. (2004). *Stärke und Stärkederivat*. Hamburg: Behr’ s GmbH.

Tharanathan, R. N. (2005). Starch — Value Addition by Modification. *Critical Reviews in Food Science and Nutrition*, *45*(5), 371–384. https://doi.org/10.1080/10408390590967702

Tian, S., Chen, Y., Chen, Z., Yang, Y., & Wang, Y. (2018). Preparation and Characteristics of Starch Esters and Its Effects on Dough Physicochemical Properties. *Journal of Food Quality*, *2018*, 1–7.

https://doi.org/10.1155/2018/1395978

Tran, T., Lin, C., Chaurasia, S., & Lin, H. (2019). Elucidating the relationship between states of water and ion transport properties in hydrated polymers. *Journal of Membrane Science*, *574*, 299–308. https://doi.org/10.1016/J.MEMSCI.2018.12.059

Van Hung, P., Maeda, T., & Morita, N. (2006). Waxy and high-amylose wheat starches and flours—characteristics, functionality and application. *Trends in Food Science & Technology*, *17*(8), 448–456. https://doi.org/10.1016/J.TIFS.2005.12.006

Williams, P. A. ., Phillips, G. O. ., & de Vries, J. (2004). Hydrocolloid gelling agents and their applications. In *Gums and Stabilisers for the Food Industry 12* (pp. 23–31). https://doi.org/10.1039/9781847551214-00023

Wu, Y., Tahmasebi, P., Lin, C., Zahid, M. A., Dong, C., Golab, A. N., & Ren, L. (2019). A comprehensive study on geometric, topological and fractal characterizations of pore systems in low-permeability reservoirs based on SEM, MICP, NMR, and X-ray CT experiments. *Marine and Petroleum Geology*, *103*, 12–28. https://doi.org/10.1016/J.MARPETGEO.2019.02.003

Wüstenberg, T. (Ed.). (2014). *Cellulose and Cellulose Derivatives in the Food Industry*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/9783527682935

Yu, J., Wang, S., Wang, J., Li, C., Xin, Q., Huang, W., … Wang, S. (2015). Effect of laboratory milling on properties of starches isolated from different flour millstreams of hard and soft wheat. *Food Chemistry*, *172*, 504–514. https://doi.org/10.1016/J.FOODCHEM.2014.09.070

Zhang, G., Venkatachalam, M., & Hamaker, B. R. (2008). Methods to slow starch digestion rate in functional cereal products. *Technology of Functional Cereal Products*, 518–537. https://doi.org/10.1533/9781845693886.2.518

Zhu, T., Jackson, D. S., Wehling, R. L., & Geera, B. (2008). Comparison of Amylose Determination Methods and the Development of a Dual Wavelength Iodine Binding Technique. *Cereal Chemistry Journal*, *85*(1), 51–58. https://doi.org/10.1094/CCHEM-85-1-0051

Zia-ud-Din, Xiong, H., & Fei, P. (2017). Physical and chemical modification of starches: A review. *Critical Reviews in Food Science and Nutrition*, *57*(12), 2691–2705. https://doi.org/10.1080/10408398.2015.1087379

Žilić, S. (2013). Wheat Gluten: Composition and Health Effects. In D. B. Walter (Ed.),

Gluten : Sources, Composition and Health Effects (pp. 71–86). Nova Science Publishers, Inc. Retrieved from https://www.researchgate.net/publication/258820530_Wheat_Gluten_Composition_and_Health_Effects

Zisti, F., Tehrani, A. A., Alizadeh, R., Abbasi, H., Morsali, A., & Eichhorn, S. H. (2019). Synthesis and structural characterization of three nano-structured Ag(I) coordination polymers; Syntheses, characterization and X-ray crystal structural analysis. *Journal of Solid State Chemistry*, *271*, 29–39. https://doi.org/10.1016/J.JSSC.2018.12.049

5 Appendix

5.1 Non-peer-reviewed paper

1. Jakobi, S., Jekle, M., Becker, T.. Controlled flour functionality. The effect of various kinds of milling and grinding on the structural modifications of starches and consequently on the technological properties of doughs.. baking+biscuit. **2017**. 4. 62-66.
2. Jakobi, S., Schaufler, M., Jekle, M., Becker, T.. Modification of starch by high pressure. baking+biscuit. **2017**. 6. 48.
3. Jakobi, S., Schaufler, M., Jekle, M., Becker, T.. Modifikation von Stärke durch Hochdruck. Brot+Backwaren. **2018**. 4. 32.
4. Jakobi, S., Jekle, M., Becker, T.. Insects in bakery – curse and savior at the same time. Baking + biscuit international - The future of baking . **2018**. 32-35.
5. Paulik, S., Jekle, M., Becker, T.. Insekten als Rohstoff. Brot+Backwaren 1 (2020), 38-41.

5.2 Oral presentation

6. Jakobi, S., Jekle, M., Becker, T.. Funktionalität mechanisch modifizierter Stärken. 5. WIG Frühjahrstagung. 2016
7. Jakobi, S., Jekle, M., Becker, T.. Auswirkungen mechanisch induzierter Veränderungen von Stärke auf die Hydratationseigenschaften von Weizenmehlen. GDL-Kongress Lebensmitteltechnologie 2016. 2016
8. Jakobi, S., Jekle, M., Becker, T.. Influence of mechanical stress on starch structure and functionality. 15th European Young Cereal Scientists and Technologists Workshop. 2016
9. Jakobi, S., Vogel, C., Köhler, P., Jekle, M., Becker, T.. Physical modification of cereal biopolymers during milling – distinction between mechanically and thermally induced alterations. Innovations in Food Science & Technology. 2017
10. Jakobi, S., Jekle, M., Becker, T.. Mechanisch oder thermisch? Wie physikalische Kräfte bei der Vermahlung die Stärkemodifikation beeinflussen. 6. WIG Frühjahrstagung. 2017

11. Jekle, M., Jakobi, S., Becker, T.. Enzyme in Backwaren: Was wir schon wissen und was wir noch wissen müssen. 10. Wissenschaftliches Symposium des VGMS . Würzburg, Germany. 2017
12. Jakobi, S., Jekle, M., Becker, T.. Physical modification of flour - distinction between mechanically and thermally induced alterations . 69th Starch Convention . Detmold, Germany. 2018
13. Jakobi, S., Jekle, M., Becker, T.. Superfood zur Verbesserung nutritiver und funktioneller Eigenschaften von Teigen und Backwaren. 7. WIG Frühjahrstagung. Freising, Germany. 2018
14. Jakobi, S., Becker, T., Jekle, M.. Fluch und Segen der Stärkebeschädigung – Einfluss der Nachvermahlung auf die Teig- und Backwarenqualität. VGMS-Getreidetagung 2018 – Fragen und Antworten zur Zukunft des Ackerbaus. Freising, Germany. 2018
15. Jakobi, S., Jekle, M., Becker, T.. Praxisvortrag: Superfood zur Verbesserung nutritiver und funktioneller Eigenschaften von Teigen und Backwaren. 7. WIG Frühjahrstagung. Freising, Germany. 2018
16. Jakobi, S., Jekle, M., Becker, T.. Physical modification of cereal biopolymers during grinding – suitable method for decoding structure-function relationships of wheat based matrices. AACC International Annual Meeting. London, UK. 2018