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Aeration strategies and identification of foam stabilization mechanisms for gluten-free dough systems

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Aerated foods therefore create desires among consumers that producers struggle to satisfy, demands that are not trivial to supply … . It follows, then, that these most valued and most challenging of foods demand the greatest mastery of food science and technology

Grant M Campbell

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Preface

Dominique Dana Elgeti

M. Sc.

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 September 2011 to April 2016.

Publications

The following peer reviewed publications (shown in chronological order) were generated in the period of this work. Publications, which are part of this thesis are in bold. The cumulative impact factor for 2015 is 20.25 (12.95 for the publications of this thesis).

1 Elgeti, D., Nordlohne, S.D., Föste, M., Besl, M., Linden, M.H., Heinz, V., Jekle, M., Becker, T.: **Volume and texture improvement of gluten-free bread using quinoa white flour.** Journal of Cereal Science 59 (2014), 41-47.

2 Föste, M., Nordlohne, S.D., Elgeti, D., Linden, M.H., Heinz, V., Jekle, M., Becker, T.: Impact of quinoa bran on gluten-free dough and bread characteristics. European Food Research and Technology 239 (2014), 767-775.

3 Föste, M., Elgeti, D., Brunner, A.K., Jekle, M., Becker, T.: Isolation of quinoa protein by milling fractionation and solvent extraction. Food and Bioproducts Processing 96 (2015), 20-26.

4 Elgeti, D., Jekle, M., Becker, T.: **Strategies for the aeration of gluten-free bread - A Review.** Trends in Food Science & Technology 46 (2015), 75-84.

5 Verheyen, C., Albrecht, A., Elgeti, E., M., Jekle, M., Becker, T.: Impact of gas formation kinetics on dough development and bread quality. Food Research International 76 (2015), 860–866.

6 Elgeti, D., Yu, L., Stüttgen, A., Jekle, M., Becker, T.: **Interrelation between mechanical and biological aeration in starch-based gluten-free dough systems**. Journal of Cereal Sciences 76 (2017), 28-34.

7 Elgeti, D., Peng, L., Jekle, M., Becker, T.: **Foam stabilization during processing of starch-based dough systems.** Innovative Food Science and Emerging Technologies 39 (2017), 267-274.

Contents

Abbreviations

μCT	micro–computed tomography
γ	surface tension
APC	antigen presenting cells
ATI	amylase/trypsin inhibitors
BU	Brabender Units
CD	celiac disease
CMC	carboxy methyl cellulose
d	diameter
DATEM	diacetyl tartaric acid ester of mono- and diglycerides
DS	degree of substitution
ELISA	enzyme-linked immune sorbent assay
EU	European Union
FODMAPs	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
G^*	complex modulus
G'	elastic (storage) modulus
G''	viscous (loss) modulus
GFD	gluten-free diet
HLA	human leukocyte antigens (e.g. HLA-DQ)
HLB	hydrophilic-lipophilic balance
HPMC	hydroxy propyl methyl cellulose
IBS	irritable bowel syndrome
Ig	immunoglobulin
MW	molecular weight
n	number/…
NCGS	non-celiac gluten sensitivity
O/W	oil-in-water
p	pressure
pH	decimal logarithm of the reciprocal of the hydrogen ion activity
ppm	parts per million
R	gas constant
R, r	radius
R^2	coefficient of determination
Re	Reynolds number
rel.	relative
rpm	rotations per minute

S. cerevisiae	*Saccharomyces cerevisiae*
SD	standard deviation
SSL	sodium stearoyl lactylate
T	temperature
t	time
T cell	type of lymphocyte
TG	tissue transglutaminase
TPA	Texture Profile Analyzer
UK	United Kingdom
US	United States of America
V	volume
v	velocity
W/O	water-in-oil
x	distance
η	viscosity
ρ	density
Φ	gas volume fraction
$\dot{\varepsilon}_H$	Hencky strain
$tan\ \sigma$	loss factor
$\dot{\gamma}$	shear rate
Ω	speed

Summary

People with gluten-related indispositions depend on the availability of alternative food products, which in the case of bread still have major quality deficits. In starch-based dough systems, the entrapment and stabilization of gas bubbles present central challenges for gaining bread with high volume and fine pores. Thus, the goal of this thesis was to identify mechanisms of foam stabilization in the gluten-free system and to increase the level of aeration.

Initially, the suitability of different food aeration techniques was reviewed. In particular, the current heterogeneity of mixing parameters for gluten-free dough production seemed problematic and advantages of mechanical aeration similar to cake batter whipping became apparent. Moreover, centrifugation was identified as a suitable method for determining the gas-free dough density, which is a prerequisite for assessing the level of gas entrapment. Successful biological aeration depends on the ability of yeast strains to survive in specific gluten-free media and the availability of substrates for gas production.

In search of new raw materials for gluten-free bread, a previously developed fractionation process was used to obtain a white flour without bran components from the pseudocereal, quinoa. An exchange of rice and corn flour by this milling product strongly increased the gas input during fermentation. The activity of glucosidase in quinoa white flour significantly exceeded the one of rice and corn flour resulting in a higher availability of substrates for yeast fermentation. In addition to 33% volume increase, the use of quinoa white flour enhanced the gas retention, reduced the crumb hardness and improved the bread colouring, all of which was related to the removal of the bran.

Moreover, quinoa flour without bran almost doubled the gas inclusion during mixing and considerably facilitated bubble stabilization. The availability of this recipe with superior gas retention also provided the basis for consecutive mixing trials. The latter were performed without yeast by varying processing parameters and water content to maximize mechanical aeration independently from the yeast metabolism. By exchanging the dough hook by a wire whip together with an increase of the mixing speed from 200-420 rpm, 60% (rel.) more gas was entrained into the dough. The water content only affected dough viscosity, energy input and temperature, but not the level of aeration during mixing. A strong relation between energy input and dough temperature was established ($R^2 = 0.98$). In the next trials, yeast fermentation and baking enabled an evaluation of the effect of mechanical aeration on the final bread porosity. However, dough temperature variations superimposed the impact of processing parameters or medium properties, by directly influencing yeast activity and, in turn, bread volume ($R^2 = 0.88$). Thus, it was important to become independent from the substrate availability of ingredients and the energy input during mixing. A method for standardizing the biological aeration was developed.

As a result the specific bread volume was raised by 18% only through the improved mechanical aeration, when exchanging the dough hook by a wire whip and increasing the mixing speed.

Currently, the huge variety of recipes used by industries and researchers makes it difficult to compare and generalize obtained results. Therefore, the final research focus was on the identification of rheological properties that promote bubble stabilization throughout mixing, proofing and baking. A fundamental rheological program was established to simulate the prevailing temperature and shear conditions during dough processing and baking in a rotational rheometer. Flour type, water and hydrocolloid (HPMC) content were varied to obtain a broad range of viscosities (15–750 Pa s). These data were correlated with the gas volume fraction of doughs (3-21%) and the bread densities (0.42-0.21 g/ml). The best results for volume and pore structure were obtained with quinoa white flour or refined rice flour and 2% HPMC. Shear-thinning promoted gas entrapment and was more relevant for the mechanical aeration ($R^2 = 0.74$) than the absolute viscosity. As hypothesized, a higher viscosity during fermentation and the end of baking, the higher the bread volume ($R^2 = 0.72$) and smaller the pores ($R^2 = 0.68$), respectively.

In summary, successful methods to increase mechanical and biological aeration of gluten-free bread were developed and valuable insights into foam stabilization mechanisms were revealed. By combining the novel aeration method with the right choice of functional ingredients, the level of aeration was elevated to the one of wheat pan bread. For future studies, gas bubbles in dough should be monitored via μCT and the headspace atmosphere during mixing should modified. Based on promising preliminary data these techniques might further complement the valuable findings of the present thesis.

Zusammenfassung

Personen mit einer Sensitivität gegenüber glutenhaltigen Nahrungsmitteln sind von der Verfügbarkeit von alternativen Produkten abhängig. Diese weisen besonders im Fall von Brot noch immer deutliche Qualitätsdefizite auf. In stärkebasierten Teigsystemen stellen der Gaseintrag und die Stabilisierung von Gasblasen zentrale Herausforderungen dar, um Brot mit hohem Volumen und feiner Porung zu erhalten. Daher war es Ziel dieser Arbeit Mechanismen der Schaumstabilisierung in glutenfreien Teigen aufzuklären und den Gaseintrag zu erhöhen.

Anfangs wurde die Eignung von verschiedenen Methoden des Gaseintrags in Lebensmittel evaluiert. Speziell die aktuelle Heterogenität von Mixparametern schien bei glutenfreien Broten problematisch und die Vorteile eines mechanischen Gaseintrags, ähnlich des Aufschlagens von Kuchenteig wurden deutlich. Darüber hinaus wurde die Zentrifugation als geeignete Methode identifiziert um die gasfreie Teigdichte zu bestimmen, was eine Voraussetzung für die Bewertung des Gaseintrags darstellt. Eine erfolgreiche biologische Lockerung hängt zum einen von der Fähigkeit von Hefestämmen ab in spezifischen glutenfreien Medien zu überleben und zum anderen von der Verfügbarkeit der Substraten für die Gasproduktion.

Der Austausch von Reis- und Maismehl durch Mahlprodukte mit höherer Verfügbarkeit an Hefesubstraten, wie Quinoamehl, führte daher zu deutlich mehr Gaseintrag während der Fermentation. Die Aktivität von Glukosidase war in Quinoa signifikant höher als in Reis- und Maismehl. Zusätzlich zu 33% Volumensteigerung, förderte der Einsatz dieser fraktionierten Pseudocerealie die Gasrückhaltung, verminderte die Krumenhärte und verbesserte die Färbung der Brote. Alle Effekte hingen mit dem Entfernen der Kleie zusammen.

Des Weiteren bewirkte Quinoamehl ohne Kleie einen nahezu verdoppelten Gaseintrag während des Mixens und erleichterte die Gasblasenstabilisierung. Dieses Rezept mit hoher Gasrückhaltefähigkeit stellte die Basis für nachfolgende Aufschlagversuche dar. Die Versuche wurden ohne Hefe mit verschiedenen Prozesseinstellungen und Wassermengen durchgeführt um den mechanischen Gaseintrag unabhängig von der Hefeaktivität zu maximieren. Durch den Austausch des Knethakens durch einen Schneebesen und eine Erhöhung der Mixgeschwindigkeit von 200-420 rpm wurde 60% (rel.) mehr Gas in den Teig eingeschlagen. Der Wassergehalt beeinflusste die Viskosität, den Energieeintrag und die Temperatur, aber nicht den Gaseintrag beim Mixen. Es zeigte sich eine starke Abhängigkeit zwischen Energieeintrag und Teigtemperatur (R^2 = 0.98). In den folgenden Versuchen wurde im Anschluss an die Fermentation mit Hefe und den Backprozess der Einfluss des mechanischen Gaseintrags auf die finale Brotporung analysiert. Allerdings überlagerten die Variationen der Teigtemperatur die Effekte der Prozessparameter und Medieneigenschaften. Die Teigtemperatur hatte einen direkten Einfluss auf die Hefeaktivität und folglich auf das Brotvolumen (R^2 = 0.88).

Daher war es wichtig von der Substratverfügbarkeit der Inhaltsstoffe und dem Energieeintrag während des Mixens unabhängig zu werden. Es wurde eine Methode entwickelt um den biologischen Gaseintrag zu standardisieren. Als Ergebnis wurde alleine durch den verbesserten mechanischen Gaseintrag, mittels Austausch von Knethaken gegen Schneebesen und schnellerem Mixen, das spezifische Brotvolumen um 18% erhöht.

Aktuell behindern die weitreichenden Rezeptvariationen, welche von der Industrie und Forschern verwendet werden, die Übertragbarkeit und Allgemeingültigkeit von Ergebnissen bei glutenfreien Broten. Daher lag der finale Forschungsfokus auf der Identifizierung von rheologischen Eigenschaften, welche die Stabilisierung von Gasblasen während Mixen, Fermentation und Backen unterstützen. Ein fundamentales rheologisches Programm wurde entwickelt um die Temperatur- und Scherbedingungen, welche bei der Teigverarbeitung und dem Backen vorherrschen in einem Rotationsrheometer zu simulieren. Die Art des Mehles, sowie der Gehalt an Wasser und Hydrokolloid (HPMC) wurden variiert, um eine große Bandbreite an Viskositäten zu erhalten (15–750 Pa s). Diese Daten wurden mit dem Gasvolumenanteil der Teige (3-21%) und den Brotdichten (0.42-0.21 g/ml) korreliert. Die besten Ergebnisse bezüglich Volumen und Porung wurden mit Quinoaweißmehl oder raffiniertem Reismehl und 2% HPMC erhalten. Die Scherverdünnung der Teige förderte den Gaseintrag und war für den Erfolg beim Aufschlagen von größerer Bedeutung als die absolute Viskosität. Je höher die Viskosität während Fermentation und am Ende des Backprozesses war, desto höher war das Volumen der Brote ($R^2 = 0.72$) und desto kleiner waren die Poren ($R^2 = 0.68$).

Zusammenfassend wurden erfolgreiche Methoden entwickelt um den mechanischen und biologischen Gaseintrag in glutenfreie Teigsysteme zu erhöhen und es wurden wertvolle Einblicke in die Mechanismen zur Schaumstabilisierung aufgedeckt. Durch die Kombination aus einem neuartigen Aufschlagprozess zusammen mit der richtigen Auswahl an funktionellen Inhaltsstoffen wurde das Volumen der glutenfreien Brote auf den Level von Weizenkastenbroten angehoben. Für zukünftige Untersuchungen, sollten die Gasblasen im Teig mittels μCT untersucht werden und die Atmosphäre im Kopfraum des Mixers sollte modifiziert werden. Basierend auf vielversprechenden Vorversuchen könnten diese Techniken die wertvollen Erkenntnisse dieser Arbeit ergänzen.

1 Introduction

The size of the gluten-free packaged food market is expected to continue growing with a compound annual growth rate of 8.9% from 2016 to 2020 (Euromonitor, 2017). This trend reflects the steadily rising demand of gluten-free products, of which bread constitutes to over 33% of the global sales (Euromonitor, 2017). Thus, the development of innovative strategies for the improvement of current product deficits presents an important research topic. Indeed, also the number of publications in the area of gluten-free bread making has substantially increased in recent years (Figure 1). Gas entrapment and stabilization are among the most critical challenges for the production of gluten-free bread. Without a viscoelastic protein network, the retention of gas bubbles is strongly impaired. Consequently, the products often suffer from lower volume and irregular pores, when compared to wheat pan bread. The present study aimed at improving these features by means of ingredient and processing innovations as well as by new insights into gas stabilization mechanisms of starch-based cereal foams.

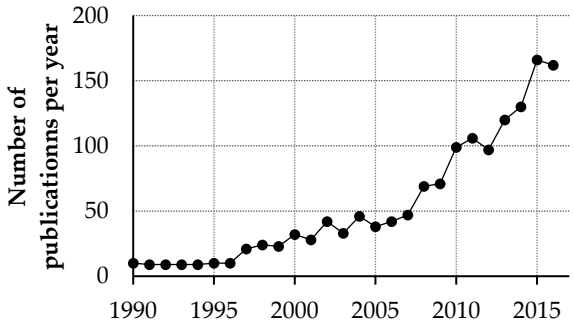

Figure 1. Development of annual gluten-free research publications. The graph presents the number of publications on gluten-free bread making per year that were found by searching journal articles containing "gluten-free" in title, abstract or key words within the scientific platform, sciencedirect.com.

In the following chapters the reasons for choosing a gluten-free diet are discussed prior to a presentation of processing challenges and current deficits of gluten-free bread, in particular regarding its porosity. A subsequent evaluation of the functionality of ingredients focuses on the recipes used in this study and merges with an outline of medium properties relevant for gas stabilization. Finally, the thesis outline is presented.

1.1 Disorders related to cereal consumption

Typically, people choose gluten-free to improve their health and well-being. Unfortunately, a considerable disconcertion prevails in the population regarding the benefits of gluten-free nutrition. In his popular book "Wheat Belly" from 2011, William Davis relates widespread diseases, like obesity, to wheat consumption. A gluten-free diet has been connected to weight-loss, despite the fact that respective products typically contain more lipids and carbohydrates (Hager *et al.* 2011). In some cases wheat has been blamed for illnesses without scientific proof, creating a mostly irrational fear of gluten (Jones 2012).

In fact, wheat contributes to a healthy diet, since it is rich in B-vitamins, minerals and trace elements (Belitz *et al.* 2001). Thus, for the majority of the population the benefits of a cereal-free diet are questionable.

Nevertheless, there are evidently several diseases with mild to severe manifestations, for which a gluten-free diet is the only effective treatment. A distinct diagnosis is often problematic because of many overlapping symptoms and gaps in knowledge. However, in recent years, research has contributed significant new insights into prevalence, diagnosis and pathomechanisms that are summarized in the following section.

Celiac disease, gluten ataxia and dermatitis herpetiformis

In patients with celiac disease (CD), the consumption of gluten triggers chronic inflammation of the small intestine through an autoimmune reaction (Feighery 1999). The high content in proline and glutamine impairs the enzymatic digestibility of gluten. In a healthy individual, this merely affects the bioavailability, but in case of a reduced barrier function of the small intestine, gluten peptides are able to reach the *lamina propria*. The initial absorption of gluten peptides is not completely understood, yet (Wieser *et al.* 2014). Figure 2 summarizes the pathomechanism of coeliac disease. Ultimately, the gluten uptake may lead to an autoimmune response involving epithelial apoptosis and substantial reduction of the mucosal surface (Compilato *et al.* 2010). In turn, an impaired absorption of essential nutrients can lay the foundation for a wide variety of symptoms and nearly four-fold higher mortality (Rubio–Tapia *et al.* 2009, West *et al.* 2004). In addition to gastrointestinal problems, such as diarrhea, vomiting and bloating, the deficient uptake of e.g. iron and calcium can cause anemia, osteoporosis and dental defects (Fasano and Catassi 2012).

CD is a multifactorial disease strongly correlating to the expression of genes coding for HLA-DG (Wieser *et al.* 2014). This explains the ten-times elevated prevalence of CD in first-degree relatives. While 30% of the general population have the necessary genes, which can be identified with HLA-DQ markers, this predisposition is not sufficient to cause CD. Several other factors contribute to the onset of the disease, such as the age and dose at the initial introduction of infants to gluten and the overall health condition (Norris *et al.* 2005, Stene *et al.* 2006). Historically, CD, which is also known as sprue, was thought to especially occur during childhood (Gee *et al.* 1888). Today, the prevalence in adults has risen, giving an overall prevalence of 1:160 for all ages, but with a higher ratio for women (Biagi *et al.* 2010). According to the iceberg model, first drafted by Logan in 1992, only a very small proportion of patients, the tip of the iceberg, have been diagnosed with CD, while the majority remain undiagnosed, partly, due to absent or uncommon symptoms. Both, glutenin and gliadin molecules were found to trigger CD, but several gaps in knowledge exist regarding single peptide sequences and their activities (Wieser *et al.* 2014).

Rubio-Tapia *et al.* (2009) analyzed celiac markers in over fifty year old blood samples, revealing that the rate of undiagnosed CD has then been only 0.2%, which indicates a four-fold increase until today. Consequently, also a future accumulation of cases is likely.

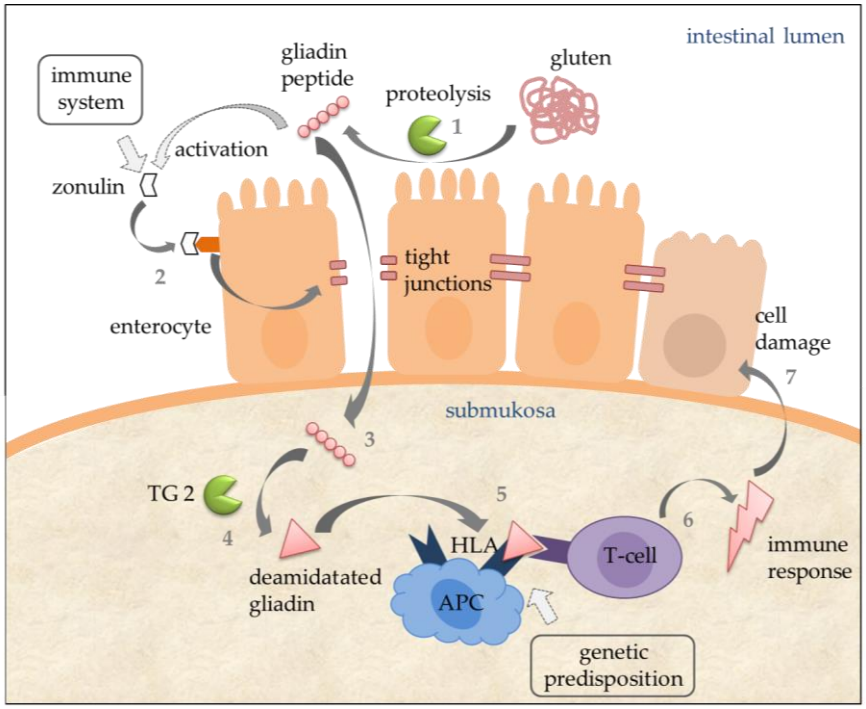

Figure 2. Pathomechanism of coeliac disease. (1) Dietary gluten entering the intestinal lumen is partly digested by proteases. Resulting gliadin peptides cannot enter a healthy mucosa. Only when zonulin production is triggered through gliadin or an activated immune system, tight junctions are degraded (2) and gliadin peptides reach the submucosa (3). Here, tissue transglutaminase 2 (TG2) deamidates the peptide (4), which increases its affinity towards human leukocyte antigens (HLA) on top of antigen presenting cells (APC) (5). Specific HLA-DQ requires a genetic disposition. APCs activate $CD4^+$ T cells with fitting receptors, which in turn produce cytokines and trigger an innate immune response (6) that may lead to cell damage (7). (Fasano 2009, Gujral *et al.* 2012; Wieser *et al.* 2014)

In addition to intestinal disorders, CD can also occur in other forms. Several less prevalent neurological manifestation, including gluten ataxia as well as the cutaneous counterpart, called dermatitis herpetiformis, have been diagnosed (Hadjivassiliou *et al.* , Marks *et al.* 1966). The syndromes of dermatitis herpetiformis comprise skin itching and burning, while in gluten ataxia antibodies against gluten attack the cerebellum, affecting muscle coordination (Borroni *et al.* 2013, Hadjivassiliou *et al.* 2003). Currently no alternative treatment other than a gluten-free diet (GFD) is available for celiac disease, gluten ataxia and dermatitis herpetiformis.

Allergic reactions, irritable bowel syndrome and non-celiac gluten sensitivity

Food allergies are Type I-hypersensitivity reactions caused by IgE-mediated immune reactions to dietary proteins. They are diagnosed by patch/prick tests, serum IgE and exclusion challenge (Majamaa *et al.* 1999). Depending on the diagnosis method, conflicting data on the prevalence of wheat/cereal allergies exist, ranging from 0.25-3.6% (Gilissen *et al.* 2014, Zuidmeer *et al.* 2008).

In theory, every protein is a potential allergen but only a small number of them is responsible for the majority of food allergies. Cereal allergies are mostly caused by wheat, wherein the allergenic protein may or may not belong to the gluten family (Pulido 2010).

The antigen, ω5-gliadins from gluten, can trigger wheat-dependent exercise induced anaphylaxis, while amylase/trypsin inhibitors (ATIs) are the main cause for baker's asthma (Gómez *et al.* 1990). Unfortunately, modern breeding methods have drastically increased the ATI content in cereals, because of their functionalities as pest and parasite repellants (Brouns *et al.* 2013, Ryan 1990). The high resistance to intestinal digestion and the initiation of autoimmune reactions via a toll-like receptor pathway makes the accumulation of ATIs problematic (Junker *et al.* 2012, Schuppan and Zevallos 2015).

In the cases of irritable bowel syndrome (IBS) and non-celiac gluten sensitivity (NCGS) causal relationships are more obscure. Both are comparably widespread gastrointestinal disorders, connected with many unresolved questions. While some estimations of the prevalence suggest that IBS and NCGS are far more common than CD, a realistic number is not available at present, since the majority of cases are undiagnosed and specific biomarkers are missing (Hungin *et al.* 2003, Volta *et al.* 2014). The intestinal symptoms resemble those of CD, comprising abdominal pain, bloating and constipation, but without positive endoscopic or serologic test results. In addition, gluten-sensitivity has been associated with several neurological disorders, which may also occur in the case of CD and gluten ataxia (Lundin and Alaedini 2012). Unfortunately, a direct diagnosis is impossible so that only the combination of certain symptoms and the absence of other diseases qualify for labeling a condition as IBS or NCGS (Catassi *et al.* 2015). It is challenging to identify toxic substances, since a diet lacking common cereals is not only gluten-free but also free of compounds causing IBS. Sensitivity to fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs), the above mentioned ATIs, high intake of insoluble dietary fiber, caffeine, yeast and have been named as possible causes for IBS (Halmos *et al.* 2014). For a distinct diagnosis, a gluten-free diet would have to be separately supplemented by other suspicious substances. Although the majority of patients relate their symptoms to food, there is not always an improvement after changing the diet. For both, IBS and NCGS, a gluten-free diet is often recommended, however, with variable success (Pietzak 2012).

All of the presented indispositions are usually treated by an exclusion diet. Although the prevalence of each disease may be low, in combination numerous people depend on a life-long GFD with regular follow-up. In comparison to an average daily uptake of 20 g gluten per day, a strict GFD limits this value to 20 mg, corresponding to an estimated one-hundredth of a bread slice (Wieser *et al.* 2014).

Gluten is not restricted to bakery products, pasta and cereals; it also serves as a sometimes hidden functional additive for water binding, thermosetting, thickening, vitamin binding and crispiness for textured meats, ice cream, sauces, cornflakes and coffee creamers (Wieser *et al.* 2014). Due to contaminations, high costs and social inconvenience this restriction presents a great burden, which can be relieved by improving the current portfolio of gluten-free products.

1.2 Processing challenges and current deficits of gluten-free bread

The demand of gluten-free bread has been growing noticeably in recent years, but its production is still not trivial. Bakeries that decide to expand their portfolio with gluten-free products, have to consider several challenges and limitations. Foremost, the Codex Alimentarius published a regulation intended for "…foods for special dietary use for persons intolerant to gluten", in 2008. In accordance with EU regulations, the sold food must not exceed a maximum limit of 20 mg/kg (20 ppm) gluten to be termed "gluten-free".

Implementing allergen management and avoiding gluten contamination

For the labeling of gluten-free products, national organizations provide a purchasable annual license for the Crossed Grain symbol. The license is associated with several conditions in terms of analytical method, monitoring and certificates by accredited laboratories (Deutsch *et al.* 2008). The current method of choice for standardized gluten detection is the enzyme-linked immunoassay (ELISA) sorbent R5 Mendez (Wieser *et al.* 2014). In addition to the analysis method, an exhaustive gluten extraction and a representative reference are of essential importance for an accurate quantification (García *et al.* 2005). Despite big research efforts, it is not yet applicable to detect both prolamins and glutelins from all cereals with comparable sensitivity.

To guarantee conformation to the gluten limit, a disciplined allergen management has to predominate (Pulido 2010). The risk of gluten contamination has to be avoided during all production stages, including cultivation, milling, processing, storage and distribution. For a higher degree of segregation, the management of allergens benefits from an implementation of a separate processing line or a detached raw material storage (Kelly *et al.* 2008). The construction of new or modified production plants constitutes major investments. As an example, the Swiss Jowa AG modified an old plant for €4.6 million to enable packaging of their gluten-free products in a clean room (brot+backwaren 2013). Among the most important measures are an extensive personnel training, accurate labeling and regular sampling, for instance, with rapid gluten test sticks (Deutsch *et al.* 2008).

Processing challenges

In conventional wheat bread, gluten is involved in several fundamental processes: water absorption, formation of a viscoelastic network, gas retention and crumb texture formation (Goesaert *et al.* 2005).

Without these properties, several processing challenges emerge and the final bread quality might be compromised in various aspects. Settings during proofing and baking of gluten-free products can resemble conventional ones, so that only few adaptions are necessary at first. However, the handling during mixing, molding and transportation cannot be implemented without modifications. As discussed in Section 1.3, gluten-free flours typically require higher water addition (He and Hoseney 1991).

The lacking gluten network, high water content and hydrocolloid addition increase stickiness and reduce elasticity of the dough so that molding and sheeting are not feasible (Nishita *et al.* 1976). Moore *et al.* (2004) demonstrated that even with the same water content, gluten-free dough is noticeably softer than wheat dough. Although this applies to the vast majority of recipes, there are exceptions, like recipes containing zein, which allow for better dough handling, but only above the glass transition temperature (Lawton 1992). In general, cleaning of equipment and surfaces is more time- and cost intensive for gluten-free processes. Thus, direct molding after mixing, as is typical for the similarly sticky cake batter, might be favorable to reduce cleaning operations. However, no detailed instructions on how to plan a gluten-free production line are available, because of the wide range of dough consistencies, depending on the specific recipe composition.

Higher raw material and production costs obviously translate into more costly products. Indeed, two studies from 2007 and 2008 revealed that in the U.S. and Canada prices for gluten-free products were about 240% higher than for their regular counterparts (Lee *et al.* 2007, Stevens and Rashid 2008). Similarly, in a publication from the UK, gluten-free bread was 360% more expensive than conventional bread (Singh and Whelan 2011). The long list of ingredients does not only increase the costs, it may also convey an unnatural impression for consumers. For specialty products with unique selling propositions, higher prices might be acceptable, if the quality is sufficient. Confirmative, about 15% of Europeans would pay more for gluten-free food (Nielsen, 2016). However, sensoric and textural aspects do not allow for many cutbacks without compromising the quality and the current range of gluten-free bread products still displays a large variety of deficits.

Quality deficits of gluten-free bread

Considering the malnutrition caused by coeliac disease, it becomes obvious that a treatment diet should not only exclude gluten, it should also reintroduce nutrients (Hager *et al.* 2012b). Unfortunately, as other restrictive diets, the gluten-free diet is associated with nutritional deficits. Even though gluten has a relatively low biological value, wheat is an important source for carbohydrates, B vitamins, minerals and trace elements. Moreover, traditional products are often enriched with several nutrients, which are often not incorporated into their gluten-free counterparts (Thompson 1999). The replacement of wheat flour by pure starch or refined flours notably reduces the amount of micronutrients, proteins and fiber (Hager *et al.* 2012b).

In combination with the intestinal malnutrition, this accounts for nutrient deficits, even after several years of a GFD (Shepherd and Gibson 2013). While nutrients can be easily fortified, this does not solve the problem of a higher glycemic index. Since CD is connected to diabetes, the higher bioavailability of glucose from starch breads is counter-productive (Berti *et al.* 2004).

The high starch content not only causes biofunctional but also technological deficits (Schober 2009). For the shelf-life of bread, the staling rate is a limiting factor. The specific mechanism of staling is yet unknown, but associations to starch retrogradation and water migration from crumb to crust are likely (Gray and Bemiller 2003). Higher proportions of starch with less other water-binding components seem to enhance and accelerate the mechanism of staling (Demirkesen *et al.* 2013). Even fresh samples of gluten-free bread often appear dry and crumbly. The missing gluten-network makes it especially difficult to retain gas bubbles during fermentation. He and Hoseney demonstrated in 1991 considerably higher gas release during fermentation, proofing and baking of rice and corn dough than for wheat or rye dough. As a result, the bread volume is low and the crumb has an irregular pore structure.

1.3 Functionality of selected ingredients in gluten-free bread

For the production of gluten-free bread, wheat flour has to be replaced by one or more ingredients with similar properties. Selection criteria for raw materials should focus on functionality, costs and availability. Additional considerations may address ethical and regulatory requirements. When choosing a new ingredient, it might be necessary to revise the overall recipe because of interactions with other components. Each additive might affect volume, texture, aroma, mouth-feel, storability and nutritive value of the product. To achieve a certain quality, a combination of ingredients with different functionalities is required. The cheapest ingredient, gas, is fundamental for the aerated foam-like crumb. Starch and water enable stabilization of the gas through rheological aspects, thermal gelation and retrogradation. Further components and their effects are summarized in Table 1. The following section provides an overview on different flours, starches, proteins and hydrocolloids that can be used for gluten-free bread.

Gluten-free flours and starches

While it is difficult to find a suitable alternative for the functional protein of conventional cereal flours, it is easier to find alternatives for cereal starches. Starch is the main component of flours, and because of its gelatinization and retrogradation properties it is fundamental for structure formation during bread production (Abdel-Aal 2009, Witczak *et al.* 2015). Different grain sizes and amylose-amylopectin ratios are mainly responsible for variations in swelling, gelatinization, retrogradation and enzyme susceptibility (Jane *et al.* 1999, Lindeboom *et al.* 2004). Houben *et al.* (2012) have provided further information on the role of starch in gluten-free bread. A huge variety of gluten-free starches is available from different plant sources.

Apart from the members of the Triticae, all cereal and non-cereal flours are gluten-free. Other suitable sources for gluten-free flours and starches are listed in Table 2.

Table 1. The functionality of substances involved in gluten-free bread production. The assignments are based on interpretations from experiments performed in the curse of this thesis and further inspired by literature data. + positive effect, – negative effect. γ: surface tension, TG: transglutaminase.

compound	volume and porosity	sensory aspects	shelf-life	nutrition
gas	+ foam structure			
starch	+ rheology, gelation		− staling	
water	+ − rheology, hydration	+ juicy crumb	+ staling	
protein	+ − rheology, γ	+ browning	− +	+
fiber	− volume	+ juicy crumb	+	+
hydrocolloids	+ rheology, γ			
lipids	+ γ		− staling	−
short saccharides	+ fermentation	+ browning		−
micronutrients/flavors		+ taste		+
acids		+ taste	+ microbiology	
enzymes	+ TG: network + lipase: γ + amylase: fermentation	+ browning	+ staling	

The pseudocereals buckwheat, amaranth and quinoa produce seeds with a composition that is similar to the one of cereals, although they do not belong to the cereal family of Poaceae and are safe for a gluten-free diet (Zevallos *et al.* 2014). Especially amaranth and quinoa, which are both members of the Amaranthaceae, are gaining popularity in North America and Europe, because of their special nutrient profiles (Alvarez-Jubete *et al.* 2010a). While quinoa is considered an ancient grain in Peru and Chile, its consumption is relatively new in Europe. The Food and Agriculture Organization of the United Nations declared 2013 to be the International Year of Quinoa because of its praised nutritious qualities as well as its ability to grow in harsh environments. However, controversial political debates have emerged because the recent production has not been able to meet the demand, so that the world prices for quinoa have increased dramatically (Parker-Gibson 2015, Small 2013). An expansion of the cultivation region and sustainable methods might improve the future supply and make quinoa more affordable for lower-income communities (Graf *et al.* 2015).

In many aspects, the amino acid, lipid and micronutrient profiles of quinoa surpass the ones of traditional cereals, including the most popular gluten-free flours from rice or corn (Hager *et al.* 2012b, Alvarez-Jubete *et al.* 2010a, Schoenlechner *et al.* 2010). In addition, more and more studies about the beneficial effect of secondary metabolites in quinoa, such as triterpenoids, phenolics and betalains, emerge (De Carvalho *et al.* 2014, Farinazzi-Machado *et al.* 2012, Zevallos *et al.* 2014).

Table 2. Sources for gluten-free flours and starches. Members of the *Triticeae* tribe can induce gluten related indispositions (×), while their extracted starches may be gluten-free (✓) - depending on their purity.

cereals		pseudocereals	tubers	legumes	others
× rye		✓buckwheat	✓cassava	✓soy	✓chestnut
× barley	Triticae	✓amarant	✓potato	✓chickpea	✓tigernut
× wheat		✓quinoa	✓yam	✓pea	✓chia
✓oat	✓sorghum			✓carob	
✓rice	✓millet				
✓corn	✓teff				

However, bitter saponins in the pericarp, which serve as valuable predator protection, have to be removed by abrasion and /or washing before consumption (Prego *et al.* 1998). In comparison to a mechanical removal, washing poses the problem of excessive water usage and pollution and requires sufficient drying to provide microbial safety (Small 2013). The concentration of saponins can range from 0.03-1.1 g/100 g for sweet varieties and up to 1.4 g/100 g in bitter types (Taylor and Parker 2002). Apart from the bitterness, saponins can have positive and negative physiological effects. Although featuring antiviral, anticancer and antithrombic activities, they also impair the absorption of iron through complex formation and may be responsible for hemolytic reactions (Zevallos *et al.* 2014). As shown in Figure 3, the fruit color of quinoa varies broadly. Surrounded by the embryo, the perisperm forms the starchy center. Cereal grains, in contrast, store starch in their endosperm. The weight of the embryo is about 35-40% of the seed weight and contains nearly all of the proteins, lipids and minerals (Föste *et al.* 2015, Chauhan. *et al.* 1992)

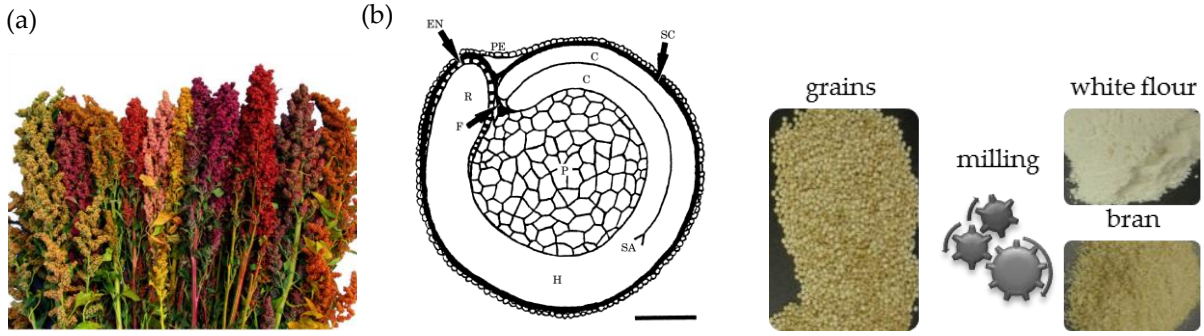

Figure 3. Quinoa plants and grain structure. (a) *Chenopodium quinoa* plants with varying fruit colors (Graf *et al.* 2015). (b) Longitudinal section of a *Chenopodium quinoa* grain. PE: pericarp, H: hypocotyl radicle axis, C: cotyledons, EN: endosperm, F: funicle, P: perisperm, PE: pericarp, R: radicle, SA: shoot apex, SC: seed coat. Bar = 500 µm (Prego *et al.* 1998). (c) Fractionation of quinoa into white flour and bran by milling fractionation.

Despite of the high popularity of quinoa, only very few publications have analyzed its baking performance. First results were promising, when potato starch was exchanged by quinoa flour, since a slight volume increase was observed without compromised acceptability (Alvarez-Jubete *et al.* 2010b). Nevertheless, the volume of gluten-free bread with quinoa flour is significantly lower than the volume of wheat bread.

Insufficient aeration and an undesirable pea-like aroma were the main challenges for increasing the content of quinoa in gluten-free bread (Burešová *et al.* 2014, Hager *et al.* 2012a, Rosell *et al.* 2009). While the typically used rice or corn flour may also cause strong deviations from traditional bread aroma profiles, they are mostly less intense. Thus, Wolter *et al.* (2014a,b) attempted to create a more pleasant aroma profile by fermenting quinoa dough with lactobacilli, however, without success. Subsequently, Axel *et al.* (2015) revealed that a specific quinoa sourdough had antifungal activity and slightly improved the overall gluten-free bread quality, but the volume was below the one of wheat bread. The acceptability of the bread was not addressed. In comparison to other gluten-free flowers, slower staling was observed and attributed to the low amylose content in quinoa flour (Hager *et al.* 2012a).

Apart from lactobacilli fermentation, the only other pretreatment that has been evaluated to improve the baking performance of quinoa, is milling fractionation. Föste *et al.* (2015) successfully optimized parameters during conditioning, roller milling and sieving to separate the seed tissues. In a consecutive study, the resulting quinoa bran, with high protein, lipid and mineral content was added to gluten-free dough, to improve the nutritional value (Föste *et al.* 2014). Similar to other fibers, increasing amounts of quinoa bran reduced the bread volume, but at 10% addition, all quality attributes were positively influenced. Milling fractionation provides several advantages:

- the functionality of separate seed tissues can be analyzed,
- saponins can be removed without the requirement of preliminary washing and drying,
- lipids can be removed, which improves the oxidative stability and
- the flour composition can be standardized to compensate raw material fluctuations.

Thus, this study analyzes the baking performance of the promising novel ingredient, quinoa white flour, for the first time. Flours and starches provide the basis of the bread recipe, which is further complemented by water, yeast and salt. Moreover, additives, such hydrocolloids, are essential to achieve an acceptable product.

Hydrocolloids as functional additives

Hydrocolloids are hydrophilic polysaccharides or proteins, which increase the viscosity of a solution (BeMiller 2009). This definition may overlap with dietary fiber, a group of substances resisting human digestion. Dietary fiber comprises soluble plant parts, such as inulin or agar and insoluble components like lignin or xanthan. A health promoting effect can derive from prebiotic or bulking actions. Fiber is naturally abundant in many plant materials, such as bran or pomace. While the latter are typically added to gluten-free bread to improve the nutritional value, the addition is often limited by their negative impact on dough structure and bread volume (Föste *et al.* 2014, Ronda *et al.* 2015).

In contrast to the destabilizing effects of some fibers, comparatively small proportions of certain hydrocolloids are able to stabilize gas bubbles without considerably affecting other bread attributes (Lazaridou *et al.* 2007). Depending on their branching index, molecular weight and ionic charge, hydrocolloids can strongly influence the dough rheology (BeMiller 2009). Examples are gelatin, carrageenan, alginate, tragacanth gum, locust bean gum, guar gum, xanthan gum and modified celluloses.

Big price differences with strong fluctuations exist for food hydrocolloids: gellan is the most expensive with ~ $40/kg in comparison to native starches that cost ~ $0.40/kg. The price for HPMC has been estimated as ~ $10-12/kg (Wüstenberg 2014). Considering the strong effect and small dosage, the price becomes less significant.

The most frequently used hydrocolloid for gluten-free bread production is hydroxypropyl methylcellulose (HPMC, E464), also called hypromellose (Masure *et al.* 2016). As nonionic cellulose ether, it is synthesized from wood pulp or cotton linters by reacting with propylene oxide and methyl chloride (Wüstenberg 2014). This yields a more reproducible, uniform and well-characterized product than natural alternatives (Bauer *et al.* 2006). The backbone of the HPMC molecule consists of 70-1100 β-D-glucose units with (1→4)-linkage resulting in a molecular weight (MW) of 13,000-200,000 g/mol (Wüstenberg 2014). As shown in Figure 4, the free hydroxyl groups are partially substituted by hydroxypropyl and methyl groups through ether bridges. The degree of substitution (DS) and the ratio of methoxy to hydroxypropoxy groups can be varied. Like other hydrocolloids, HPMC retains water and increases the viscosity of solutions. A wide range of viscosities from 3-30,000 mPa s can be obtained by solving 2% HPMC in water and the solutions are shear-thinning. With a DS of 1.5-2.0 HPMC is soluble in cold water and undergoes reversible thermal gelation (BeMiller 2009). Gelation is thought to be facilitated by the dehydration of the molecules with rising temperature, because it enables the interaction of hydrophobic methoxy-groups.

A typically used product for gluten-free bread is Methocel K4M. The designation 4M, indicates that this HPMC has a viscosity of 4,000 mPa s, an average MW of 86,000 g/mol and a DS of 1.4 in 2% concentration in water at 20 °C (information provided by manufacturer). The gelation temperature of a 2% HPMC solution is 85 °C, but this value can be reduced to below 50 °C in the presence of other solutes. Because of its amphiphilic nature, HPMC strongly decreases the surface tension of water to 45-55 mN/m (0.05% at 25°C) (Wüstenberg 2014). With its dominant hydrophilic nature and HLB value of 10-12, HPMC is an oil-in-water emulsifier, which can gelate at interfaces and increase interface stability. Because it is nonionic, HPMC does not form complexes and is stable over a pH range of 2.0-13.0. Thus, this hydrocolloid can be used for the creation of foams and emulsions (Bauer *et al.* 2006, BeMiller 2009). Further information about interfacial phenomena is given below.

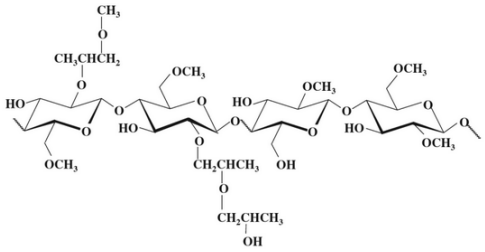

Figure 4. Molecular structure of HPMC (Wüstenberg 2014).

With 2-4% HPMC, the bread volume increased in various recipes, mostly in parallel to an elevated water content (Bárcenas and Rosell 2006, Bárcenas and Rosell 2005, Mariotti *et al.* 2013, Nishita *et al.* 1976, Sabanis and Tzia 2011, Sivaramakrishnan *et al.* 2004). In some of these cases, also improved bread texture and delayed staling were reported. BeMiller (2011) explained that hydrocolloids can influence the onset and extent of starch gelatinization in different ways depending on the type of raw materials and on the preparation and evaluation methods. Similar to the observed gluten layer around starch granules by Jekle *et al.* (2016), some gums have been shown to completely wrap native starch, so that less swelling and gelatinization occurs (Chaisawang and Suphantharika 2006). The complex nature of this interaction is visualized in Figure 5, where Kobylañski *et al.* (2004) demonstrated that rising HPMC levels accelerate T_0 at low water content, while it has the opposite cause when more water is available. Water retained by HPMC might delay the onset of starch gelatinization, but there is no obvious explanation for the contrary effect with lower water content. Already in 1976, Nishita *et al.* compared different HPMC types in gluten-free bread based on rice flour. As can be seen from Figure 5, they revealed considerable differences depending on the type, concentration and water ratio, with the best result obtained for 3% K4M or E4M with 75% water. As for other substances, the improvement of bread quality by HPMC addition is limited and for each recipe an optimum concentration has to be identified to prevent adverse effects (McCarthy *et al.* 2005). Nevertheless, its well-defined physico-chemical characteristics, including water retention capacity, viscosity impact and surface activity, make HPMC an ideal functional additive for gluten-free bread. Consequently, it will also be used and studied in the present thesis.

Because of the broad range of raw materials and additives, also the water addition in gluten-free bread varies extensively from 50-220% (flour base) (Masure *et al.* 2016). As a rule, gluten-free recipes contain more water than their conventional counterparts. In wheat dough, water addition is adapted until reaching a target dough resistance. This method compensates fluctuations regarding the water retention capacity of raw materials. In contrast, it is largely unknown which material properties will provide aspired product characteristics in gluten-free bread.

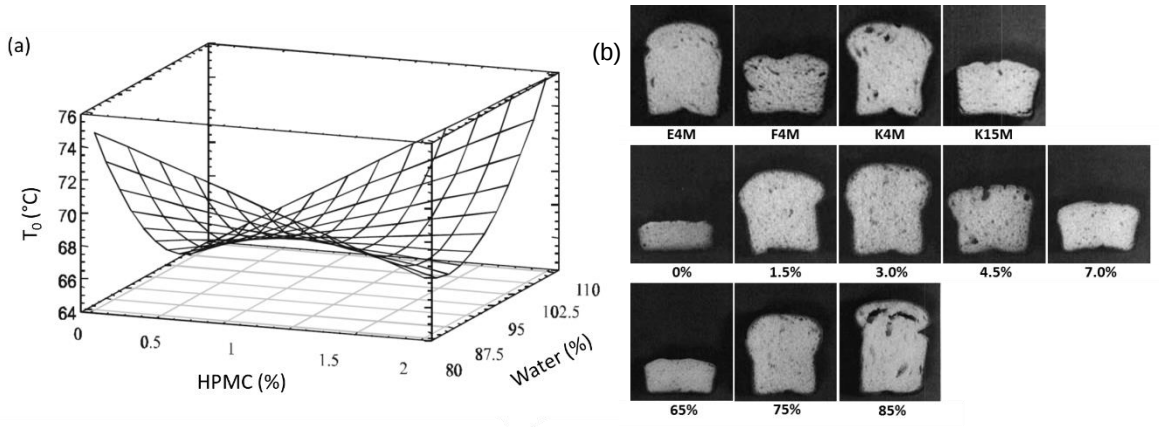

Figure 5. Influence of HPMC on starch gelatinization and gluten-free bread. (a) Effect of HPMC and water content on starch gelatinization onset T_0 in gluten-free dough with corn/cassava starch (3/1), 5% egg white, 5% shortening, 2% salt and 14% sugar (Kobylañski *et al.* 2004). (b) Gluten-free bread based on rice flour, 3% yeast, 2% salt, 7.5% sucrose, 6% oil and 75% water addition (flour base). First line: 3% addition of different types of HPMC Methocel Premium. Second line: Different concentrations of K4M. Third line: Different water concentrations with 3% K4M (Adapted from Nishita *et al.* 1976).

1.4 Material properties relevant for gas stabilization in gluten-free dough

For an aerated product, incorporated gas bubbles have to be stabilized throughout processing. Because of differences in density and chemical potential between gas and other dough components, bubbles are unstable and phases tend to separate. Moreover, the gas volume increase during fermentation and baking creates radial expansion stress on the matrix surrounding the gas bubbles (Menjivar 1990). According to the ideal gas law with the gas constant R, heating by dT elevates the volume dV of n gas molecules (N_2 from mixing and CO_2 from yeast), assuming constant pressure p (Formula 1).

$$\mathrm{d}V = \frac{n\,\mathrm{R}\,\mathrm{d}T}{p} \qquad \textbf{Formula 1}$$

The viscoelasticity of wheat dough and the viscosity of cake batter are important factors for protecting the bubbles' integrity. Additionally, during baking, starch gelatinization is crucial for obtaining an aerated end product. In contrast, foam stabilization in gluten-free dough requires either additional functional ingredients or new processing approaches. It is important to note that for traditional and gluten-free dough different mechanisms apply for the stabilization and destabilization of bubbles. While for wheat dough the prevailing destabilizing mechanisms are coalescence and disproportionation, in the case of cake batter and gluten-free dough buoyancy is an additional factor. According to Formula 2, which is based on Stokes' law, the terminal velocity v of a sphere depends on its radius r and density ρ_s as well as on the fluid density ρ_f and viscosity η, with the gravitational constant g. The fact that the radius contributes to the formula in a quadratic way, highlights the importance of the bubble size. Thus, bubble breakup during mixing and an increase of the viscosity are obvious strategies to counteract destabilization. The production of small bubbles during mixing is the basis for a stable foam.

$$v = \frac{2\,g\,r^2(\rho_s - \rho_f)}{9\,\eta}$$ **Formula 2**

Another parameter, which directly influences the bubble stability, is the surface tension γ, as can be derived from the Young-Laplace Equation (Formula 3). Despite the fact that smaller radii are favorable to prevent buoyancy, they cause higher internal capillary pressure and, in turn, higher chemical potential.

As a result, gas can migrate from small to big cells until the smaller cells disappear. Several experiments and simulations, confirmed that the mechanism of disproportionation (Ostwald-Ripening) is responsible for the transfer of gas between neighboring bubbles (Dickinson *et al.* 2002, Dutta *et al.* 2004, Jang *et al.* 2005, Kloek *et al.* 2001). The rate of diffusion depends on the solubility, the size of the adjacent bubbles as well as on the bulk and interfacial rheology. Especially in the case of gluten-free systems, a more intense evaluation of foam stabilization mechanisms is required.

$$\Delta p = \frac{2\,\gamma(r)}{r}$$ **Formula 3**

Surface-activity

The addition of surface-active substances can reduce the tension prevails at the bubbles' interface. Various ingredients with more or less foamability are worth considering: proteins, peptides, hydrocolloids, secondary plant metabolites and polar lipids. The mechanism of surface activity depends on the structure of the molecule; particularly important are its size and its hydrophilic-lipophilic balance (HLB value). Substances with low HLB, indicating the tendency for water-in-oil (W/O) emulsions, typically have more impact on reducing the surface tension, as presented in Figure 6a (Elgeti *et al.* 2015). While polar lipids rapidly arrange at the interface, proteins of high molecular weight are slower but form a stable film by unfolding and interacting with each other. Since the presence of lipids can disrupt protein foams, it can sometimes be contra-productive to add typical lipid-based emulsifiers. The effect of four emulsifiers, typically used for bakery products, on dough rheology, loaf volume and cell distribution was evaluate by Nunes *et al.* (2009). They found a concentration dependent positive effect of the emulsifiers on the volume as well as the cell size and distribution. Similarly, Elgeti *et al.* (2015) revealed that especially oil-in-water emulsifiers improve the volume of gluten-free bread (Figure 6b). However, the effect is much less pronounced in gluten-free bread than in wheat bread.

Rheology

Surface activity is not the only important factor for dough aeration. Dough rheology determines the extent of aeration during mixing as well as the ability of the system to retain gas bubbles. For most rheological values, broad ranges exist in literature - even for the homogeneous wheat dough recipes. It is not surprising that for gluten-free dough, with its infinite number of possible ingredient compositions, the range of rheological data is by far wider.

Particularly, the missing gluten network is responsible for noticeable deviations in the flow behavior. To characterize batters and doughs, various empirical and fundamental methods have been established.

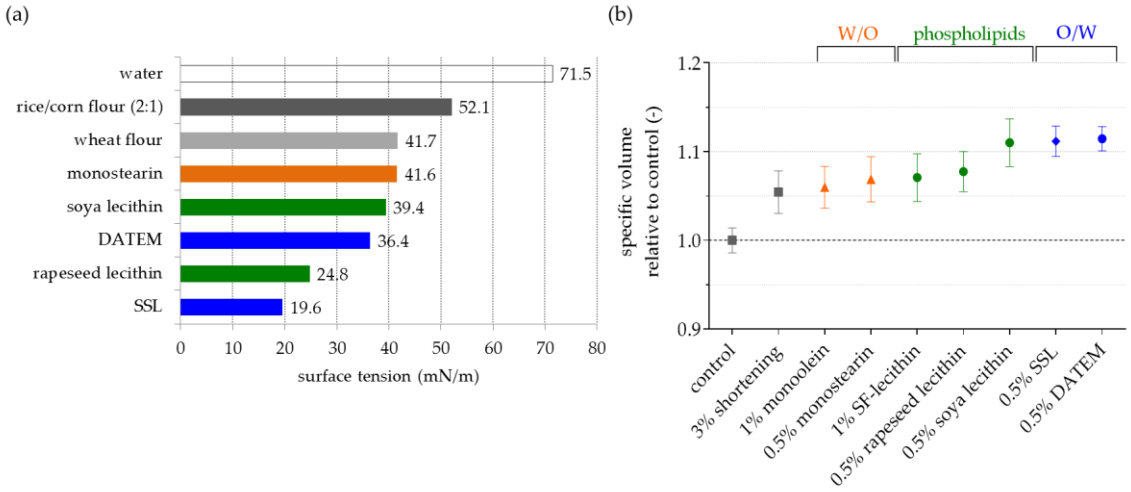

Figure 6. Effect of emulsifiers on surface activity and gluten-free bread volume. (a) Surface activity of 3% flours and 0.6% lipid emulsifiers on the tension between water and air measured in a tensiometer with the Wilhelmy-plate method. (b) Impact of lipids on the specific volume of gluten-free bread relative to a control without lipid addition baked on the same day. Lipid concentrations are based on the mixture of rice flour, corn flour and corn starch (2:1:1). Means are shown with SD. DATEM: diacetyl tartaric acid ester of mono- and diglycerides. SSL: sodium stearoyl lactylate. W/O: tendency for water-in-oil emulsions for low hydrophilic-lipophilic balance. O/W: tendency for oil-in-water emulsions. Modified from (Elgeti *et al.* 2015).

Typically, millers, providers of baking agents and bakers use empirical systems to predict the baking performance of flours. For this purpose, the Farinograph, the Doughlab and the Mixolab measure the torque of dough over the mixing time to assess the kinetic and stability of the viscoelastic structure formation. Several attempts have been made to adapt the rheology of gluten-free dough to the one of wheat dough, but Lazaridou *et al.* (2007) demonstrated that this may not always be the best strategy. An addition of xanthan increased a value that the authors termed dough elasticity and shifted the Farinograph curve to resemble the one of wheat dough. However, the gas volume fraction of the resulting bread was reduced. Significant variations were obtained when changing the type of hydrocolloid or its concentration, which stresses the sensitivity of the gluten-free dough structure. Carboxymethylcellulose (CMC) and pectin significantly improved the gas retention despite of strong divergence from the typical farinograph curve. Interestingly, Mariotti *et al.* 2009 modified the farinograph target consistency to 200 BU instead of the typical 500 BU, because it provided an "adequate condition to properly form a gluten-free dough able to sustain further transformations". Considering the fundamental structural differences of gluten-free dough and wheat dough, it is not surprising that so far no unambiguous insights resulted from these torque-measuring techniques.

To simulate the radial extension of bubbles during proofing and baking, large uniaxial or biaxial deformations can be applied in a Kieffer-Rig, extensograph, alveograph or TPA extrusion cell.

Figure 7 (a) shows the extensional viscosity η_{ex} resulting from biaxial tension for both, wheat dough and several gluten-free doughs with comparatively high viscosity (Demirkesen *et al.* 2010a). The authors observed a correlation between η_{ex} and the specific bread volume. The lower extensional viscosity of gluten-free dough was confirmed by Moore et al (2004) and might account for impaired structure stability during bubble growth. Kieffer-Rig analysis of gluten-free dough is mostly restricted by the possibility to form a cohesive strand. In Figure 7 (b), quinoa produced the strongest gluten-free dough, but wheat dough endured significantly more stress and strain.

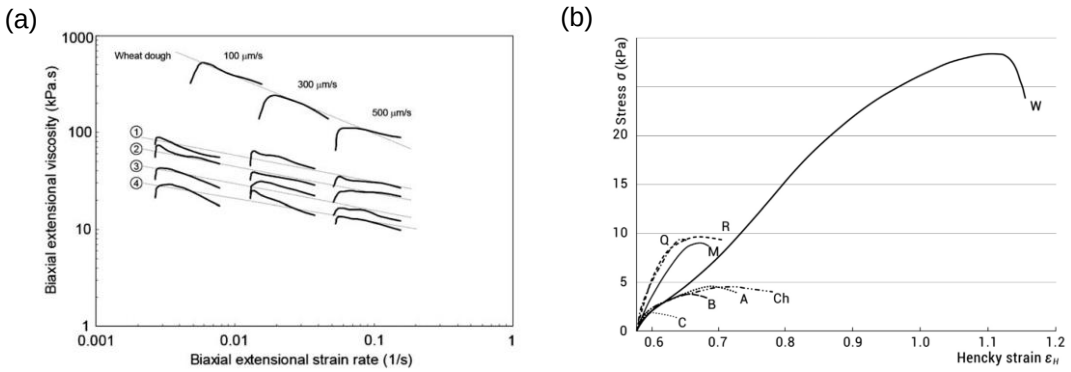

Figure 7. Comparison of viscoelasticity of gluten-free and wheat dough. (a) Extensional viscosity resulting from strain measured in a rheometer when compressing dough between two parallel plates to 80% thickness with different compression velocities (100 µm/s – 500 µm/s). Upper curves: wheat dough, lower curves (1-4): gluten-free dough with different hydrocolloids (Demirkesen *et al.* 2010a). (b) Stress-strain curves of wheat (W) and gluten-free flours with 2% salt and adapted water content to reach 500 BU. The relative deformation in uniaxial extension is represented by the Hencky strain $\dot{\varepsilon}_H$ in a Kieffer-Rig (Burešová *et al.* 2014). A: amaranth, B: buckwheat, C: corn, Ch: chickpea, M: millet, R: rice.

In comparison to the so far mentioned empirical methods, fundamental dough rheology is more time consuming and complex, but it can help to understand the structural causes for the observed behavior. Only fundamental results can be used to characterize independent physical material properties (Menjivar 1990). In addition to the viscosity, which describes the resistance to deformation and the "thickness" of a material, also other rheological parameters, such as the so called extensional viscosity, the viscoelasticity, the thixotropy and the creep recovery contribute to the success of the overall aeration process. For wheat dough most of the fundamental rheological measurements are performed in low-shear dynamic tests (Lefebvre 2006). Small amplitude oscillatory shear enables an assessment of the dough rheology without altering the structure. The resulting elastic (storage) modulus G' and viscous (loss) modulus G'' are frequency dependent. Typically, wheat dough moduli are considerably higher than the ones of gluten-free dough, as presented in Figure 8. Since the gluten network is responsible for the cohesiveness and viscoelasticity, these results are not surprising. Moreover, gluten-free dough appears more frequency dependent and thus less elastic than wheat dough (Demirkesen et al. 2010a). The ratio of G' to G'' yields tanδ, which characterizes the viscoelasticity of a material.

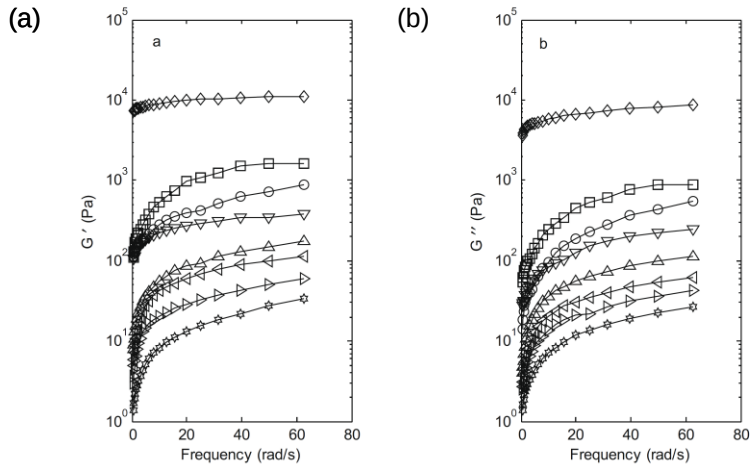

Figure 8. Comparison of viscoelasticity of gluten-free and wheat dough. Frequency sweep experiments were performed in a rheometer with 0.5% strain rate resulting in (a) the *G′*: storage modulus and (b) the *G′′*: loss modulus. ◇: wheat dough, other symbols: gluten-free dough with different hydrocolloids (Demirkesen *et al.* 2010a).

For wheat and gluten-free dough, as well as for wheat cake batter, the elastic component dominates the behavior, since tanδ was always below 1 in oscillatory rheometry. The range of tanδ in different gluten-free formulations is extremely broad: 0.1-0.9 (Peressini *et al.* 2011, Föste *et al.* 2014, Lazaridou *et al.* 2007, Mariotti *et al.* 2009, Ziobro *et al.* 2013). The complex modulus *G** comprises information about *G′* and *G′′* and estimates the stiffness and strength of a dough. However, for predicting dough behavior during processing, the significance of oscillatory measurements is limited (Dobraszczyk and Cauvin 2000). This derives from the restriction of the time scale, the linear domain and the deviation of the tested frequency range from the much higher strain rates of 10-100 s^{-1}, which are actually occurring during the production process (Bloksma 1990). Because of a typically higher water content and no gluten network, lower G* values can be expected for gluten-free dough than for wheat dough.

Particularly at large deformations, the so-called strain hardening seems to stabilize gas bubbles during baking (Kokelaar *et al.* 1996). In contrast, Berta *et al.* (2015) reported that in gluten-free dough (with zein) strain hardening, as measured in a hyperbolic contraction flow rig, did not significantly affect bubble structure formation. In order to assess the ability of dough to recover from previously applied stress, creep-recovery tests are performed. However, it is difficult to compare results from different publications, because of largely inconsistent shear rates and durations. In general, large strains might improve the understanding of the baking performance, since they provide deformations or strains that are in a similar order as those experienced during baking (Dobraszczyk and Morgenstern 2003, E.L. Sliwinskia 2004).

In comparison to the above mentioned dynamic properties, rotation measurements reveal the flow behavior of dough. High shear or extensional viscosity might prevent cell collapse during stretching conditions, such as gas cell expansion in the fermentation and baking stages. In case of non-newtonian systems, the apparent viscosity η_s is a function of the shear rate $\dot{\gamma}$.

The former can depend on the molecular weight (MW), the MW distribution and concentration as well as on intermolecular interactions (Mezger 2010). For cake batter, many rheological studies were performed after mechanical aeration. However, gas bubbles strongly influence the results, for example by increasing the apparent viscosity, the elastic modulus and the extent of shear-thinning (Chesterton *et al.* 2013, Chesterton *et al.* 2011a, Llewellin *et al.* 2002). This makes it difficult to identify the medium properties, which facilitate aeration and gas stabilization. For this purpose, measurements are performed with minimum gas content in the present thesis.

In cake batter, the extent of bubbles rising due to buoyancy is inversely proportional to the viscosity (Sumnu and Sahin 2008). Rheological analysis under shear is more difficult for wheat dough than for gluten-free dough or cake batter (Lefebvre 2006). Since high shear expels wheat dough from the measuring gap, its viscosity has only been measured at small shear rates of up to 5 s^{-1} resulting in 320 Pa s (Bloksma and Nieman 1975, Lindborg *et al.* 1997, Muller 1962, Sivaramakrishnan *et al.* 2004). The Herschel-Bulkeley, power law or Casson model have been used to fit data for dough and batter (Demirkesen *et al.* 2010a, Demirkesen *et al.* 2010b, Sahin 2008, Sivaramakrishnan *et al.* 2004). In all cases, shear-thinning behavior was observed, with flow behavior indices of 0.3-0.9. A yield stress was not always identified for gluten-free dough. Shear-thinning occurs, either if structure disintegrates in a reversible or non-reversible way or if the apparent viscosity decreases because the structure aligns with the flow direction (Songa *et al.* 2006). Especially gluten-free dough with gel-forming hydrocolloids should be evaluated under different shear conditions to elucidate its structure during simulated processing conditions.

Although most rheometers enable accurate temperature control, so far only few studies have used this tool to mimic the conditions during processing (e.g. Bloksma and Nieman, 1975). Mostly temperature sweeps have only been performed to analyze gelatinization (Peressini *et al.* 2011, Nunes *et al.* 2009). The Mixolab provides the only empirical method to analyze dough during heating. It has been used to compare the effect of various gluten-free and gluten-containing flours. However, often only flour-water mixtures are analyzed without other dough components and with the water content differing from the recipe used for baking (Torbica 2010, Hadnađev *et al.* 2011, Kahraman *et al.* 2008).

In conclusion, rheological properties play a fundamental role for gas retention in bakery products. Up to date, it was not possible to relate specific medium properties to the gas retention capacity of gluten-free dough. It seems unsuitable to apply empirical systems, which have been specifically adapted to wheat dough properties. Instead, a fundamental measuring program should be developed to determine relevant dough characteristics. Since several rheological characteristics of gluten-free dough are more similar to cake batter than to wheat dough, it might be beneficial to accordingly modify processing procedures.

1.5 Thesis outline

Awareness and diagnosed cases of gluten-related indispositions are steadily increasing. Thus, research and industries are currently focusing on improving the deficient quality of gluten-free bakery products, but entrainment and stabilization of gas bubbles are still major challenges. Many new ingredients have been tested, resulting in complex and obscure compositions with unpredictable material properties. Particularly, the interplay between dough rheology and baking performance is largely unknown. This thesis aims at developing new approaches for the aeration of gluten-free dough and at gaining insights into bubble stabilization. Based on the current state of knowledge, the following hypotheses form the focus of the studies:

- A critical review of aeration strategies for food foams should reveal new suitable approaches for the aeration of gluten-free dough
- An optimization of the substrate availability for yeast fermentation should increase the biological gas input
- Because of several rheological similarities of gluten-free dough and cake batter, the orientation towards batter processing should improve mechanical aeration and processability of gluten-free dough
- Bread should benefit from less bitterness and structure destabilization, when using quinoa white flour without bran components
- Studying the viscosity of different recipes throughout processing via fundamental rheology should reveal which type of flow behavior yields maximum aeration and gas retention
- A combination of optimized aeration strategies should yield a bread density similar to one of wheat pan bread

Figure 7 summarizes the procedures that are performed in order to evaluate these hypotheses. Initially, possibilities for the entrainment of gas into dough-like systems are reviewed with the goal of identifying new strategies that seem promising for the gluten-free medium. Different methodologies for wheat dough and cake batter serve as templates and are compared to the ones that are presently used for gluten-free dough. In order to be able to assess the success of aeration, also suitable techniques for analyzing the gas volume fraction in dough are reviewed.

The first experimental study focuses on increasing the biological aeration in gluten-free dough. The recipe is based on a mixture of rice flour, corn flour and corn starch, complemented by the hydrocolloid HPMC. As a novel ingredient, refined quinoa white flour replaces rice and corn flour in rising concentrations. In comparison, whole grain quinoa flour was used to enable an evaluation of the functionalities of the grain compartments. In particular, the suitability of different raw materials for yeast fermentation is assessed.

Since, biological aeration depends on the availability of substrates, special attention is given to mono- and disaccharides as well as amylolytic enzymes. As a prerequisite for subsequent studies, the dough composition with the highest bubbles retention is identified.

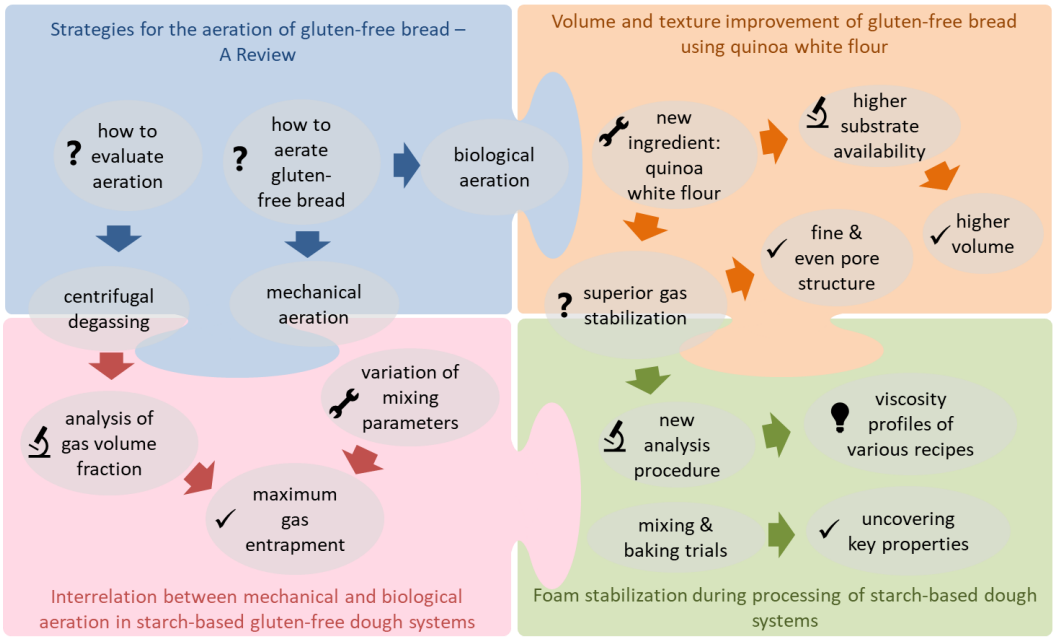

Figure 7. Thesis outline. Four publications were combined to identify gas stabilization mechanisms and to maximize the aeration of gluten-free bread.

Following the characterization and optimization of biological gas entrainment, mechanical aeration is addressed. Up to date a considerable divergence exists regarding the processing settings that are used for producing gluten-free dough. Thus, mixing time, speed and geometry are varied with the goal of maximizing the gas volume fraction in dough without yeast. Separate baking trials analyze the effect of mixing variations on biological gas entrainment and finally evaluate the interrelation between mechanical and biological aeration.

To finally close the gap in knowledge between structure and function, a new fundamental rheological test program is developed, which covers all processing stages in one measurement. The selected range of recipes provides a variety of structure-function relationships. Biological aeration is standardized to make sure that the assessment of gas input and stabilization can occur without falsification by differences in energy input and substrate availability. In summary, this thesis aims at improving gluten-free bread by providing more knowledge about key material properties and by compensating the missing protein network through the development of innovative raw materials and aeration stages.

2 Results (Thesis Publications)

2.1 Summary of thesis publications

Strategies for the aeration of gluten-free bread – A review **Pages 28-37**

Low volume and irregular pores remain major deficits of gluten-free bread. While previous studies have mainly focused on different ingredients or material properties, this review presents strategies for maximizing the gas input into dough. Owing to fundamental differences in medium properties of gluten-free and wheat dough, a strict adherence to the traditional kneading process is counterproductive. Thus, various approaches for the aeration of dough and batter are divided into (1) biological, (2) chemical, (3) mechanical and (4) physical methods and assessed critically. For biological aeration through microorganisms, the substrate availability of gluten-free raw materials plays an important role, since it determines the efficiency of gas production. Chemical reagents as an alternative or additive to biological systems might increase the gas input, but require extensive ingredient and processing adaptions. Utilization of the conventional kneading with a dough hook is only suitable for homogenization or aeration if the medium is more cohesive than adhesive, which is rarely the case for sticky and fluid gluten-free dough. Mechanical aeration through a high-speed beating process, similar to cake batter mixing, is identified as promising strategy to substantially elevate the level of gas in gluten-free bread. A successful implementation of this strategy requires knowledge of the influence of single processing parameters on the success of aeration and a recipe providing sufficient gas retention.

Volume and texture improvement of gluten-free bread using quinoa white flour **Pages 38-44**

In order to optimize the aeration of gluten-free bread, a recipe with extensive gas retention capacity is a prerequisite. The second study of this thesis shows that the use of quinoa white flour as an innovative gluten-free raw material, meets this requirement. The pseudocereal is a suitable substrate for dough aeration using yeast, since considerably more glucose and a higher activity of α-glucosidase were found in comparison to rice and corn flour. Moreover, the biological gas input benefits from an exchange of standard rice and corn flour by quinoa flour, since it provides more yeast substrates and amylolytic enzyme activity. A replacement of rice and corn flour by quinoa white flour enhanced the specific volume by 33%. Adding sucrose or fungal amyloglucosidase to the control recipe elevated the volume in a similar way; however, the pore structure became coarse and inhomogeneous. This highlights the superior gas retention of quinoa white flour. While several explanations for the gas stabilizing properties of quinoa white flour are possible the identification of key properties, which are relevant for bubble retention in gluten-free dough remain unknown.

Interrelation between mechanical and biological aeration in starch-based gluten-free dough systems **Pages 45-51**

For the production of bread without gluten, alternative aeration and gas stabilization strategies are required. In the present study, traditional wheat dough kneading was iteratively adapted towards high-speed beating, similar to cake batter production. The effect of different parameters on the density and temperature development during mixing was analyzed without yeast. With a wire whip and a speed of 420 rpm the dough temperature increased from 20 °C to 32 °C and the gas volume fraction in dough rose from 6% to 21%. In consequent baking trials with yeast, all of the approaches produced acceptable crumb structures, except for the recipe based on rice/corn flour. This confirms the revelation of the previous study that dough with quinoa white flour stabilizes gas throughout fermentation and baking in a wide range of water addition and processing conditions. Water reduction elevated dough viscosity and temperature without affecting the level of aeration. Common rheological methods, such as non-destructive oscillation trials, were not suitable to predict gluten-free dough behavior during mixing. When varying the energy input, carbon dioxide production by yeast is affected, because its metabolism strongly reacts to temperature differences. Thus, influences of the mixing settings on the bread volume were superimposed by a varying degree of yeast fermentation. In summary, the beating process adopted in this study increased dough aeration by 60% (rel.), while the traditional wheat dough kneading stage is less suitable for the homogenization of sticky gluten-free dough and does not serve to incorporate high gas volumes (see Figure 8). A dependency of the bread volume on the level of mechanical aeration independent from the associated dough temperature increase has to be validated.

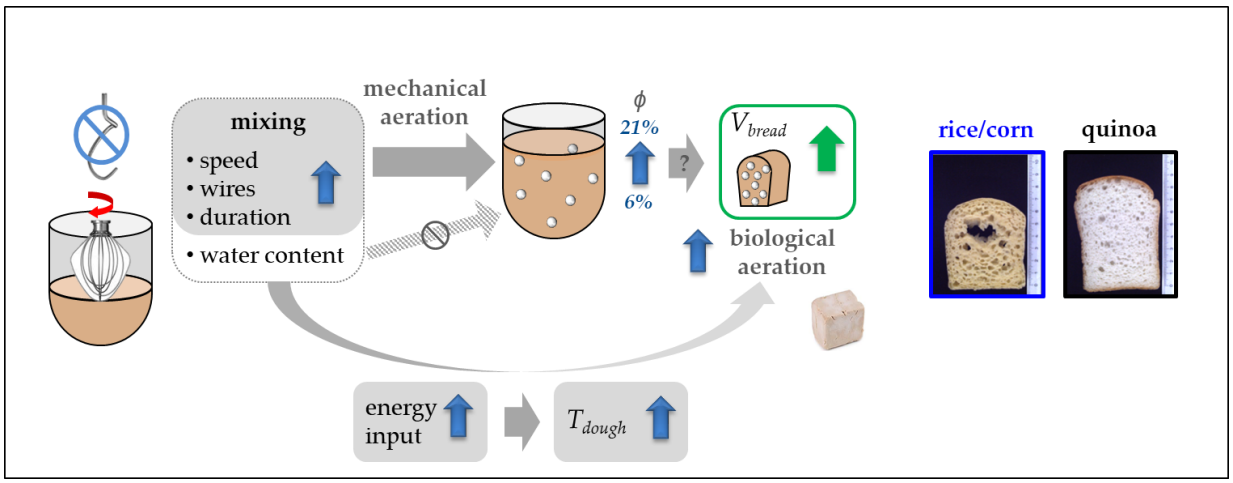

Figure 8. Graphical abstract of the third publication. V_{bread}: Bread volume, T_{dough}: Dough temperature after mixing.

Foam stabilization during processing of starch-based dough systems Pages 52-59

Currently, the huge variety of recipes used by industries and researchers makes it difficult to compare and generalize glen-free research. Thus, the final study focus was on the identification of rheological properties that promote bubble stabilization throughout mixing, proofing and baking. In order to allow for correlations between the bread volume and the mixing process or the rheological properties, yeast fermentation was standardized. Indeed, high speed beating increased the bread volume by 18%, independent from the positive effect of dough heating on yeast activity (see Figure 9). Dough viscosity of various recipes was monitored in a novel rheometer program, simulating shear stress and temperature from the processing stages. During mixing, higher shear-thinning and lower viscosity promoted aeration but bread density was not directly related to dough density. High gas retention capacity is fundamental for the development of new aeration strategies, since bubble stabilization seems to be of special importance for gluten-free dough aeration. The retention of gas was improved if the viscosity during fermentation and at the end of baking was higher. Fractionated quinoa and rice flour resulted in the lowest dough density as well as the highest bread volume. In conclusion, a new method to predict the baking performance of different recipe compositions was developed, which led to new insights into the mechanisms for the production of gluten-free bread with high volume and fine pores.

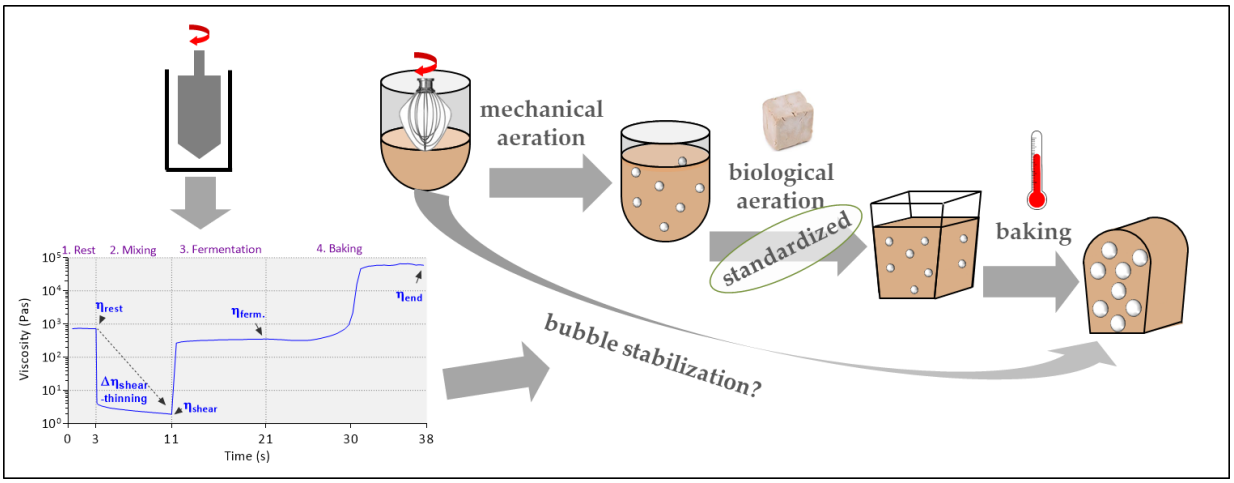

Figure 9. Summary of the fourth publication.

2.2 Strategies for the aeration of gluten-free bread - A Review

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Review

Strategies for the aeration of gluten-free bread – A review

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ABSTRACT

Background: Deficient gas retention properties and consequent low loaf volume are major issues in the production of gluten-free bread. Owing to fundamental differences in medium properties of gluten-free and wheat dough, a strict adherence to traditional techniques is counter productive.
Scope and approach: The present study reviews analysis tools that enable the monitoring of single bubbles as well as the aeration state with regard to spatial and temporal resolution. Various methods used for the aeration of conventional dough and batter are evaluated and compared with those used for gluten-free dough production. Promising strategies and processing parameters that might improve the incorporation and stabilization of gas in gluten-free dough are presented.
Key findings and conclusions: The substrate availability of gluten-free raw materials plays an important role for biological gas production through microorganisms, which can additionally improve the gas retention capacity by synthesizing hydrocolloids. Moreover, the deficient volume of gluten-free dough might be substantially improved by optimizing mechanical aeration via beating. High-speed mixing can provide a homogeneous distribution of small gas bubbles. Computed tomography is the method of choice to monitor gas bubbles if structure-conserving preparations and sufficient resolution are provided. To replace the traditional kneading stage, processing adaptions should provide maximum gas entrapment by mixing.

1. Introduction

Aerated foods such as cake and bread owe their distinctive texture and appearance to the presence of bubbles (Campbell & Mougeot, 1999). The incorporation of gas into dough is a central challenge for the bakery industry because volume and cell structure are particularly relevant quality attributes that can vary depending on the type of product. In the case of traditional bread production, initial gas cell nuclei are incorporated by kneading. The bubbles grow initially by chemical or biological carbon dioxide formation and subsequently through evaporation and gas expansion caused by baking heat. To achieve a low crumb density, both the incorporation and the stabilization of gas bubbles are crucial. Thus, the evolution of bubbles during kneading, proofing, and baking in traditional wheat-based systems has been investigated (Chiotellis & Campbell, 2003; Shah, Campbell, McKee, & Rielly, 1998). However, owing to differences in composition and structure, such results are only partly transferable to the mechanisms in gluten-free formulations. Because the absence of gluten makes it challenging to stabilize and retain gas, improvements of current aeration methods are required to fulfill steadily rising demands and expectations regarding these products.

Various approaches for bread dough aeration are possible. After the incorporation of gas and throughout all further processing steps, destabilizing mechanisms must be suppressed as far as possible to maintain the foam structure. While for traditional dough rheological properties such as strain hardening, high viscosity, and extensibility aid in gas retention (Bloksma, 1990; Mills, Wilde, Salt, & Skeggs, 2003; Stauffer, 2007), dough made from gluten-free flour and water typically lacks all these qualities. Therefore, new strategies for gluten-free products must include ingredients and production methods other than those used in conventional bread making. Merely modifying the recipe composition is insufficient because of the fundamental differences in the dough structure. The entire bread-making process comprising preconditioning steps, mixing, resting, proofing, and baking must be adapted to the gluten-free medium.

Previous reviews have mainly focused on ingredient interactions; in contrast, this study summarizes recent strategies for the aeration of gluten-free bread. Methods for assessing the gas

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volume fraction and the bubble size distribution in dough are critically compared, and the impact of the aeration method on bubble growth and stabilization is assessed. Finally, chemical, biological and physical aeration methods are presented with a stress on those suitable for gluten-free dough. This may encourage the development and improvement of new approaches for the production of gluten-free products.

2. Bread as food foam: how gas adds value to bread

The controlled and steady production of aerated food is challenging and requires the interaction of industrial experience and scientific research. Although most cereal-based products such as breakfast cereals, popcorn, croissants and bread attain most of their value and functionality from aeration, comparatively little research has been conducted on this process. Since the macrostructure of bread can be described as cellular, the entrapment and stabilization of gas bubbles play a crucial role. Cellular solids comprise a cluster of enclosed spaces that can differ in size, shape, orientation, and connectivity (Cafarelli, Spada, Laverse, Lampignano, & Del Nobile, 2014). Such structures are present in natural and man-made sponges, corks, etc., the uses of which have increased in popularity because of superior thermal insulation and cushioning properties (Gibson & Ashby, 1999). Cell-like structures in food facilitate biting, chewing, and digestion. For example, the crisp and crunchy textures that are desired in snack products result from the cellular honeycomb structure formed by extrusion (Barrett & Peleg, 1992). Moreover, the heat transfer during baking strongly depends on the gas volume fraction in the product such that increasing the porosity by 20% results in a 7 min reduction in baking time (Mack, Hussein, & Becker, 2011). Thus, by considering the spongy, porous crumb as a cellular solid new perspectives and insights can be gained.

The number and size distribution of gas pores substantially differ among bread types. While the quality of ciabatta and baguettes is strongly related to the presence of large pores, consumers expect white pan-baked bread to feature small, homogeneously distributed pores. The mechanical behavior and overall quality of cellular foods are mainly influenced by the degree of aeration and bubble size distribution. In addition, the geometry of the cells as well as the thickness and strength of their wall material are essential factors (Dogan & Kokini, 2007).

3. Evaluation of gas volume fraction and gas-free density

To evaluate the various aeration methods, the level of gas entrapment must be defined. However, several challenges need to be overcome to obtain this value. In this section, the different methods and formulas employed to determine the amount of air in cake and wheat dough will be discussed with regard to their applicability for gluten-free dough.

3.1. Determination of the gas-free density

A problem often neglected is the determination of the gas-free density, often termed “true density”, representing the continuous phase of the foam structure of a dough or bread sample. Particularly in the case of gluten-free dough, extensive recipe variations presuppose the awareness of the gas-free density to enable a comparison of the aeration level. Applied methods and their results for various dough and cake formulations are summarized in Table 1. A theoretical approach is the estimation of the gas-free density by summing the densities of single ingredients in their corresponding ratios, also referred to as the rule of mixtures. However, it is not understood how the density of a powder such as flour can be used to estimate the density of hydrated particles in dough without considering volume changes. Moreover, interactions among salt, polymers, and water are neglected. Further factors reported to influence the gas-free density of dough independently from its formulation include oxygen availability during mixing, mixer design, mixing speed, and shear history (Campbell, Rielly, Fryer, & Sadd, 1993; Chin & Campbell, 2005).

In previous experiments, the gas-free density of dough or batter has been measured by carefully stirring the ingredients to obtain a homogeneous mixture without air inclusion (Massey, Khare, & Niranjan, 2001), by mixing the dough under vacuum (Baker & Mize, 1937), or by degassing the samples (Richardson, Langton, Fäldt, & Hermansson, 2002). Campbell et al. (1993) obtained a gas-free wheat dough density of 1.28 g/cm^3 by mixing samples at various pressures and extrapolating the graph of dough density versus mixing pressure back to zero pressure. This labor intensive method is based on the assumption that mixing at zero pressure (vacuum) results in dough without gas, featuring the same chemical properties as the continuous phase of aerated dough.

Alternatively, the density with and without gas as well as the gas volume fraction can be evaluated with the aid of computed tomography or other imaging techniques. These methods are discussed in Chapter 4. Lassoued, Babin, Della Valle, Devaux, and Réguerre (2007) reported a correlation of $r^2 = 0.91$ between the gas-free density as determined by calculation and X-ray analysis for bread. Similarly, Bellido, Scanlon, Page, and Hallgrimsson (2006) presented a difference less than 1% when comparing both methods, although they noted that this led to a larger error for the respective gas volume fractions. No degassing step is required if the density of the continuous phase derives from image analysis, but the validity of the result strongly depends on the resolution of this method. If gas bubbles are smaller than the detection limit, they will falsely decrease the corresponding gas-free density. Richardson et al. (2002) used centrifugation for the purpose of degassing cake batter; however, they did not give the centrifugation parameters. For gluten-free dough, probably all of the methods are applicable, although no data have been reported thus far. Due to the large number of recipes, a time-efficient technique would be convenient. Degassing by (ultra-) centrifugation might be a suitable technique because it is rapid and lacks the aforementioned disadvantages.

3.2. Methods used to evaluate the dough density

In 1993, Campbell et al. devoted an entire study to the measurement and interpretation of dough density. Their labor-intensive method included freezing the dough and adding water to a high density calcium chloride solution until buoyancy was reached. In 2001, a more convenient double-cup buoyancy technique was developed, allowing calculation of the density by comparing the sample weight in air with that in xylene (Campbell, Herrero-Sanchez, Payo-Rodriguez, & Merchan, 2001). However, because gluten-free dough is usually more sticky and fluid than wheat dough, which can easily be formed into a ball, this method might not be applicable.

During fermentation, aeration through a microorganism such as *Saccharomyces cerevisiae* can be monitored in a Rheofermentometer. With this method, however, the initial aeration through kneading and fermentation prior to to the measurement is neglected. Gómez, Talegón, and De La Hera (2013) reported that the lack of consistency causes an overflowing of the gap between the rheofermentometer basket and the probe when measuring gluten-free dough, even without the addition of resistance weights. As a consequence, the interpretation of the obtained curves may be challenging. Verheyen, Jekle, and Becker (2014) compared the density of wheat dough in different analysis devices and

Table 1
Gas-free density of dough and batter determined by various methods. Deviations between gas-free dough densities may derive from different recipes. µCT: micro-computed tomography.

Refs.	Method	Water content (g/100 g flour)	Medium	Gas-free density (g/cm^3)
Baker & Mize, 1937	Mixing under high vacuum	Not defined	Wheat dough	1.250
Bellido et al., 2006	3D analysis via µCT	67	Wheat dough	1.188
Campbell et al., 1993	Mixing at different pressures and extrapolating to zero pressure	61	Wheat dough	1.276
Elmehdi et al., 2004	Mixing at different pressures and extrapolating to zero pressure	63	Wheat dough	1.285
Richardson et al., 2002	Centrifugation	n. d.[a]	Cake batter	1.250
Shehzad et al., 2010	Rule of mixtures	60–65	Wheat dough	1.21–1.25
Trinh et al., 2013	Mixing at different pressures and extrapolating to zero pressure	58	Wheat dough	1.262

[a] In case of cake batter the recipe contained approximately 34% water (incl. egg).

determined strong variations owing to alterations in handling and applied pressure. Defloor, De Geest, Schllekens, Martens, and Delcour (1991) monitored the increasing volume of gluten-free dough during fermentation in a graduated glass cylinder located in a tempered water bath. However, this method requires fluid dough that can be poured easily.

3.3. Gas volume fraction

The density gives the first indication of the amount of gas in dough. However, the validity of the correlation is impaired, if the density of the continuous phase changes due to processing or ingredient variations, which is often the case when developing gluten-free formulations. To describe the amount of gas in the product more specifically, the gas volume fraction, which is referred to alternatively as void fraction, gas hold-up, overrun, or porosity, can be measured. Through mixing, the dough density has been reported to decrease from 1.22 to 1.01 g/ml (Shehzad et al., 2010). As presented in Table 2, the amount of gas entrained after mixing wheat dough without yeast strongly depends on the mixing conditions such as atmosphere, speed, and time resulting in a gas volume fraction of 5.5–20% and up to 71% in the case of cake batter with respective emulsifiers. In the course of fermentation, the proportion of gas in dough can rise to 80% (Shehzad et al., 2010). The level of aeration through mixing in gluten-free dough is yet unknown.

Usage of the term “porosity” can be ambiguous because it also defines the permeability of a material. According to Campbell and Mougeot (1999), porosity is valid only when the air phase is continuous as in the case of bread. In contrast, various studies use porosity ε interchangeably with gas volume fraction Ø or void fraction α to describe the volume of gas V_{gas} in relation to the total dough volume V_b (Equation (1)). This value can also be calculated by determining the bulk and gas-free densities, ρ_b and ρ_{gf}, respectively, assuming that the weight of gas is negligible (Besbes, Jury, Monteau, & Le Bail, 2013; Demirkesen et al., 2014; Hicsasmaz, Yazgan, Bozoglu, & Katnas, 2003; Massey et al., 2001). The fact that a 0.5% deviation elevates the gas volume fraction by 10% highlights the importance of the correct determination of the gas-free density (Chin & Campbell, 2005).

$$\varepsilon = \phi = \alpha = \frac{V_{gas}}{V_b} = 1 - \frac{\rho_b}{\rho_{gf}} \quad (1)$$

Gibson and Ashby (1999) preferred to evaluate cellular solids by using the relative density (1−ε), which has also been used for bread (Besbes et al., 2013). A separate value, the overrun OR or the specific volumetric content V_a, exists for systems with very high gas content (Campbell & Mougeot, 1999; Germain & Aguilera, 2014). With the aid of Equation (2) the degree of batter aeration can be expressed (Tan, Chin, & Yusof, 2011). The gas fraction is related to the volume of the continuous phase instead of the total volume, resulting in higher values.

$$OR = V_a = 100 \times \left(\frac{\phi}{100 - \phi}\right) = 100 \times \left(\frac{\rho_{gf}}{\rho_b} - 1\right) \quad (2)$$

When using 2D or 3D image analysis for assessing the porosity of bread, area/volume of voxels per area/volume of interest is typically employed (Van Dyck et al., 2014). Richardson et al. (2002) obtained a linear relation between the area fraction of a binary dough image and the dough density.

In summary, various techniques and formulas used to describe the proportion of gas in dough have been previously reported. As a prerequisite, a time-efficient reference method for assessing the gas-free density, such as high-speed centrifugation, should be employed. While “porosity” is an ambiguous term, “gas volume fraction” is a more accurate description. The following chapters focus on the gas cell sizes and distributions in addition to various assessment methods in current usage.

Table 2
Gas volume fraction of dough and batter after mixing without yeast. µCT: micro-computed tomography.

Refs.	Method/Treatment	Water content (g/100 g flour)	Medium	Gas volume fraction (%)
Baker & Mize, 1937	Comparing bulk density to gas-free density	Not defined	Wheat dough	20
Bellido et al., 2006	3D analysis via µCT	63.0–67.4	Wheat dough	7.6–9.5
	Comparing bulk density to gas-free density			7.6–10.4
Campbell et al., 1993	Comparing bulk density to gas-free density	61	Wheat dough	
	➔ Strong flour, 150 rpm mixing speed			~5.5
	➔ Weak flour, 300 rpm mixing speed			~9.2
Richardson et al., 2002	Comparing bulk density to gas-free density	n. d.[a]	Cake batter	
	➔ without emulsifier			20
	➔ with best emulsifier (Aroplus)			71
Shehzad et al., 2010	Comparing bulk density to gas-free density	60–65	Wheat dough	17
Trinh et al., 2013	Comparing bulk density to gas-free density	58	Wheat dough	6.4

[a] In case of cake batter the recipe contained approximately 34% water (incl. egg).

4. Monitoring of the bubble size distribution in dough

The previously described gas volume fraction of dough is an important value for assessing the success of an aeration method. Additionally, it is beneficial to know how the gas is distributed within the continuous phase. Two dough samples with the same gas content may produce fundamentally different crumb structures owing to differences in the initial bubble sizes and their size distributions. The opacity and instability of dough makes it difficult to observe the distribution and the behavior of incorporated gas bubbles. Because gluten-free dough is often more fluid and less elastic than wheat dough, coalescence buoyancy and disproportionation might play large roles in its foam stability. Thus, suitable techniques for evaluating gas bubbles throughout the processing in gluten-free dough are required. Images of gluten-free and wheat dough and batter with visible gas bubbles are presented in Fig. 1. Among others, the methods used to obtain these pictures are compared critically in the following section.

One strategy for analyzing bubbles in food microstructures is the use of image analysis. In the case of cake batter, microscopy has been coupled with a digital or charge-coupled device camera for analyzing gas bubbles; however the bubble shape can be altered when the sample is squeezed between the glass plates (Hicsasmaz et al., 2003; Massey et al., 2001). Other researchers analyzed physically sectioned samples of frozen dough under a microscope or through cryo-scanning electron microscopy for higher resolution (Carlson & Bohlin, 1978; Trinh, Lowe, Campbell, Withers, & Martin, 2013). Unfortunately, freezing affects the dough density; thus, the gas cell distribution might be altered (Campbell et al., 1993). Furthermore, Trinh et al. (2013) reported that starch granules can be lost during the fracturing process, and their recesses might be mistaken for air bubbles. A summary presented by Bellido et al. (2006) included studies using microscopy either with or without physical sectioning. They detected considerable variations in the gas volume fraction (3.5–10%), the bubble sizes (35–112 μm) and the number of bubbles ($2500–10^8$ cm^{-3}), which strongly depended on the observed bread slice thickness.

Confocal laser scanning microscopy (CLSM) enables a 3D visualization of the internal microstructure of dough after staining specific ingredients (Jekle & Becker, 2011; Richardson et al., 2002). For the analysis of gas bubbles, the components of the surrounding matrix have to be visualized. Insufficiently stained ingredients and Becke lines, which make bubbles appear smaller, can falsify the results (Richardson et al., 2002). Similarly, interactions between dyes and dough components might have an influence on the visible structure. Larger gas bubbles (500–2000 μm) are difficult to detect via CLSM because they occupy most or all of the visible area.

A strategy that does not require staining agents or other preparatory steps is magnetic resonance imaging. This method has been applied for analyzing the structure of wheat bread and dough, with possible temperature treatments required to simulate fermentation and baking (e.g., Bajd & Serša, 2011; Takano, Naito, Ishida, Koizumi, & Kano, 2002). However, with this technique it is challenging to find a compromise between temporal and spatial resolution. For example, Bajd and Serša (2011) used high-field 3D magnetic resonance microscopy for monitoring the evolution of gas bubbles in wheat dough. By using an image resolution of $0.23 \times 0.23 \times 1.5$ mm^3 and a scan time of 7 min, they were able to followed the complete fermentation and baking process. Surprisingly, no image blurring was reported during the lengthy measurements; only a minor proportion of bubbles was detected. In a dough volume of 4.2 cm^3, the number of bubbles increased from approximately zero to 500 during 110 min of fermentation, which resulted in a final pore concentration of 0.1 cells/mm^3. These bubbles likely did not emerge during proofing; it is plausible that the bubble size gradually increased and bubbles which were initially too small exceeded the detection limit.

Similar to the previously described methods, X-ray analysis with computed tomography is another non-invasive technique. This method is steadily gaining popularity and has been used for the analysis of the gas bubble distribution in dough and bread in various studies (e.g., Besbes et al., 2013; Demirkesen et al., 2014; Van Dyck et al., 2014). Table 3 gives an overview on the X-ray systems and settings used and the corresponding results of the gas bubble analysis in wheat dough. A limiting factor for determining the size distribution of gas bubbles is the resolution. For example, the laboratory X-ray device was not able to distinguish bubbles smaller than 20 μm (Trinh et al., 2013). Furthermore, the lengthy duration of the measurement makes it impossible to analyze yeasted dough. In the case of the medical system used by Bellido et al. (2006) or the Synchrotron system, rapid measurements with high resolution enable monitoring of the fermentation and baking processes. Because of the high amount of energy involved, influence on the dough structure is likely. Although Babin et al. (2006) reported that yeast activity is not influenced by X-rays, the impact of such radiation should be further observed. All the aforementioned examples required the formation of miniature dough samples, which counteracts the non-invasive nature of the method.

A different approach is to use the different responses of gases and solids to sound pressure waves in the ultrasound frequency range of 20 kHz up to several gigahertz. While high-energy ultrasound (20–50 Hz) can be used to modify food in a physical, mechanical, or (bio-)chemical way, lower intensities (50–500 kHz) are non-destructive and are thus applicable to analyze processes and end products by sensors (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). The most striking advantage of ultrasound analyses is the possibility of cost-efficient on-line monitoring during

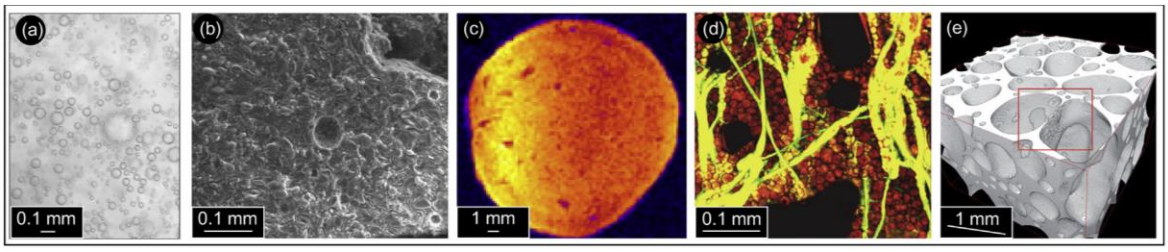

Fig. 1. Images of gas bubbles in dough and batter resulting from different acquisition methods. (a) Microscopy image of cake batter by Hicsasmaz et al. (2003); (b) Cryo-SEM image of wheat dough without yeast after high pressure mixing by Trinh et al. (2013); (c) MRM image of wheat dough without yeast by Bajd and Serša (2011); (d) CLSM image of gluten-free dough with the addition of defatted zein (yellow strands) by Johansson, Krona, and Stading (2012); (e) X-ray image of fermented (125 min) wheat dough by Turbin-Orger et al. (2015). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Studies employing X-ray tomography to analyze the gas bubbles in dough. x_g: geometric mean; $\bar{x}$: arithmetic mean.

Refs.	X-ray system	Resolution	Sample preparation	Yeast/ Fermentation	Mean bubble diameter	Scan time	Cell count
Babin et al., 2005, 2006	Synchrotron ESRF	15 μm	7 mm tube/dough balls: $(9 \times 9 \times 4\ \text{mm})^3$	Yes	180 μm ($\bar{x}$)	30 s	–
Bellido et al., 2006	Scanco Medical VIV-ACT 40	10 μm	Dough plate 2 mm × 20 mm	No	100–109 μm (x_g)	420 s	30–57 N/mm^3
Turbin-Orger et al., 2012	Synchrotron ESRF	5 μm	9 mm tube	Yes	220 μm^{-1} mm ($\bar{x}$)	9 s	–
Trinh et al., 2013	Nikon Xtek	43 μm	10 mm tube	No	~70–115 μm ($\bar{x}$)	45 min	45–91 N/mm^3

food production (Coupland, 2004). Initial attempts to characterize wheat dough using ultrasonic pulses have been made (e.g., Elfawakhry, Hussein, & Becker, 2013; Elmehdi, Page, & Scanlon, 2004; Garcia-Alvarez et al., 2006; Létang, Piau, Verdier, & Lefebvre, 2001). Leroy et al. (2008) reported that a pulsed transmission technique is suitable for detecting the sizes of gas cells in unyeasted wheat dough. Unfortunately, knowledge of various other material properties such as the gas volume fraction and rheological features, some of which are more difficult to obtain, is required. Furthermore, Leroy et al. (2008) were not able to measure samples without considerably compressing the dough, which resulted in significant differences in the particle size distribution measured through X-ray tomography.

When comparing the available techniques, subjection to computed tomography appears to be the most promising. However, this method has to be optimized to ensure sufficient temporal and spatial resolution without interfering with the dough structure through preparation steps or radiation. While many approaches for analyzing the gas bubble distribution wheat dough have been conducted, such analysis is lacking for gluten-free dough. Due to differences regarding the gas retention capacity and rheology the gas bubble distribution in gluten-free dough might fundamentally differ from that of wheat dough. Therefore, targeted adaption might be key for improving the foam stability. From this perspective, the success of various aeration methods can be evaluated.

5. Adaption of aeration methods for food foams to gluten-free formulations

While Campbell and Mougeot (1999) gave a comprehensive overview on methods used for the aeration of foods, the present study focuses on methods that can be employed specifically for gluten-free dough. In Fig. 2, the various aeration stages for the production of bread and cake are shown with their respective mechanisms. For clarity, the aeration methods are divided into the three categories: biological, chemical, and physical. However, this classification is not strict because many of the methods presented in previous studies actually belong to more than one group and the limits may be blurred. In this section, the applicability of biological aeration will be discussed first, since it is associated with highest quantitative impact. Although chemical leavening agents are less frequently used for bread, they play an important role in cake and possibly in gluten-free bread. Finally, physical aeration is discussed as a decisive qualitative factor with manifold potential for improvement.

5.1. Biological aeration

To achieve a fine crumb structure with low density, the size and amount of bubbles incorporated through mixing should increase evenly during the subsequent processing stages. For this purpose, biological aeration through microorganisms, such as baker's yeast, is applicable, because it continuously produces carbon dioxide as long as the surrounding conditions are favorable. The rate of carbon dioxide formation depends on choice, viability, and vitality of the microorganisms and in addition to on temperature, humidity, substrate availability, pH-value, ionic strength, and the presence of nutrients. The resulting growth of bubbles during proofing and baking can be effectively simulated (e.g., Chiotellis & Campbell, 2003; Fan, Mitchell, & Blanshard, 1999; Shah et al., 1998). For correct calculation of the diffusion of carbon dioxide into air bubbles, it is challenging to determine its concentration in the liquid dough phase. Among the results of previous studies, it was reported that the final bubble size depends on the initial size at the beginning of fermentation (Chiotellis & Campbell, 2003). This finding stresses the importance of the preliminary mixing process for producing small nuclei with a narrow size distribution. A suitable model applicable to gluten-free dough would be helpful for identifying the right choices of fermentation and baking settings.

Although the gas incorporated by mixing primarily comprises nitrogen and oxygen, bubble growth through a raising agent is limited to carbon dioxide (Cauvain & Young, 2007). Soluble carbon dioxide enters the gas bubbles if the concentration in the medium exceeds that in the bubbles. The different solubilities of gases in the liquid dough phase are of particular importance because they depend on temperature, pressure, pH-value, and the presence of solutes. At saturation, 1 L of water in contact with air (at 1 bar) contains 11 ml nitrogen, 6 ml oxygen and 0.3 ml carbon dioxide. However, if pure gases are used instead of the gas composition of air, up to 878 ml carbon dioxide, 31 ml oxygen, and 16 ml nitrogen can be solved (Roland, 2006). Campbell and Mougeot (1999) discussed the physical behavior of bubbles in food regarding gas solubility, mass transfer, and diffusivity and reported that the evolution of the bubble size distribution in food is complex, particularly that in bread dough during proofing. It becomes even more complex when exchanging the familiar structure of wheat dough or cake batter for a heterogeneous gluten-free dough matrix.

Of the many different microorganisms available for the production of carbon dioxide in the dough medium, *S. cerevisiae* yeast is the most common. A mixture of various yeasts and lactic acid bacteria is used to produce sourdough. The use of sourdough for gluten-free bread has been comprehensively reviewed by Gobbetti, Rizello, Di Cagno, and De Angelis (2007) and O'Shea, Arendt, and Gallagher (2014). Since sourdough is a very heterogeneous material and its manufacturing conditions vary significantly, the range of effects on the textural and sensory properties is extensive. While several functions such as the proteolytic degradation of gluten, microbiological stability through acidification, and generation of flavor compounds may be employed, the present study focuses on the improvement of aeration and gas retention. An interesting feature of several bacteria is the production of exopolisaccharides (EPS) with a structure and function similar to that of hydrocolloids. Rühmkorf et al. (2012) demonstrated a positive effect of EPS produced by four different strains of lactobacilli on the specific volume of gluten-free bread.

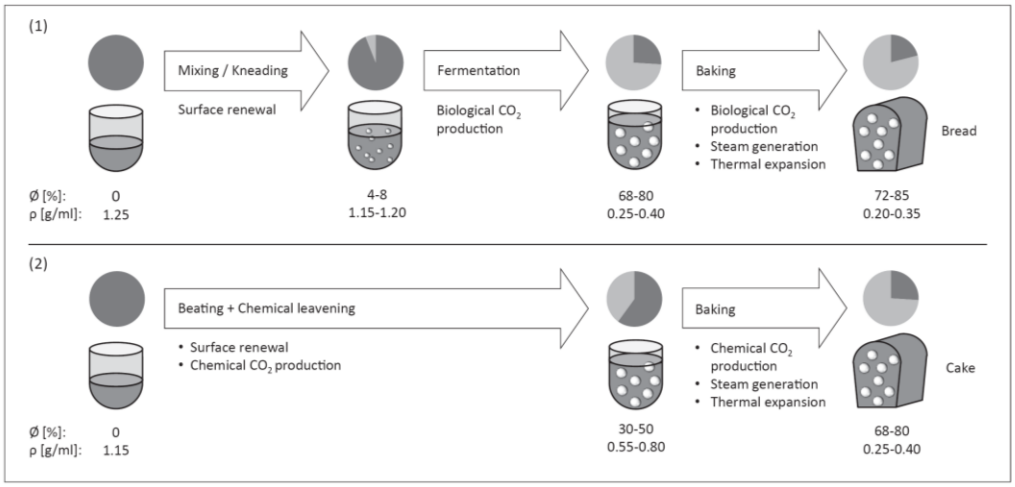

Fig. 2. Aeration stages during bread (1) and cake (2) production. The aeration stages are indicated in the arrows with the respective mechanisms listed below. Pie charts illustrate the gas volume fraction (light grey) in the respective material. The values for the gas volume fraction Ø and the density ρ originate from Campbell and Mougeot (1999).

Previous studies do not indicate the use of sourdough as a universal remedy for the improvement of gluten-free bread. In particular, the effect on the loaf volume can be positive or negative depending on the combination of ingredients. For improving aeration through microorganisms, all essential nutrients must be available and the optimum pH and temperature region should be maintained. The most important substrates for the production of carbon dioxide through yeast are mono- and disaccharides. Since typically used flours and starches in gluten-free formulations gerenally lack these substrates (Elgeti et al., 2014), the addition of sugar is common. As alternatives, pre-gelatinization, mechanic damage or amylolytic enzymes can favor starch degradation. Elgeti et al. (2014) determined that quinoa white flour improved the volume of gluten-free bread by significantly elevated α-glucosidase activity in comparison with rice and corn flour. Similarly the addition of 10% quinoa bran improved aeration by providing substrates for yeast (Föste et al., 2014).

5.2. Chemical aeration

One strategy for improved aeration of gluten-free bread is the use of chemical raising agents as a replacement or in accompaniment to yeast. Similar to biological leavening, aeration through chemical raising agents depends on the rate of carbon dioxide formation and the ability of the dough to retain the gas. Although the application of chemical raising agents is rare for traditional bread, they are employed for many gluten-free products in addition to yeast. Segura and Rosell (2011) compared 11 gluten-free breads from a Spanish market, of which 7 included raising agents. Moreover, Sinelli, Casiraghi, and Downey (2008) reported that chemical raising agents are common in gluten-free and wheat bread formulations. Careful selection of the type, combination, and concentration of the baking powder is important as is the consideration of the specific properties and requirements of the product. Thus, it is surprising that no study has been conducted on the application of chemical leavening agents.

By combining two different acid carriers instead of a single or two-component baking powder, two separate aeration events can occur during processing. This enables a rapid carbon dioxide release during mixing through the first acid and retarded gradual aeration at higher temperatures during baking through the second acid (Bode, 2009). The disadvantages of chemical raising agents include legal restrictions and possible negative effects on the taste. Sodium hydrogen carbonate with sodium pyrophosphate is reported to cause a soap-like taste in products with a low amount of sugar and fat, and an additional astringent mouth feel is possible (Bode, 2009). The potential of chemical raising agents for more controlled aeration in gluten-free bread should be evaluated in future studies.

5.3. Physical aeration

Biological and chemical aeration are usually preceded by mixing or kneading, which assumes a special role in the resulting bubble size distribution. Depending on the amount and distribution of initial gas nuclei, the kinetics of the consecutive bubble growth and stability during proofing and baking are affected. This process might be the most important target adaption of traditional processing settings to the requirements of gluten-free dough.

5.3.1. Aeration through kneading or mixing

For the production of food, a huge variety of mixing mechanisms is available, the choice depends on the purpose, the material properties of the product, and the desired throughput (Vyakaranam & Kokini, 2011). In traditional bread making, the mixing process serves mainly to homogeneously distribute the ingredients, and the subsequent kneading incorporates gas nuclei and formes a 3D gluten network.

When kneading gluten-containing dough, a spiral or hook is sufficient for ensuring the inclusion of the entire material due to the cohesiveness of the dough. Because batter and gluten-free dough are generally stickier and less cohesive, stagnant zones should be avoided to provide homogeneous distribution of the components (Todd, 2004). Consequently, the integration of dough scrapers during mixing seems necessary (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010; Schober, Messerschmidt, Bean, Park, & Arendt, 2005).

The forces acting upon the gas bubbles during kneading and mixing are complex and difficult to analyze. Thus, only few studies have been conducted on the flow characteristics or shear rates in various constructions and in different media. Particle image velocimetry and laser Doppler anemometry have been used to

visualize the flow of particles in a mixer (Vyakaranam & Kokini, 2011). In addition, numerical simulation techniques have been developed to observe and predict the flow behavior in a non-intrusive manner (Connelly & Kokini, 2006). In contrast to the extensional stress during proofing and baking, shear stress in a dough mixer is dominant because the mixing device causes a strain parallel to the wall material (Stauffer, 2007). This laminar stretching and folding are non-dispersive mixing mechanisms (Vyakaranam & Kokini, 2011). During kneading, sheets of dough are placed upon each other, trapping air through the action of surface renewal (Campbell & Mougeot, 1999). The choice of the mixing machine and the processing parameters strongly influences the number and size of the entrained gas bubbles (Cauvain & Young, 2006). Although this technique has been carefully adapted to the requirements of gluten-containing formulations, it is questionable whether it is suitable for gluten-free dough.

While the network formed by gluten can be destabilized by excessive shearing (Peighambardoust, Fallah, Hamer, & van der Goot, 2010), dispersive mixing used to aerate cake batter benefits from high shear stress, and more importantly, intense deformation for the disruption of bubbles (Chesterton, de Abreu, Moggridge, Sadd, & Wilson, 2013 and Todd, 2004). Dispersive mixing is a promising alternative for the aeration of gluten-free dough because it lacks a gluten network and has a consistency similar to that of cake batter. Mixing at high speed in a planetary mixer is also commonly reffered to as beating. In a planetary mixer, the revolution (rotary motion around vertical axis of beating arm) is always slower than the rotation (rotary motion of mixing tool around its own axis) and is in opposite direction, which means that the local speed exceeds the total speed (Cheng, 1992). As in traditional kneading, the beating process entrains gas through surface aeration; however, the dispersion and disruption of generated bubbles in high shear zones play key roles. These shear zones can be created by forcing the material through narrow passageways; e.g. between mixing geometry and wall (Massey et al., 2001). As a secondary effect, the constant creation of new surfaces during mixing induces cooling based on evaporation, although the simultaneous input of energy increases the dough temperature through friction depending on the rheology. In turn, the dough temperature affects gas volume, gas solubility, viscosity, and the rate of carbon dioxide formation through a raising agent. This effect plays a bigger role for high-speed mixing than for the traditional kneading.

Table 4 lists examples of mixing methods employed for gluten-free dough and roughly divides them according to their energy input. Two obvious factors are noted: large heterogeneity regarding devices and settings and the low level of importance given to mixing parameters. In many studies, gluten-free dough is referred to as batter, and planetary mixers replace traditional kneaders (e.g., Mariotti, Ambrogina, Pagani, & Lucisano, 2013; Nunes, Moore, Ryan, & Arendt, 2009). However, only Gómez et al. (2013) actually compared the effects of different mixing parameters (two mixing speeds, three durations and three geometries) on proofing and specific bread volume.

Furthermore, dough development during proofing strongly depends on the extent of agitation. Although for wheat dough several studies determined that greater aeration is related to higher mixing speed (Chin & Campbell, 2005), no correlation was drawn between mixing speed and specific volume for gluten-free dough. To determine the energy input, the mixing speed in addition to the duration must be considered. Chesterton et al. (2013) determined that extended mixing produced fewer and larger bubbles in batter. It might be possible to compensate for destabilizing mechanisms by providing more surface active ingredients or by increasing the dough viscosity. For gluten-free dough it was observed that longer mixing periods of 4 or 8 min rather than 2 min, accelerated the dough aeration during fermentation. It is likely that the higher yeast activity contributed to the effect through an elevated dough temperature resulting from the longer mixing period. Thus, monitoring the dough temperature is advisable for interpreting mixing trials.

Sciarini, Ribotta, León, and Pérez (2012) integrated an additional mixing stage after proofing and before baking in order to add more gas to the gluten-free dough, redistribute the grown air cells, and disperse the yeast substrates. This technique merits attention, because it simulates the traditional step of manual preparation prior to the molding of wheat dough. Although it is likely that gas is lost in this process since additional processing might cause destabilization of the foam structure, the fine redistribution might promote the retention of gas during the consecutive baking stage.

Another important parameter is the agitator geometry, which includes spiral hooks, straight hooks, blades, floats, or whisks. The latter can be pear shaped, cylindrical, or cruciform composed of thick or thin wire, depending on the medium properties and the desired function (Cheng, 1992). Gómez et al. (2013) used a higher flour to water ratio of 1:1.1 instead of 1:0.8 when using a wire whip because this geometry requires a more fluid dough. The results showed that a wire whip is more suitable for gluten-free dough than the flat or kneading geometry, but the impact of the higher dough hydration was unknown. Alternatively, many have used flat beaters, as shown in Table 4. It may be impossible to identify optimum processing parameters applicable for all recipes and mixing devices. Clearly, additional research should be dedicated to identify suitable mixing systems for gluten-free dough aeration.

5.3.2. Aeration through pressure or ultrasound mixing

In 1962, the Chorleywood Bread Process was developed as an alternative for traditional kneading, which combines high-speed mixing with the application of pressure or a vacuum (Cauvain & Young, 2006). Because this process allows for indirect modulation of bubble sizes by varying the headspace pressure, it might be suitable for the requirements of gluten-free dough. Massey et al. (2001) determined that an increase in pressure during the mixing of cake batter increased aeration and reduced the bubble size. In their study, however, the improved gas fraction was attributed to the expansion of bubbles after releasing the pressure rather than enhanced gas retention. Generally, pressure beating produces fine bubbles that expand as soon as the pressure is released, which can save time and ingredients (Cheng, 1992).

As explained in Chapter 4, ultrasound can be employed to analyze dough properties after mixing. However, it can also be used as a processing technique by generating pressure, shear, and temperature gradients in the medium in which they propagate (Dolatowski, Stadnik, & Stasiak, 2007). Tan et al. (2011) patented a method combining a mixer with a power ultrasonic bath tank for improved aeration of cake batter through acoustic cavitation (Chin, Tan, Che Pa, & Yusof, 2012). Ultrasound aeration is considered to be a good method for the aeration of batter at the small scale, although its adaption to industrial processes might substantially increase operating costs.

In summary of the reviewed methods, the adaption of the mixing process to the requirements of gluten-free dough appears to be a promising strategy. Future trials should further evaluate the impact of mixing parameters on the temperature, structure, and gas volume fraction of gluten-free dough.

5.3.3. Aeration through evaporation and gas expansion

During the baking of bread and cake, the rise in temperature initially amplifies carbon dioxide production through the raising agent. As shown in Fig. 1, further increases in the gas volume fraction are provided by thermal expansion of the gases in the

Table 4
Examples for mixing settings employed for gluten-free dough. Initial low-speed homogenization steps were neglected. If not provided, the rotational speeds were approximated from manufacturer specifications and the number of rotations calculated for a rough classification into low- and high-energy mixing.

Estimated energy input	Refs.	Device	Geometry	Speed (level)	Speed (rpm)	Duration (min)	Rotations
Low/medium	Alvarez-Jubete et al., 2010	Hobart mixer A120	–	2	194	3	582
	Föste et al., 2013	Laboratory Mixer SP 12 A^{-3}, Diosna	Spiral hook	–	200	2	400
	Gómez et al., 2013	Kitchen Aid KPM5	Dough hook, flat beater, wire whip	2	~80	2	~160
	Hager and Arendt, 2013	Kenwood Chef Classic	Batter attachment	Medium	~450	1.5	675
	Moore, Schober, Dockery, & Arendt, 2004	Planetary mixer Hunt 30	Batter attachment	–	135 (shaft 270)	1	135
	Phimolsiripol, Mukprasirt, & Schoenlechner, 2012	Kitchen Aid KPM5	–	2	~80	6	480
	Schober et al., 2005	Kenwood Major mixer	(Flat/paddle) K beater	2	331	2	662
	Sciarini et al., 2012[a]	Planetary mixer (Arno)	–		214 + 156	2.1	584
High	Defloor et al. 1991	Kitchen Aid 5K45SS	Flat beater	6	~150	10	1500
	Gómez et al., 2013	Kitchen Aid KPM5	Dough hook, flat beater, wire whip	4	~170	12	2040
	Sabanis, Lebesi, & Tzia, 2009	Kenwood KM400	Spiral hook	–	180	6	1080
Undefined	Eggleston, Omoaka, & Ihedioha, 1992	Kenwood mixer KM201	Flat K beater	6	–	10	–
	Ziobro, Korus, Juszczak, & Witczak, 2013[a]	Laboratory Mixer SP 12, Diosna	Spiral hook	–		8	–

[a] Marked references contain two separate mixing steps before and after fermentation.

bubbles, which are mainly carbon dioxide and nitrogen, and by the evaporation of components such as water, dissolved carbon dioxide, and ethanol. Although these mechanisms are powerful tools for physical aeration, optimum conditions for gluten-free bread such as temperature, duration, number of baking stages, and amount of steam must be identified. In contrast to the slow rate of bubble growth during fermentation, bubbles expand rapidly during baking (Fan et al. 1999). Bell, Daniels and Fisher (1981) developed a method for observing the effects of rapid bubble expansion independently from heat by monitoring the dough volume in a tube with steady evacuation. This technique might also be used to observe the stabilization of physically entrained gas in gluten-free dough. Depending on the gas retention capacity, it might be advisable to reduce the speed of bubble growth because in gluten-free dough stabilization through strain-hardening is unknown. Computed tomography in combination with a heating chamber might be an adequate technique to directly observe the effects of different baking conditions on bubble expansion and crumb formation (Babin et al., 2006).

6. Conclusion

A great variety of techniques is available for the aeration of fluids. For highly viscous media such as dough or batter several restrictions limit the number of available methods. The entrainment of bubbles in bread dough is a complex procedure comprising several stages that require careful adaption of their process parameters and strongly depend on the gas retention capacity of the medium. Thus, the replacement of wheat flour in the production of gluten-free bread poses challenges in developing new recipes and in processing.

For biological aeration, the combination of substrates and microorganism determines the kinetics of gas production during fermentation. Although modeling can help predict the growth of bubbles, the identification of suitable yeasts or lactobacilli for any type of gluten-free raw material must be tested in practice. For example, the concentration of short saccharides as well as amylolytic activities in flours can significantly influence the gas production by yeast. Aeration might be further improved by a controlled bacterial production of hydrocolloids (EPS) through elevated gas retention. The potential of chemical raising agents has been previously demonstrated in commercial products, but this topic is neglected in research studies.

Utilization of the conventional kneading process with a dough hook is suitable only if the medium is more cohesive than adhesive so that homogenization is possible. This is rarely the case for sticky and fluid gluten-free dough. Therefore, several alternative strategies that appear to be suitable for this challenging medium were identified in the present study. These methods include: an exchange of the agitator arm; variation of speed, temperature, and time; the application of vacuum or pressure; the use of ultrasound; employment of an additional mixing stage; and the addition of sourdough or chemical raising agents. The strategy showing the greatest potential is the implementation of a mixing stage similar to the whipping of a cake batter with a beating geometry and high energy input, which can be categorized as dispersive mixing. Although various research groups currently employ this process, knowledge of the influence of single processing parameters on the success of aeration remains largely incomplete.

When selecting a recipe composition for gluten-free dough that allows for maximum gas entrapment and stabilization, the extreme heterogeneity of the formulations presents a significant challenge. Thus, it might be more expedient examine relevant medium properties instead. The necessary shear/flow behavior required to enable aeration during mixing and the optimum resistance and deformation behavior providing stable growth of the gas nuclei during fermentation and baking must be determined. Similarly, techniques for analyzing the surface activity of single ingredients should be further developed.

The construction of a low-cost aeration strategy with efficient energy and time parameters might facilitate the production of gluten-free bread, which may in turn increase the attractiveness of this product for industry and consumers. Gluten-free bread with a crumb structure similar to wheat bread and a short list of ingredients is a goal that can be reached only by combining the adapted processing conditions with suitable medium properties.

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2.3 Volume and texture improvement of gluten-free bread using quinoa white flour

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Volume and texture improvement of gluten-free bread using quinoa white flour

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ABSTRACT

Patients suffering from coeliac disease have to avoid traditional cereals-based products and depend on the availability of gluten-free alternatives. The gluten-free bread matrix and its foam stability are strongly affected by the choice of ingredients. In this study, the impact of quinoa white flour on bread quality parameters, in particular volume, has been investigated. The pseudocereal proved to be a suitable substrate for dough aeration using yeast, since considerably more glucose and a higher activity of α-glucosidase were found in comparison to rice and corn flour. Consequently, quinoa white flour was used to replace 40–100% of the rice and corn flour in a gluten-free control recipe. As a result, quinoa white flour enhanced the specific volume by 33%, which was related to the absence of bran components and the increased α-glucosidase activity. The significance of the latter was proven by separately adding sucrose and fungal amyloglucosidase to the control recipe. Moreover, the crumb featured homogeneous and finely distributed gas bubbles and the taste was not compromised. Thus, it was possible to improve the quality of gluten-free bread by using quinoa white flour, which might be a relief for coeliac patients.

1. Introduction

Due to improved analytical methods, a growing number of people are being diagnosed with coeliac disease. The prevalence rates of this lifelong disorder lie between 0.40 and 0.75% in Europe, with a tendency towards higher values (Catassi and Fasano, 2008). Unfortunately, the range of gluten-free products available in stores offers only poor quality and low nutritional value (Houben et al., 2012). The lack of gas-holding properties in dough without gluten can only be overcome by means of an extensive adaption of the composition and processing parameters. However, the contribution and interaction of the various single ingredients in the formation of the final bread matrix is not fully understood yet.

Thanks to their abundance in regard to bio- and techno-functional substances and their lack of allergenic proteins, pseudocereals such as quinoa afford new perspectives. To date, research has been focused on blends of pseudocereals with wheat (Schoenlechner et al., 2008; Chauhan et al., 1992), and only a few attempts have been made to integrate quinoa whole grain flour into gluten-free bread (Alvares-Jubete et al., 2010). Concerning quinoa, milling fractionation primarily had the purpose of facilitating the examination of single grain components, or else it was done in order to remove saponins (Becker and Hanners, 1990; Reichert et al., 1986). Further advantages of fractionation are the increased homogeneity as well as the possibility of targeted employment of functional ingredients. As far as the white flours of pseudocereals are concerned, baking properties mainly depend on their essential component, starch. Quinoa starch is especially interesting because of its low gelatinization enthalpy, high viscosity and high water-binding capacity in comparison to wheat starch (Atwell et al., 1983; Inouchi et al., 1999; Lorenz, 1990).

Thus, the use of quinoa white flour provides a promising opportunity for the improvement of gluten-free bread. In this study, its impact on volume, texture and sensory parameters in comparison to unfractionated quinoa whole grain flour has been evaluated. Initially, free mono- and disaccharides were quantified simultaneously with enzyme activities, which are responsible for starch degradation. Sucrose and amylolytic enzymes were used in order to investigate the effect of substrate availability on the carbon dioxide production of yeast.

Until now, only scant information about the foaming properties of ingredients in gluten-free bread has been available. Insights into

Abbreviations: QF, Quinoa white flour; QWG, Quinoa whole grain flour.
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the aeration and foam stabilization of the gluten-free dough and crumb are essential. With the aid of new ingredients and experiments which clarify their influence on the final crumb porosity, successful strategies can be generated. This paper presents a novel approach for improving the quality of gluten-free bread by the application of quinoa white flour.

2. Material and methods

2.1. Ingredients for dough and bread preparation

German commercial wheat flour Type 550 was obtained from Rolandmühle (Bremen, Germany). Finely-ground whole grain rice flour of the plant *Oryza sativa* L., finely-ground corn flour *of Zéa mays* L. without sperm, and corn starch produced from washed and dried corn were purchased from Davert (Senden, Germany). Organic Royal Quinoa grains (*Chenopodium quinoa*, freed of saponins) originating from Bolivia were purchased from Ziegler & Co. GmbH (Wunsiedel, Germany). Whole grain flour (QWG) was produced in an ultra-centrifugal mill (Retsch ZM 200, Haan, Germany) with a 500 μm mesh. QWG was composed of: 63.3% starch (AACC 76-13), 11.7% proteins (AACC 40-16, $N \times 5.45$), 5.9% lipids (AACC 30-25), 2.4% ash (AACC 08-12) on a dry basis and 12.6% water (AACC 44-01). Quinoa white flour (QF) was obtained by the removal of bran components in a Quadrumat Junior mill (Brabender, Duisburg, Germany) with a 200 μm mesh. Before milling, water was added to the seeds to attain a moisture content of 15%, and conditioning was carried out in an airtight box for 20 h at 20 °C. The resulting QF contained 87.0% starch, 3.9% proteins, 2.0% lipids, 0.7% ash (all on a dry basis) and 14.7% water (analyzed with the same methods as for QWG).

Further materials for dough and bread formulations were baking margarine (CSM Deutschland GmbH, Bingen am Rhein, Germany), food grade hydroxypropyl methylcellulose (HPMC, K4M, The Dow Chemical Company Midland, USA), NaCl (Esco, Hannover, Germany), sucrose (EEC2, Südzucker AG, Mannheim, Germany) and dry yeast of the species *Saccharomyces cerevisiae* (Casteggio Liveti, Casteggio, Italy). Fungal α-amylase (NS27238) and amyloglucosidase (NS27253), both produced in the organism *Aspergillus oryzae*, were kindly provided by Novozymes (Bagsvaerd, Denmark).

2.2. Quantitation of water soluble mono- and disaccharides in flours

In order to inactivate enzymes, flour samples were heated (120 °C, 60 min) prior to a quantification of sugars. All samples were analyzed with and without this treatment to enable a preliminary evaluation of amylolytic enzyme activities. For the aqueous extraction of mono- and disacchrides, 6 g flour was diluted with 60 mL distilled water, heated at 70 °C for 15 min and stirred for a further 30 min at room temperature. Afterward, 2 mL of Carrez solutions I and II (360 mM $K_4[Fe(CN)_6]_3$ and 1 M $ZnSO_4$, respectively), purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany) were added. After 2 min of stirring, samples were diluted to 100 g with distilled water and filtered. Subsequently, 300 μL of the filtrate was mixed with 700 μL acetonitrile and centrifuged for 5 min at 2522× *g*.

Sugars in the supernatant were separated in a 250/4 Nucleosil 100-3 NH_2 column (Macherey–Nagel AG, Oensingen, Switzerland) and quantified using a refractive index detector (Waters Corporation, Milford, USA) under the following conditions: eluent: 77% acetonitrile in water; flow: 1.0 mL/min; oven temperature: 35 °C; detector temperature: 40 °C. For calibration, five concentrations of glucose and fructose in distilled water from 0.01 to 1.00% (w/v) and four concentrations of maltose and sucrose in distilled water from 0.02 to 1.00% (w/v) were used as external standards.

2.3. Analysis of the endogenous enzyme activities in flours

The activity of endogenous α-amylase was assayed using the enzyme extraction protocol for wheat and barley flours of the Ceralpha Method for the measurement of plant and microbial α-amylases (AACC 22-02) (Megazyme, Ireland). One unit of activity (Ceralpha Unit, CU) is defined as the amount of enzyme required to release 1 μmol of p-nitrophenol from the substrate in 1 min under the defined assay conditions in the presence of excess thermostable α-glucosidase. To determine the endogenous α-glucosidase activity, 1.0 g of the sample was extracted for 20 min at 40 °C with 10 mL 200 mM NaAc (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) pH 4.5. After centrifugation (10 min, 30,790× *g*, 20 °C), the supernatant was analyzed with the assay of amyloglucosidase using p-nitrophenyl-β-D-maltoside plus thermostable β-glucosidase (Megazyme, Ireland). One unit is defined as the amount of enzyme that releases 1 μmol of p-nitrophenol from the substrate per minute at the defined pH and temperature.

2.4. Preparation of bread samples

For baking trials, rice flour, corn flour and corn starch were kept in the proportion 2:1:1 for the control formulation. Further recipe components were 80.0% water, 3.0% margarine, 2.0% HPMC, 2.0% NaCl and 1.5% dry yeast (percentages are based on 100% of the flour-starch mixture). The final water content was adjusted to the flour moisture content according to the AACC approved method 44-01.01. When varying this recipe, the amount of corn starch was kept constant, since it was considered to be a functional ingredient, while the milling products rice flour and corn flour were replaced by QWG or QF. The relation of rice to corn flour always remained at 2:1. In the case of sugar or enzyme addition, 100 g flour (14% moisture) were supplemented with either 2 g sucrose, 2.5 U α-amylase or 450 U amyloglucosidase.

For dough preparation, water was tempered to produce a dough of 28 °C and the amount was adapted to standardize the water content of all flours to 14%. All ingredients were mixed for 2 min at 25 Hz and for a further 2 min at 50 Hz in a spiral kneader type SP 12 A-3 (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). Prior to weighing 250 g into each baking tin, the dough was kept in a proofing chamber at 30 °C with 80% relative humidity for 15 min.

After 30 min of fermentation (45 min for experiments with elongated fermentation) with the same conditions, samples were placed into a deck oven (Matador MD 120, Werner & Pfleiderer, Dinkelsbühl, Germany) and baked for 35 min at 220 °C with 0.5 L steam. Per recipe, two independent batches, resulting in four bread loaves each, were analyzed 2.5 h after baking.

2.5. Evaluation of bread characteristics

In order to obtain the specific bread volume (mL/g), the volume, as measured with a laser-based volumeter (BVM-L370, Perten Instruments, Hägersten, Sweden), was divided by the weight of each loaf. Texture profile analysis was performed on central bread slices of 2.5 cm with a TVT-300 XP texture analyzer (Perten Instruments, Hägersten, Sweden) equipped with a 20 mm aluminum cylindrical probe. To resemble two bites, the crumb was compressed by 40% in two subsequent cycles with 15 s intermediate rest. Downward speed was 1.7 mm/s and upward speed 1.0 mm/s with a trigger force of 0.05 N. Firmness was recorded as the peak force of the first compression and adhesiveness as the negative area under the curve after the first decompression. Additionally, elasticity (equivalent to

resilience, as calculated by the software) was defined as the percentage of the area under the curve for decompression divided by the area under the curve for compression. A trained sensory panel evaluated the bread with respect to appearance, texture, flavor and aroma. The intensity of each sensory parameter was rated on a scale from 0 to 10 (with 10 as the highest intensity) by at least 10 non-coeliac panelists on two different days ($n = 20$).

2.6. Statistical analysis

The statistical analysis was performed with the aid of Prism 5 (Version 5.03, GraphPad Software, Inc.). To detect significant differences between samples, a one-way analysis of variance (ANOVA) with separation of means by the Tukey–Kramer test ($p < 0.05$) was applied.

3. Results and discussion

3.1. Mono- and disaccharides in flours

The abundance of mono- and disaccharides is of fundamental importance for the baking performance of flours. Upon heating, reducing sugars and amino acids provide the reactants for the non-enzymatic browning, called Maillard reaction. Additionally, the yeast metabolism depends on the availability of its preferred substrates, which are mono- and disaccharides. Together, the Maillard reaction and the yeast metabolites are decisive for volume, texture, color and flavor of the resulting bread (Cauvain, 2007). A comparison of the saccharides quantified after an aqueous extraction in flours of the gluten-free control recipe, quinoa and wheat is provided in Table 1. A heat treatment for enzyme inactivation made it possible to assess the impact of starch degradation on the concentration of the detected sugars.

The amount of all mono- and disaccharides in corn starch was below the detection limit and is therefore excluded from the Table. Maltose was the main sugar source in wheat flour, while quinoa white flour (QF) was rich in glucose. Sucrose was found in all samples and dominated in the outer grain parts of quinoa. Overall, inconsistent values concerning free sugars in quinoa have been reported. The presented results are contradictory to the glucose-fructose-sucrose content and ratio (4.55%, 2.41% and 2.39%, respectively) as reported by González et al. (1989). Fundamentally different analysis techniques, varying species and origin of quinoa as well as high amylolytic activities may contribute to this variation. As soon as water is added to the flour samples, enzymes become active and the degradation of polysaccharides into smaller saccharides is initiated. In this study, the preceding heat treatment made it possible to minimize this process. Overall, the amount of simple sugars was lower after heat inactivation. The considerable loss of saccharides in the case of both quinoa flours and wheat flour indicates high amylolytic enzyme activities in comparison to rice and corn flour. In wheat flour, a maltose-producing enzyme such as α-amylase seems to predominate, while a glucose-forming glucosidase seems to be especially active in quinoa.

3.2. Activity of amylolytic enzymes in flours

The comparison of free saccharides with and without heat treatment for enzyme inactivation provided a first clue about starch degradation in the various flours. In order to validate these findings, an actual analysis of the specific enzyme activities was performed by monitoring the release of their substrates. The results can be seen in Fig. 1.

Hydrolysis by α-amylase seems to be the prevailing mechanism for starch degradation in wheat. In contrast to this, a considerably higher activity of α-glucosidase was found in quinoa, which converts maltose as well as long chain polysaccharides into glucose as soon as water is available. Since glucose is the preferred substrate for yeast and its production of carbon dioxide, the results suggest a good baking performance of quinoa. Both amylolytic enzymes were less active in flour than in whole grain quinoa (QWG), which indicates an accumulation in outer grain parts (probably the embryo). In both rice flour and corn starch, the enzyme activities were below detection limit. Together with the minor contribution of corn flour, the mixture of the control flours (rice flour: corn flour: corn starch 2:1:1) lacked yeast substrates. All measured enzyme activities were in accordance with the free sugars quantified with and without heat treatment in chapter 3.1. Similarly, Fleming and Galwey (1995) found low amylase activity in quinoa, and Lindeboom (2005) also analyzed lower enzyme activities when removing bran. However, the latter author measured an extremely high level of α-amylase activity and simultaneously drew attention to significant alterations in different quinoa varieties. The exceptionally active α-glucosidase found in this study might have contributed to the low Brabender Units detected in the Amylograph by Lorenz and Nyanzi (1989).

In order to simulate the endogenous activity of α-glucosidase in quinoa, different concentrations of a fungal amyloglucosidase were added to the control flours. Fig. 1(C) reveals that approximately 5000 mg/kg are required to measure the same activity as in QF. This concentration (diluted by the additional amount of starch into 3500 ppm) was used to investigate the effect of α-glucosidase in subsequent baking trials. It is widely accepted that α-amylase has a relevant impact on the texture and shelf life of (gluten-free) bread (Rosell, 2009). Since less α-amylase activity was found in QF compared to wheat flour, 0.025 units per g flour of a fungal α-amylase were added to the control recipe.

3.3. Specific volume of bread loaves

In the case of pan breads, high specific volume is desired since it indicates proper aeration and porosity of the respective bread loaves (Gould, 2007). Gluten-free bread recipes typically feature bad gas-holding properties, leading to low volume (Houben et al.,

Table 1
Amount of free mono- and disaccharides in different flours. Quantification via HPLC. The table lists means of duplicates. Left: Untreated flour. Right: Inactivation of enzymes by heating the samples prior to HPLC analysis at 120 °C for 60 min. QF: Quinoa white flour; QWG: Quinoa whole grain flour.

Flours	Free saccharides (g per 100 g flour)							
	No treatment				Inactivated enzymes			
	Glucose	Fructose	Maltose	Sucrose	Glucose	Fructose	Maltose	Sucrose
Wheat	<0.1[a]	<0.1[a]	4.71	0.20	<0.1[a]	<0.1[a]	0.65	0.20
Rice	<0.1[a]	<0.1[a]	<0.3[a]	0.62	<0.1[a]	<0.1[a]	<0.3[a]	0.56
Corn	0.18	0.10	<0.3[a]	0.57	<0.1[a]	<0.1[a]	<0.3[a]	0.50
Quinoa (QF)	2.16	<0.1[a]	<0.3[a]	0.51	0.16	<0.1[a]	<0.3[a]	0.37
Quinoa (QWG)	1.5	<0.1[a]	<0.3[a]	2.26	0.18	<0.1[a]	<0.3[a]	2.04

[a] Below detection limit.

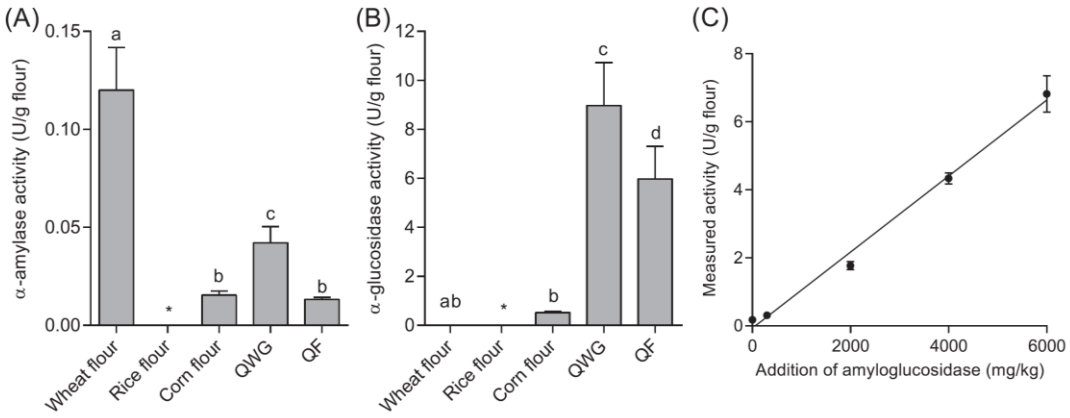

Fig. 1. Endogenous activity of α-glucosidase and α-amylase in different kinds of flour. (A) Activity of α-amylase. (B) Activity of α-glucosidase. (C) Measured enzymatic activity of α-glucosidase/amyloglucosidase after addition of fungal amyloglucosidase to the control flour mixture (rice flour to corn flour 2:1) with linear regression ($R^2 = 0.9848$). Means with standard deviation ($n = 4$). Different letters indicate significant differences between means (ANOVA, $p < 0.05$). * Below detection limit. QF: Quinoa white flour; QWG: Quinoa whole grain flour.

2012). The impact of quinoa on the specific volume of a gluten-free control formulation has been tested in the present study. Formulations which used varying proportions of QF (40–100%) to replace rice and corn flour, were baked and analyzed. Additionally, 100% QWG, amylolytic enzymes (α-amylase and amyloglucosidase) as well as sucrose were employed and compared with the effect of QF. When exchanging rice and corn flour with QF or QWG, the amount of corn starch was never altered. This means that recipes indicated as 100% additionally contained 25 g corn starch per 100 g of the respective flour (so that in total 75% of the rice flour/corn starch mixture had been replaced). The enzymes were added in order to assess the impact of substrate availability for yeast on the resulting bread volume. With regard to amyloglucosidase, the amount was adapted to the endogenous activity in QF. The influence of different amounts of QF and QWG on the specific volume of gluten-free bread is summarized in Table 2. All ingredients and measures enhanced the specific volume compared to the control bread. A total substitution of rice and corn flour by QF increased the volume by 33%.

In comparison to whole grain flour, the removal of hull components and the embryo had a positive effect on the volume. This might be related to the lack of bran particles, which are known to interfere with gas cells (Moore et al., 2004; Schober, 2009). With 2.19 mL/g, the specific volume of bread with 100% QF approaches the value of standard wheat bread (2.36 mL/g) as by Verheyen et al. (2012). In comparison, commercial wheat and gluten-free bread can only achieve higher porosity by adding various supplements, which results in a negative impact on nutrition and consumer acceptance (Moore et al., 2004).

When elongating the fermentation by 15 min (long proofing), the volume of the standard recipe was not influenced, possibly due to the lack of yeast substrates. In contrast, QF seems to provide excessive glucose and amylolytic activity, since longer fermentation enabled more time for gas production. The bread loaves gained volume when sucrose, amyloglucosidase or α-amylase were added to the control recipe. Although α-glucosidase seems to be the main reason for the higher porosity when using QF, the separate addition of amyloglucosidase to the control bread achieved 7.5% less volume increase. This might be facilitated by the high enzyme susceptibility of quinoa starch, related to its small granule size and low amylose content (Ruales and Nair, 1994). To sum up, rice and corn flour resulted in low bread volume due to a lack of mono- and disaccharides. This was overcome by the direct addition of sucrose or enzymes producing yeast substrates. However, due to its inherent substrate availability, QF is particularly suitable for increasing the volume of gluten-free bread and might lead to better customer satisfaction.

Table 2
Specific volume of bread loaves. Presented are means ± standard deviation. % Increase corresponds to the mean value of the control recipe. Long proofing: 60 min instead of 45 min fermentation. Amount of sucrose, α-amylase and amyloglucosidase: 2 g, 2.5 U and 450 U per 100 g flour. Number of replicates: $n \geq 8$. Different letters indicate significant differences between means (ANOVA, $p < 0.05$). QF: Quinoa white flour; QWG: Quinoa whole grain flour.

	Specific volume (mL/g)	Increase (%)
Control	1.65 ± 0.04 a	–
Long proofing	1.65 ± 0.03 ab	+0.1
Sucrose	1.82 ± 0.06 c	+10.3
α-amylase	1.75 ± 0.06 bc	+6.1
AMG	2.06 ± 0.07 d	+25.5
Quinoa (QWG)		
100%	1.81 ± 0.10 c	+9.8
Quinoa (QF)		
40%	1.85 ± 0.11 c	+12.4
80%	2.12 ± 0.14 d	+29.1
100%	2.19 ± 0.04 d	+33.0
Quinoa (QF) 100 %		
Long proofing	2.50 ± 0.17 e	+51.9

3.4. Texture of bread crumbs

Unfortunately, gluten-free bread usually features a hard and friable crumb with low elasticity. Since QF had a significant impact on the specific volume of the control formulation, modifications in the texture parameters were also likely. As presented in Table 3, an increase in volume generally went together with softer crumbs and less adhesiveness, because lower density resulted in less sample-probe-interaction, as measured via texture profile analysis (TPA). Likewise, Miñarro et al. (2012) reported a negative correlation of firmness and specific volume.

In contrast to the texture parameters that correlate with the density of a sample, elasticity was used to describe the crumb's recovery from deformation; i.e. as the percentage recoverable work after deformation (Gil et al., 1999). The more control flour was replaced by QF, the more elastic the crumb became. In wheat bread, elasticity positively correlates with protein content (Rao et al.,

Table 3
Texture profile analysis of the crumb. Presented are means ± standard deviation. Number of replicates: $n \geq 8$. Long proofing: 60 min instead of 45 min fermentation. Amount of sucrose, α-amylase and amyloglucosidase: 2 g, 2.5 U and 450 U per 100 g flour. Different letters indicate significant differences between means (ANOVA, $p < 0.05$). QF: Quinoa white flour; QWG: Quinoa whole grain flour.

	Firmness (N)	Adhesiveness (N m)	Elasticity (%)
Control	14.75 ± 1.69 ab	0.23 ± 0.08 a	35.4 ± 2.06 a
Long proofing	15.98 ± 1.32 a	0.36 ± 0.11 b	34.71 ± 1.47 a
Sucrose	11.08 ± 1.33 c	0.22 ± 0.05 a	34.34 ± 0.04 a
α-amylase	13.66 ± 1.39 b	0.20 ± 0.11 a	33.83 ± 3.76 a
AMG	6.89 ± 1.48 ef	0.09 ± 0.05 c	30.05 ± 3.51 b
Quinoa (QWG)			
100%	8.9 ± 1.8 cd	0.02 ± 0.01 c	41.22 ± 3.00 c
Quinoa (QF)			
40%	8.41 ± 1.97 de	0.07 ± 0.03 c	46.26 ± 2.24 d
80%	5.81 ± 0.89 fg	0.03 ± 0.02 c	56.07 ± 0.79 e
100%	4.89 ± 0.30 fg	0.03 ± 0.01 c	58.61 ± 2.90 ef
Quinoa (QF) 100%			
Long proofing	4.02 ± 0.73 g	0.00 ± 0.01 c	61.10 ± 2.33 f

1992). However, without a gluten network, this explanation becomes improbable, and QF had less protein than rice and corn flour (data not shown). According to Rao et al. (1992), the elasticity of wheat bread was ascribed to the cellular ability to recover from structural damage. The same authors demonstrate that neither surface active components nor starch have an effect on the elasticity of wheat bread. Despite the missing microstructural explanation, QF renders the product more appealing, since higher elasticity goes hand in hand with less rigidity and less crumbliness (D'Appolonia and Morad, 1981).

3.5. Sensory evaluation of appearance and taste of bread loaves

High volume is not a quality factor in itself. The distribution of gas bubbles plays a major role in consumer acceptance. Just as for ideal wheat bread of the aspired category (tin or pan loaf), the pores are supposed to be small and homogeneous (Cauvain, 2000). Through longer fermentation, sucrose addition and amylolytic enzymes, the bread loaves gained in volume. However, as evident in Fig. 2 and the sensory evaluation in Fig. 3, the pores were coarser and less uniform, thereby rendering the crumb structure less desirable.

Although the specific volume of control bread with amyloglucosidase was lower compared to bread with 100% QF, the fungal enzyme was apparently more active than the endogenous one. This conclusion is based on the higher sweetness together with the darker crust and crumb when adding AMG (Fig. 3). The darker crust indicates more saccharides, which are substrates for the Maillard reaction, and confirms the degradation of starch. It is likely that although amyloglucosidase strongly enhanced the gas production during fermentation, the increase in volume was limited, because of the bad gas-holding properties of the control recipe.

Furthermore, QF improved the color of crust and crumb. While the substitution of yellowish corn flour by QF resulted in a bright white crumb, the crust became darker. Thus, gluten-free bread with

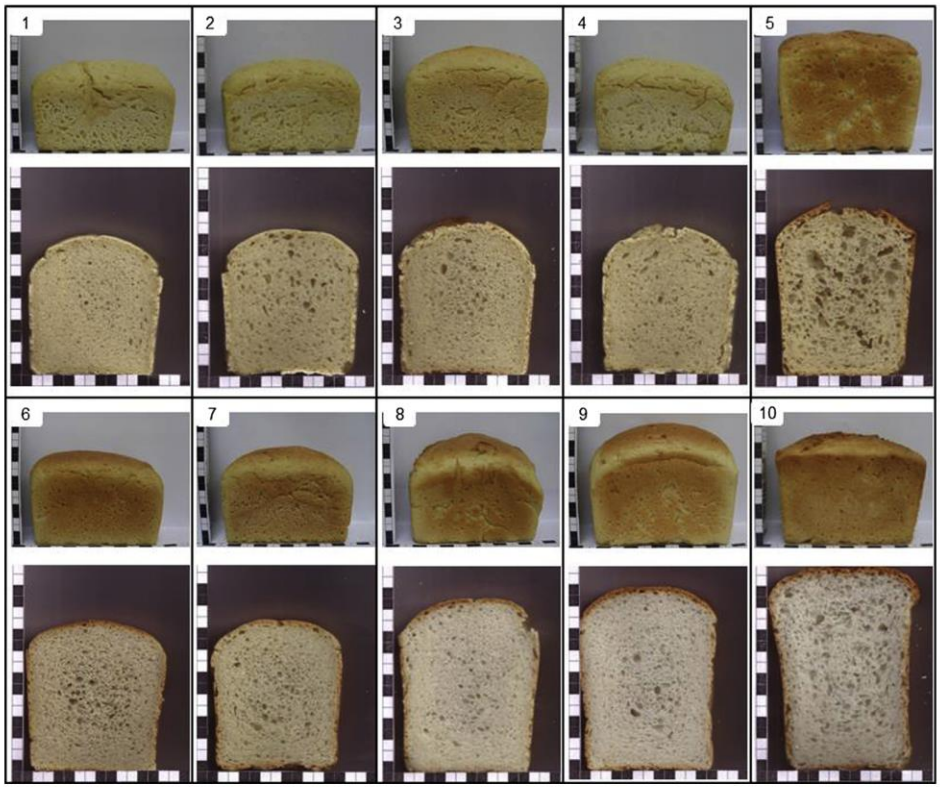

Fig. 2. Pictures of bread loaves and slices. 1: Control. 2: Long proofing (15 min extra). 3: Control + 2 g sucrose in 100 g flour. 4: Control + 2.5 U α-amylase in 100 g flour. 5: Control + 450 U amyloglucosidase in 100 g flour. 6: 100% of rice and corn flour replaced by quinoa whole grain flour. 7: 40% of rice and corn flour replaced by quiona white flour. 8: 80% of rice and corn flour replaced by quinoa white flour. 9: 100% of rice and corn flour replaced by quinoa white flour. 10: 100% of rice and corn flour replaced by quinoa white flour + long proofing (15 min extra). Pictures were taken 2.5 h after baking. One square of the scale is 0.5 cm.

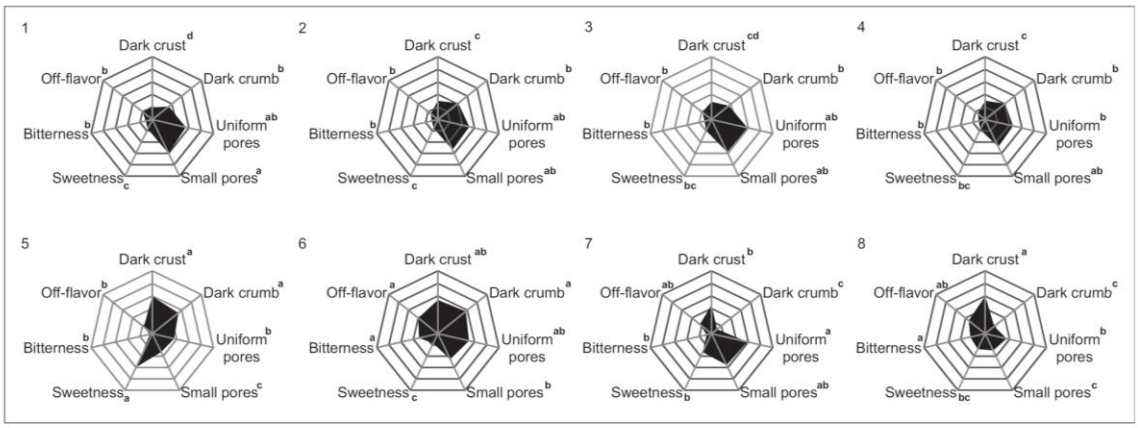

Fig. 3. Sensory evaluation of the gluten-free bread recipes. The intensity of each sensory parameter was rated on a scale from 0 to 10 (with 10 as the highest intensity) by at least 10 panelists on two different days ($n \geq 20$). The network charts imply means with 0 as the center and 10 as the edge. 1: Control. 2: Long proofing (15 min extra). 3: Control + 2 g sucrose in 100 g flour. 4: Control + 2.5 U α-amylase in 100 g flour. 5: Control + 450 U amyloglucosidase in 100 g flour. 6: 100% of rice and corn flour replaced by quinoa whole grain flour. 7: 100% of rice and corn flour replaced by quinoa white flour. 8: 100% of rice and corn flour replaced by quinoa white flour + long proofing (15 min extra). Different letters indicate significant differences between means (ANOVA, $p < 0.05$).

QF had more resemblance to traditional tin loaf. Regarding taste and flavor, the removal of bran components strongly reduced bitterness and off-flavor in QF in comparison to whole grain flour. The negative bitterness observed by Lorenz and Coulter (1991) by the addition of 30% quinoa to wheat bread was not observed here.

4. Conclusion

Without a viscoelastic network, the production of high-quality gluten-free bread is challenging. In this study, a typical gluten-free recipe based on rice and corn flour was used as a control formulation. Although this control formulation was supplemented by functional hydroxypropyl methylcellulose and corn starch, the resulting bread still featured typical deficits leading to low sensory popularity. Encouraging results for the replacement of rice and corn flour by Quinoa white flour (QF) were obtained.

Dough with QF was able to stabilize a high amount of gas via an unknown mechanism. The replacement of rice flour, whose proteins are supposedly unsuitable for gas retention (Rosell, 2009), might have already made an impact. The elevated gas volume was stabilized throughout fermentation and baking, resulting in a homogeneous structure with fine pores. Several explanations for foam stabilization by means of QF are possible: Firstly, surface active components, such as peptides or polar lipids, might help to stabilize gas bubbles; secondly, the viscoelastic properties might be optimized for the purpose of gas inclusion and stabilization. In detail, the respective mechanisms for the stabilization of gas bubbles in products without gluten are provided by Schober (2009). Future work will be directed to assess the mechanism of foam stabilization in gluten-free bread. Furthermore, it might be worthwhile to optimize the water content as well as the processing conditions of bread with QF. While quinoa white flour was able to improve the bread-like properties of gluten-free bread, the lack in nutrients remains unaffected.

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2.4 Interrelation between mechanical and biological aeration in starch-based gluten-free dough systems

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Interrelation between mechanical and biological aeration in starch-based gluten-free dough systems

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ABSTRACT

Alternative aeration and gas stabilization strategies are required for the production of starch-based cellular food systems, such as gluten-free bread. In the present study, density and temperature were monitored in mixing experiments without yeast, aiming at maximum mechanical aeration. Additionally, the same trials were performed with subsequent biological aeration, including yeast fermentation and baking. As a result, the gas volume fraction was elevated to 21%, instead of 6% with conventional kneading. Reducing the water content from 120% to 90% (flour/starch weight base) raised dough viscosity and temperature without affecting the state of aeration. The bread volume was strongly influenced by the dough temperature after mixing ($R^2 = 0.98$), since it depended on yeast activity. The implemented process is suitable to aerate starch-based dough systems mechanically and enables the production of gluten-free bread with high volume and fine pores.

1. Introduction

Through the formation of a three-dimensional network, gluten is responsible for the stabilization of gas cells in wheat dough, resulting in a stable sponge-like crumb. Thus, it is challenging to produce gluten-free bread, with a similar volume and pore structure as conventional wheat bread. Yeast fermentation, in combination with evaporation and bubble expansion during baking, provide about 89–95% of the gas for wheat bread, while the amount of gas incorporated through kneading is minor, in comparison (values calculated from Campbell and Mougeot, 1999). In contrast, cake aeration is dominated by mechanical gas inclusion through beating, which produces 37–73% of the final gas volume (values calculated from Campbell and Mougeot, 1999). Since the consistency of gluten-free dough is often more fluid and sticky than wheat dough, aeration through beating presents a promising opportunity to improve current deficits. Moreover, the pore structure in the bread crumb is strongly related to the number and size distribution of initial air nuclei formed during kneading, which highlights the importance of the mixing stage for the overall aeration (Cauvain, 2015).

However, to date it is unknown how mechanical aeration through mixing influences gas amount and bubble distribution in gluten-free dough without yeast. While for wheat bread, the effect of single kneading parameters on dough and bread parameters has been studied extensively (e.g. Kilborn and Tipples, 1972; Martin et al., 2004; Oliver and Allen, 1992; Peighambardoust et al., 2010), this process is still a "black box" in the case of gluten-free bread. This lack of knowledge becomes obvious when comparing method sections of recent studies about gluten-free bread. There is a big variance concerning mixing parameters for dough production (Elgeti et al., 2015). While some mix gluten-free dough with a typical kneading program others refer to the dough as "batter" and use high-speed mixers (e.g. Mariotti et al., 2013). Because of major deviations between material properties of wheat-based and gluten-free dough, it is questionable, whether a conventional kneading stage is the best option for maximum gas entrapment and distribution.

For the formation of the gluten-network in wheat dough, an initial homogenization stage is followed by kneading, which can be described as a series of shearing, compressing and stretching operations. Further goals of the mixing process are the dissolution and hydration of proteins and starch as well as the incorporation and dispersion of air nuclei (Cauvain, 2003). During kneading, the resistance of the dough rises until reaching a maximum that represents the formation of the gluten network. High shear rates can impair this network and, in turn, the gas retention ability (Peighambardoust et al., 2010). By adapting mixing speed and

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duration, the energy input can be adjusted to meet specific requirements of the dough recipe and to prevent an under- or over-developed structure (Skeggs, 1985). Because of the lacking network, different selection criteria must define an optimum energy input for gluten-free dough. Gómez et al. (2013) reported that different mixing parameters have a big influence on the gluten-free dough development in a Rheofermentometer and on the bread volume.

In the present study, a conventional kneading process was adapted iteratively, by changing only one parameter per trial, towards a high-speed mixing process, similar to beating cake batter. Investigated variables were duration, speed, mixing geometry and water content. Firstly, the influence of these settings on dough density was observed in a mixing trial without yeast, to evaluate the success of mechanical aeration. In parallel, the temperature and the energy input were monitored during mixing. Secondly, baking trials with yeast fermentation were performed to determine how mixing affects the biological aeration and the final bread density. Homegeneously distributed small pores and a target density of 0.20–0.35 g/ml, which is typical for wheat pan bread, was aspired (Campbell and Mougeot, 1999). Experiments were performed with a recipe based on quinoa white flour, because of its superior gas retention (Elgeti et al., 2014). In summary, this paper aims to develop a high-speed mixing procedure for maximum dough aeration.

2. Experimental

2.1. Ingredients for dough and bread preparation

Corn starch produced by Davert (Senden, Germany) originated from ground, washed, and dried corn. Quinoa white flour was produced by removing the bran of Organic Royal Quinoa grains (Bolivian *Chenopodium quinoa*, freed of saponins) purchased from Ziegler & Co. GmbH (Wunsiedel, Germany). Milling fractionation was performed in a Quadrumat Junior mill (Brabender, Duisburg, Germany) with a 200 μm mesh, as previously described by Föste et al. (2015). The resulting flour fraction consisted of 87.0% starch, 3.9% proteins (N × 5.45), 2.0% lipids, 0.7% ash (all on dry base), and 14.7% water. This composition was determined by the following AACC approved methods: 76-13, 46-10, 30-25, 08-12, and 44-01 (AACC, 2002).

The recipe was based on a mixture of quinoa white flour and corn starch in a ratio of 3:1. Further components were 3.0% shortening (baking margarine, CSM Deutschland GmbH, Bingen am Rhein, Germany), 2.0% hydroxypropyl methylcellulose (HPMC, K4M, The Dow Chemical Company Midland, USA), 2.0% NaCl (esco, Hannover, Germany), and for baking trials 1.5% dry yeast of the species *S. cerevisiae* (Casteggio Liveti, Casteggio, Italy). Percentages are related to the flour-starch weight basis (fwb). Starting with 80% (demineralized) water, which is a common amount for wheat bread but resulted in comparably stiff dough, recipes with more water (either 90, 105 or 120%) were prepared to widen the viscosity range and to enable whisking. In order to compensate for deviations in the moisture content of starch and flours from the standard value of 14%, the actual water addition was adapted. Therefore, the moisture content of starch and flours was regularly analyzed.

2.2. Mechanical aeration through mixing

For mixing trials, no yeast was added to the dough recipes. To produced 3.00 kg dough, all dry ingredients, including shortening, were distributively blended for 1 min at the lowest speed (110 rpm) in a planetary mixer (Bear-Varimixer RN10 VL-2, A/S Wodschow & Co., Brøndby, Denmark). The temperature of this mixture was measured with a thermometer (TLC 730, Ebro Electronic GmbH, Ingolstadt, Germany) and water was tempered to obtain 20 °C initial dough temperature with the following formula: $T_{water} = 2\ T_{dough} - T_{flour}$ (adapted from Cauvain, 2015). The mixing process was started directly after water addition with a scraper and either a kneading or a whipping geometry. The speed of the mixing geometry rotating around its own axis was either 200 rpm (level 5) or 420 rpm (level 15, maximum speed). After 1, 2, 4, 6 and 8 min the mixer was stopped and a small dough sample was collected to monitor temperature and density. The temperature was detected by immediately inserting the thermometer probe, before taking small samples for density determination (see Section 2.3) and directly restarting the mixer. The duration of the interruptions was kept as short as possible (approximately 20 s). Thus, the final dough temperature with interruptions varied by less than 1 °C from the one with continuous mixing. For baking trials, density and temperature measurements were performed only after 8 min of continuous mixing to prevent affecting the extent of fermentation. Mixing trials were performed in triplicates.

2.3. Determination of aerated and gas-free dough densities

The aerated dough density ρ_{bulk} was analyzed by filling dough into two shallow glass dishes with a filling volume of 25 ml. The density was calculated as mass of the sample divided by its volume. In order to determine the gas-free density, dough samples were degassed by centrifugation. Dough was transferred into three 50 ml graduated tubes with a 1 ml scale and centrifuged for 20 min at 4500*g at 20 °C in a swing-out rotor (Rotina 420R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The gas-free density $\rho_{gas\text{-}free}$ resulted from dividing the mass of a dough sample by its volume after centrifugation. The gas volume fraction ϕ was calculated by Equation (1). Two samples were analyzed per dough batch. Each trial was performed in triplicates.

$$\varphi = \left(1 - \frac{\rho_{bulk}}{\rho_{gas-free}}\right) \times 100 \quad (1)$$

2.4. Determination of the complex shear modulus of dough

For rheological measurements, 100 g dough was produced in a z-kneader similar to the 50-g-bowl of a DoughLAB (Perten Instruments, Germany). Dry ingredients including shortening were mixed for 1 min prior to water addition and further 3 min mixing. Mixing was performed at low speed (63 rpm) to guarantee homogenization while entrapping a minimum amount of gas (~6–9%). Water amount and temperature were adapted as mentioned in Section 2.2. The fundamental rheological behavior of dough was analyzed by a time-sweep oscillatory test with controlled shear deformation. The according AR-G2 rheometer (TA instruments, New Castle, USA) was equipped with a 40 mm parallel plate and a constant gap of 2 mm and connected to a smart-swap peltier plate system to maintain a temperature of 20 °C. To protect the outer dough surface against dehydration, paraffin oil was applied after trimming the edges carefully with a spatula. Preliminary amplitude sweep tests identified 0.1% deformation as highest value in the linear viscoelastic range. After 1 min for equilibration, the rheological measurement started with a frequency of 0.5–15 Hz. The complex shear modulus G^* was evaluated to assess the stiffness of samples. Measurements were performed in triplicates.

2.5. Monitoring energy consumption during mixing

The motor-power was recorded during mixing with an external kilowatt-hour meter with 0.1 W resolution (Energy Logger 4000, Voltcraft, Wollerau, Switzerland). To be able to subtract the no-load power, the mixer was run empty. The power was recorded in 1-min-intervals, but without the possibility to control when the first measurement takes place. To avoid evaluating data from the warm-up or cool-down phase of mixing, the first and last recorded values were deleted. Instead, at $t = 0.25$ min and $t = 8$ min the power was extrapolated from the inner values of each curve. Integrating the power curves over 8 min of mixing gives the consumed energy.

2.6. Preparation and evaluation of bread samples

After weighing 190 g into each of 4 baking tins (w/h/l: 70/80/110 mm), the dough was kept in a proofing chamber at 30 °C with 80% relative humidity for 45 min. Subsequently, samples were placed into a deck oven (Matador MD 120, Werner & Pfleiderer, Dinkelsbühl, Germany) and baked for 35 min at 220 °C with initial steam increasing the humidity by 3.6 mg water vapor/l air. Each recipe was baked twice with four bread loafs per dough batch ($n = 8$). Analysis was performed 2.5 h after baking. To obtain the bread density, the weight of each loaf was divided by its volume, which was measured with a laser-based volumeter (BVM-L370, Perten Instruments, Hägersten, Sweden). The specific bread volume (ml/g) was calculated as reciprocal value of the density.

2.7. Statistical analysis

Statistical analysis was performed with Prism 5 (Version 5.03, GraphPad Software, Inc.). Variances were analyzed (ANOVA) with a separation of means by the Tukey-Kramer test ($p < 0.05$) to detect significant differences between samples. The degree of relation between two variables was quantified by correlation analysis.

3. Results and discussion

3.1. Impact of mixing parameters on dough temperature and gas inclusion

The present study initially analyzed the effect of different conditions during mixing on dough density and temperature. Through a variation of the impeller geometry, mixing duration and speed it was aspired to increase the level of mechanical aeration. Starting with conventional kneading, a spiral hook was used at 200 rpm, before increasing the mixing speed to 420 rpm. Subsequently, the water content was elevated from 80 to 105%, because high-speed mixing with a wire whip (in the consecutive trial below) was only possible with reduced viscosity. Dough density was used as an indicator for the level of mechanical aeration. Knowing the gas-free density, which was determined by degassing the dough via centrifugation, the gas volume fraction was assessed. The chosen water variation did not result in significant differences regarding the gas-free density and ranged around a mean value of 1.18 ± 0.01 g/ml. The temperature was analyzed for two reasons: (1) as an indicator for dough resistance and energy input and (2) as a decisive factor for the extent of yeast fermentation in subsequent baking trials.

As shown in Fig. 1, the dough temperature rose with prolonged mixing and with higher rotational speed. Similar results have been reported for biscuit batter by Edoura-Gaena et al. (2007). In parallel to the temperature development, the dough densities decreased continuously during mixing. After mixing for 1 min at low speed (200 rpm), dough contained only 5.8% gas, which was almost doubled to 12.3% after 8 min. The latter percentage already exceeds the 4–8% of gas that is typically incorporated after kneading wheat dough without yeast (Campbell and Mougeot, 1999). High speed mixing at 420 rpm further increased the gas volume fraction to 17%.

After duration and speed variations, also the effect of the impeller geometry was evaluated. Various mixing geometries are available for different medium properties and purposes. The typical spiral hook has been specifically designed to provide mechanical energy for the formation of a viscoelastic gluten network. This might not be the optimum geometry for a starch-based dough system without gluten, because these are typically more fluid, less cohesive and more adhesive. Thus, beating at maximum speed (420 rpm) with a wire whip was compared to kneading with a spiral hook with the same speed regarding density and temperature development (see geometries in Fig. 2(a)).

The 20 strands of the wire whisk have a total perimeter of 15.4 cm, while the perimeter of the dough hook is 4.1 cm. Thus, the wire whip introduces more energy than the spiral hook (see Section 3.3). As reported by Gómez et al. (2013), more water is required when mixing with a whip. With 80% water (fwb) dough resistance was too high and the mixer overheated. A water content of 105% (fwb) was chosen, since preliminary trials revealed that this higher water amount compensates for the temperature increase through the wire whip and produces the same final dough temperature as the spiral hook (28.2 °C). Fig. 2(b) shows that the wire whip causes considerably more heating and a slightly higher gas volume fraction of 21.3% gas instead of 18.6% (Fig. 2(c)). In comparison, sponge cake batter contains about 40% gas (Massey et al., 2001). However, in cake, gas bubbles are stabilized though a complex mixture of surface active components, as egg proteins, lipoproteins, emulsifiers and solid fat (Chesterton et al., 2013). While cake batter foam has been reported to suffer from elongated mixing (Chesterton et al., 2013; Massey et al., 2001) and from excessive speed (Tan et al., 2012), the analyzed starch-based dough system appeared more stable.

In summary, it was shown that duration, speed and geometry have a strong effect on mechanical aeration and dough temperature. Similar to the temperature rise, the gas volume fraction increased continuously during mixing. The selected starch-based recipe enabled the incorporation of more than twice the amount of gas that typically results from kneading wheat dough. The following Section 3.2 will evaluate the significance of the water content for mechanical aeration.

3.2. Impact of water addition on temperature increase and aeration during beating

The water content was varied from 90 to 120% (fwb) to assess its impact on temperature increase as well as the aeration efficiency during high-speed mixing with a wire whip. To evaluate the influence of water addition on dough stiffness, recipes were carefully homogenated, with minimum gas entrapment, and the complex shear modulus (G^*) was measured in a rheometer (Fig. 3(a)). Less water resulted in higher dough stiffness. Within the selected hydration range, big variations of G^*, from 60 to 960 kPa, were achieved. As a consequence of higher dough resistance, more heating occurred during mixing, because more energy is required to maintain a constant mixing speed (see Fig. 3(b)). A water content below 90% (fwb) caused overheating of the mixer. However, the water content had no significant effect on the dough density in Fig. 3(c).

Few theoretical explanations are available to explain the mechanism of aeration by beating for medium to high viscosity substances, since the resulting shear field is very complex (Lin,

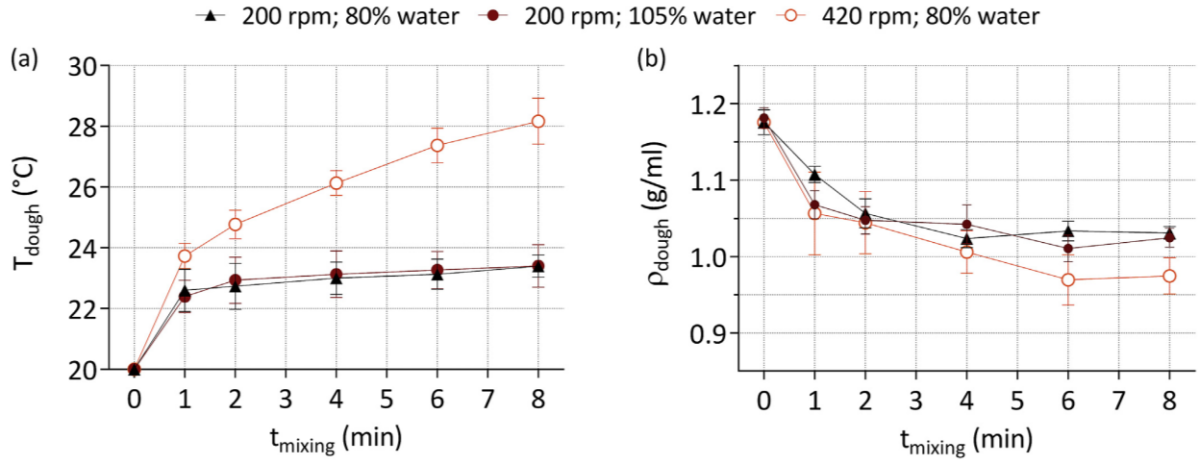


Fig. 1. Influence of the mixing speed on temperature (a) and density (b) of gluten-free dough without yeast. The dough based on quinoa white flour was mixed with a kneading geometry. The water content is given on flour weight base (fwb). The density of dough without gas at time 0 was determined by centrifugation of the aerated dough. Means are shown with standard deviation (n = 3).

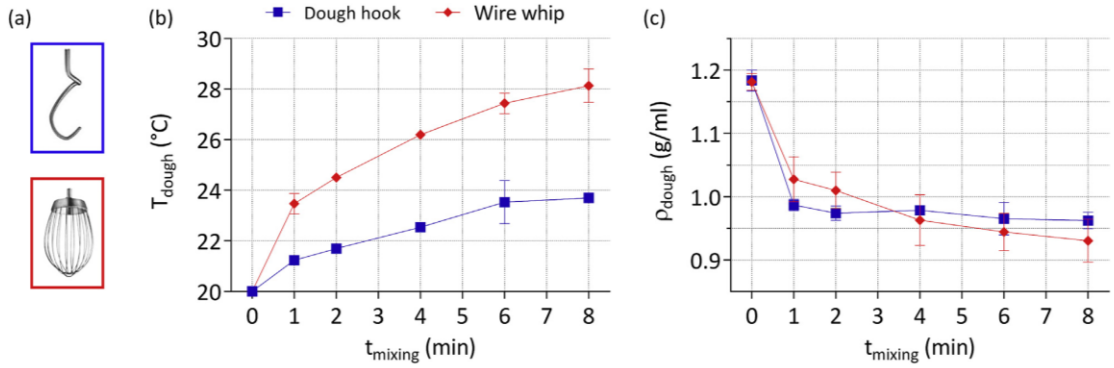


Fig. 2. Influence of the mixing geometry (a) on temperature (b) and density (c) of gluten-free dough without yeast. Dough based on quinoa white flour and 105% water (fwb) was mixed at 420 rpm in a planetary mixer. The density of dough without gas at t = 0 was determined by centrifugation of the aerated dough. Means are shown with standard deviation (n = 3).

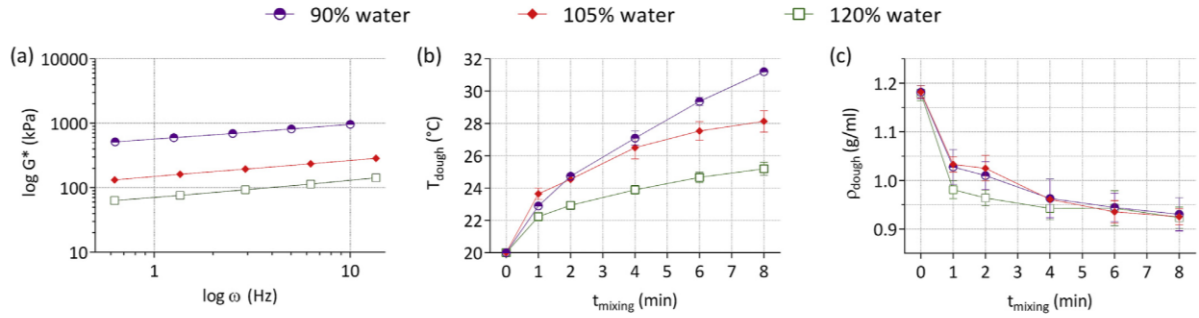


Fig. 3. Influence of dough hydration on rheology (a) temperature (b) and density (c) of gluten-free dough without yeast. The complex shear modulus G* was determined in a rheometer after producing quinoa dough with minimum gas input and shearing. For (b) and (c) the dough was mixed with a wire whip at 420 rpm in a planetary mixer. The water content is given on flour weight base (fwb). The density of dough without gas at time 0 was determined by centrifugation of the aerated dough. Means are shown with standard deviation (n = 3).

1971). Two factors seem to be decisive: (1) the movement of single wires causing the creation of cavities, new surfaces or reduced pressure regions and (2) the stabilization and dispersion of the gas cells (Delaplace et al., 2012).

3.3. Energy input during high speed mixing of gluten-free dough

Since the previous trials demonstrated that variations of the mixing process or the water level have a considerable impact on the resulting dough temperature, the energy input during mixing was evaluated. Initially, the no-load currents were measured, to detect the energy loss in motor and drive chain when running the empty mixer with different settings. Subsequently, the mixer was filled with the raw materials for 3 kg quinoa dough with 105% water (fwb) and operated with the same settings. Integrating the power curves in Fig. 4 over 8 min of mixing gives the consumed energy. The difference between the area under curve with and without dough reveals the energy required to mix a particular dough formulation and reflects the torque of the mixing arm (Shehzad et al., 2012).

The consumed energy for kneading the dough with a spiral hook at 200 rpm was negligible, since the power curve deviated only slightly from its no-load curve. In contrast, 6 kJ were required per kg of dough (= 2 Wh/kg) when using the wire whip. Although the same amount of energy was required for the no-load curves of both geometries, it appears that the whip consumes more power when mixing dough, because its numerous strands encounter higher resistance. With higher speed, this effect was more pronounced and yielded up to 34 kJ/kg (= 10 Wh/kg) at 420 rpm. In comparison, the high-speed kneading performed for the Chorleywood bread process is typically stopped after 40 kJ/kg (Axford and Elton, 1960). These observations explain the different temperature profiles that have been detected during mixing in Sections 3.1 and 3.2. In summary, it was shown that traditional low-speed kneading imparts only a negligible amount of energy into the low-viscosity starch-based dough system. More energy is consumed, when the spiral hook is exchanged for a wire whip, but only an increase of the mixing speed can distinctly elevate the power input.

3.4. Impact of different beating conditions on biological aeration and oven spring

Parameters of the mixing process influence the amount of gas bubbles, their size distribution, and the dough temperature (Chiotellis and Campbell, 2003). In turn, all of these factors are important for bubble growth and stabilization during fermentation and baking. For baking trials, the aforementioned variations of the mixing process were repeated with the addition of 2% dry yeast (fwb). After mixing, the dough was molded and fermented for 45 min prior to baking. The highest specific bread volume was obtained through high-speed beating with a wire whip at 420 rpm for 8 min with 90% water (fwb), as presented in Fig. 5(a). These settings also resulted in the highest dough temperature (33.7 °C on average) which probably triggered increased gas production during yeast fermentation. The corresponding density of 0.21 g/ml lies within the aspired range of 0.20–0.35 g/ml, which is typical for wheat pan bread. This renders the adapted beating process successful. Furthermore, the predicted capacity of quinoa dough to retain extensive gas volumes was confirmed by the fine and homogeneous pore structure for all baking trials.

To determine the significance of the dough temperature for biological aeration, a correlation analysis of the dough temperature after mixing and the resulting bread density was performed and displayed in Fig. 5(b). Hereby, higher dough temperatures were obtained because the mixer was not stopped intermittently as during the mixing trials of the previous chapters. A linear correlation ($R^2 = 0.8771$, $p < 0.0001$) between bread volume and dough temperature was determined. This indicates that carbon dioxide production by yeast was strongly affected by the dough temperature, although dough samples were directly placed into a conditioned fermentation chamber after mixing and molding.

Fig. 5(c) displays the correlation of the bread density with the level of mechanical aeration, as represented by the dough density resulting from the aforementioned mixing trials without yeast (Sections 3.1 & 3.2). The low correlation coefficient might be explained by the superposition of the biological aeration over the mechanical one. Similarly, Edoura-Gaena et al. (2007) found no clear relationship between crumb density and batter density of biscuit batter. Despite of similar dough densities after mixing, bread produced with higher water content had a higher density after baking. This is mainly related to the reduced yeast activity; however, also an impaired bubble stabilization through less viscosity during fermentation and baking might have contributed to lower bread volume. With less resistance, destabilizing mechanisms such as buoyancy, coalescence and disproportionation are favored. Experiments with wheat dough suggest that not only the gas amount, but also the bubble size distribution is affected by the mixing energy, which, in turn, dictates the end product porosity (Wilde, 2003). Thus, it would be interesting to monitor the bubble sizes throughout processing in order to further elucidate the interrelation between mixing parameters and bread pore structure.

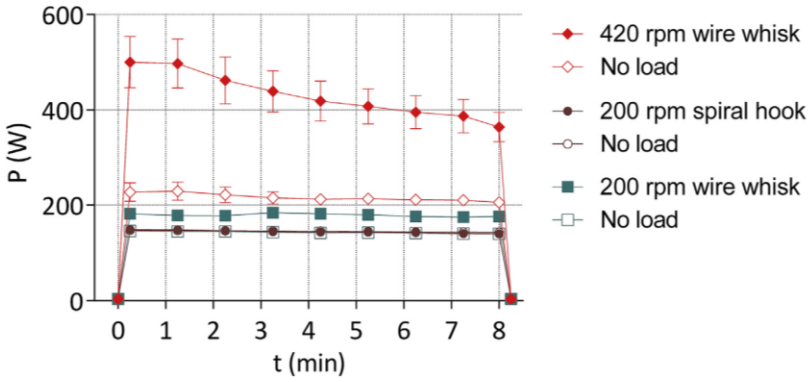
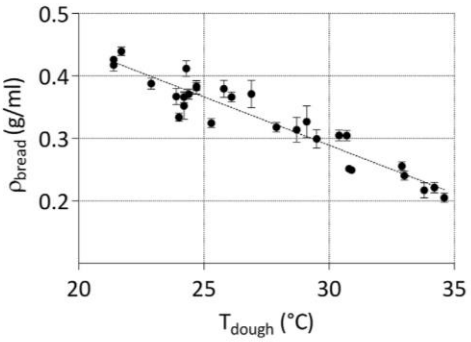


Fig. 4. Motor power consumption during mixing. Power consumption *P* in a planetary mixer was measured with an external power meter over mixing time *t*. Mixing was performed with different settings, but always included a dough scraper. Quinoa dough with 105% water (fwb) was mixed with the settings indicated in the legend. Hollow symbols represent no-load curves, resulting from empty runs with the respective settings.

(a)

	▲	○	●	■	◒	◆	□
Water (% fwb)	80	80	105	105	90	105	120
Geometry	Spiral hook	Spiral hook	Spiral hook	Spiral hook	Wire Whip	Wire Whip	Wire Whip
Speed (rpm)	200	420	200	420	420	420	420
ρ_{bread} (g/ml)	0.374 ± 0.011^{b}	0.304 ± 0.00^{d}	0.414 ± 0.011^{a}	0.343 ± 0.018^{c}	0.205 ± 0.020^{e}	0.313 ± 0.024^{d}	0.375 ± 0.018^{b}

(b)



(c)

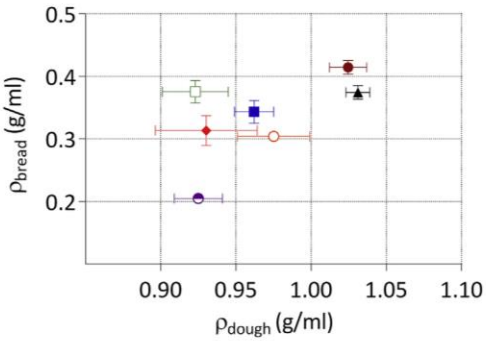


Fig. 5. Influence of mechanical and biological aeration on bread density. (a) Bread slices and densities resulting from different mixing settings and water addition levels. Different letters next to density indicate significant differences, as calculated by ANOVA with $p < 0.05$. (b) Bread density over dough temperature after mixing. Correlation of data (dotted line): $R^2 = 0.8771$, $p < 0.0001$. (c) Bread density over dough density after mixing without yeast; correlation of data $R^2 = 0.3552$, $p = 0.16$.

4. Conclusions

The present study evaluated the suitability of a beating process, similar to the production of cake batter in a gluten- and egg-free dough system based on starch and HPMC. Mixing trials without yeast revealed that use of a wire whip instead of a spiral hook and a higher mixing speed increased mechanical aeration. Through an optimization of processing parameters and water content, the volume fraction of gas in dough was elevated from 12% to 21%. However, the resulting bread volume was not only related to the level of mechanical aeration. The biological aeration as well as the dough viscosity were identified as further key factors. When varying the energy input during mixing, the temperature must be controlled, because the carbon dioxide production by yeast metabolism was strongly affected by the extent of heating during mixing. An increase from 20 °C towards 35 °C shifted the dough temperature more closely towards the fermentation optimum of *S. Cerevisiae (40–45 °C)* (Cauvain, 2015). In turn, dough temperature depends on processing conditions and water content. Monitoring of the power input during mixing enabled a direct prediction of the dough temperature. Influences of the mixing settings on the bread volume were obscured by a varying degree of yeast fermentation. Future approaches for further increasing the mechanical aeration during mixing might include a modification of the headspace atmosphere. All approaches produced acceptable crumb structures. This proves that dough with quinoa white flour stabilizes gas throughout fermentation and baking in a wide range of water addition and processing conditions. Rheology or surface activity of quinoa flour might account for superior properties regarding creation, disruption and stabilization of bubbles during the beating process.

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2.5 Foam stabilization during processing of starch-based dough systems

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Foam stabilization during processing of starch-based dough systems

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ABSTRACT

The goal of the study was to identify material properties that (1) facilitate the incorporation of gas into starch-based dough and (2) favor bubble stabilization during all processing stages. A novel rheometer program simulated processing conditions in four consecutive stages with varying shear and temperature profiles. A broad range of viscosities was obtained by various recipe compositions. In consequence, the energy consumption varied during mixing and directly dictated the dough temperature ($R^2 = 0.98$). Rheological data were correlated with the gas volume fraction of doughs (5–25%) and with the bread densities (0.21–0.42 g/ml). Pronounced shear-thinning was more relevant for mechanical aeration ($R^2 = 0.74$) than the absolute dough viscosity. In contrast, during fermentation and baking, high viscosities increased the bread volume ($R^2 = 0.72$) and reduced the mean pore size ($R^2 = 0.68$). In conclusion, valuable new insights were obtained into relevant structures of sensitive cellular food systems, such as gluten-free bread.

Industrial relevance: An extensive variety of novel gluten-free flours and additives is available for the production of bakery products. This makes it difficult to assess and compare the functionality of ingredients. The present paper offers a new method to predict the baking performance of different recipe compositions. This lays the groundwork for an improved understanding of key factors for the production of high quality aerated food structures without a dominating gluten network. Notably, the highest bread volume resulted from a combination of high-speed mechanical aeration with a recipe based on quinoa white flour or refined rice and 2% hydroxypropyl methylcellulose.

1. Introduction

For the production of starch-based food foams without egg or gluten, aeration and gas stabilization are central challenges. In the process of mechanical aeration by mixing, air bubbles are incorporated into the dough or batter. Subsequently, further mixing can lead to coalescence or kinetic disentrainment, which decreases the number of bubbles and increases their size (Jang, Nikolov, Wasan, Chen, & Campbell, 2005). The opposite effect of smaller, more numerous bubbles results from bubble breakage through shearing (Chesterton, de Abreu, Moggridge, Sadd, & Wilson, 2013; Massey, Khare, & Niranjan, 2001). The balance between entrainment, disentrainment and disruption depends on numerous internal and external factors and determines the success of the aeration process (Mills, Wilde, Salt, & Skeggs, 2003). Moreover, the created bubbles provide atmospheric oxygen for the yeast metabolism and serve as nuclei, into which carbon dioxide can diffuse during fermentation (Khatkar, 2011). The mixing process strongly influences the distribution of pores in bread and cake, since - despite the action of other leavening mechanisms - no new gas cells are generated afterwards (Baker & Mazi, 1941; Scanlon & Zghal, 2001). To produce bread with small and homogeneous pores as well as high volume, it is important to prevent gas-loss throughout processing. Therefore, the present study evaluates key properties and mechanisms that are important for aeration and gas stabilization.

For wheat bread, several fundamental and empirical rheological methods estimate the baking performance of recipe compositions. As an example, it is generally accepted that the target torque of 4.9 N•m (or 500 BU) in a 300 g Farinograph mixer or 1.1 N•m in a Mixolab is associated with preferable dough consistency and bread volume under standard conditions. In contrast to the slight variations in water content or flour composition for wheat bread, gluten-free recipes include an extensive range of flours, starches and functional additives. With few exceptions, starch-based dough does not provide enough stability or elasticity for examinations in wheat dough analysis systems. The viscoelastic properties of wheat dough are defined by the ability of gluten proteins to network in a specific manner. Gluten-free formulations are required to be absent (<20 ppm) of these proteins, so that both, the micro- and macrostructure, depend on other ingredients and their interactions. Currently, a wide variety of strategies is available to either mimic or compensate the gluten network (Masure, Fierens, & Delcour, 2016). Starch, as the main component of cereals and pseudocereals, provides the basis of most gluten-free recipes and is typically supplemented by hydrocolloids, such as hydroxypropyl methylcellulose (e.g.

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Bàrcenasa & Rosell, 2005; Mariotti, Pagani, & Lucisano, 2013; Sivaramakrishnan, Senge, & Chattopadhyay, 2004). In these systems, the water concentration is important for the rheological behavior, as has previously been discussed by Kobylañski, Pérez, and Pilosof (2004). Because of the heterogeneity of raw materials, not only dough and bread properties, but also analytical methods differ considerably. In consequence, it is often impossible to compare the baking performance of single ingredients.

This study addresses two main questions: which rheological properties are required for maximum gas input during mixing and how does dough rheology affect the gas stabilization during processing? A fundamental rheological method was developed to characterize different gluten-free formulations and to predict their baking performance. The resulting rheological data were correlated with the level of gas entrapment during mixing without yeast. This allowed for an identification of the most important dough properties for mechanical aeration. Moreover, the energy input during mixing was evaluated to estimate dough heating through viscous dissipation (Shehzad, Chiron, Della Valle, Lamrini, & Lourdin, 2012).

Subsequently, the same dough recipes were fermented with yeast and baked before measuring bread density and pore size. As a prerequisite, it had to be avoided that the bread density is influenced by the dough temperature after mixing (through yeast activity). An effect on yeast activity would also derive from the variations in substrate in different flours. Since even the use of double-wall jackets has failed to control the dough temperature in literature studies (Edoura-Gaena, Allais, Trystram, & Gros, 2007), the biological aeration was standardized, so that bread density was only related to medium properties and mechanical gas input. Finally, bread volume and pore structure were correlated with rheological dough properties, to examine which material properties are favorable for aeration and bubble stabilization.

2. Experimental

2.1. Ingredients for dough and bread preparation

Fine ground whole grain rice flour from brown rice of *Oryza sativa* L., henceforth referred to as rice (flour), fine ground corn flour of *Zea mays* L. without sperm and corn starch produced of ground, washed, and dried corn were obtained from Davert (Senden, Germany). Dry cleaned, ground and polished white rice was milled and fractionated by Müller's Mühle GmbH (Gelsenkirchen, Germany), in the following referred to as refined rice. Organic Royal Quinoa grains (*Chenopodium quinoa*, freed of saponins) originating from Bolivia were purchased from Ziegler & Co. GmbH (Wunsiedel, Germany). Quinoa white flour was produced by removing bran components in a Quadrumat Junior mill (Brabender, Duisburg, Germany) with a 200 μm mesh, as previously described (Föste, Elgeti, Brunner, Jekle, & Becker, 2015). The resulting flour fraction contained 87.0% starch, 3.9% proteins (N × 5.45), 2.0% lipids, 0.7% ash on dry base, and 14.7% water as determined by the following AACC approved methods: 76-13, 46-10, 30-25, 08-12, and 44-01, respectively (AACC, 2002). In the following sections, rice/corn refers to a 2:1 mixture/corn refers to a 2:1 mixture of the above mentioned whole grain rice flour with corn flour, quinoa refers to fractionated quinoa white flour and rice indicates whole grain rice flour.

Further ingredients for dough production were shortening (baking margarine, CSM Deutschland GmbH, Bingen am Rhein, Germany), hydroxypropyl methylcellulose (HPMC, K4M, The Dow Chemical Company Midland, USA) NaCl (esco, Hannover, Germany) and demineralized water. For baking trials dry yeast of the species *S. cerevisiae* (Casteggio Liveti, Casteggio, Italy) and anhydrous D(+)-glucose (AppliChem GmbH, Darmstadt, Germany) were added. The gluten-free recipes used for this study are listed in Table 1, in which quantities are related to the respective flour-starch weight basis (fwb). For each formulation, the respective water amount was adapted to compensate for deviations in the moister content of starch and flours from a standard value of 14%. Both, rheometer trials and high-speed mixing with a wire whip, require relatively high water content.

2.2. Density and temperature monitoring in mixing trials without yeast

For each mixing trial, 3.00 kg dough was produced without yeast in a planetary mixer (Bear-Varimixer RN10 VL-2, A/S Wodschow & Co., Brøndby, Denmark). Prior to temperature measurements, all dry ingredients, including shortening, were distributively blended for 1 min at the lowest speed (110 rpm). In order to compensate raw material and climate variations, water was tempered to produce dough of 20 °C with the formula $T_{water} = 2T_{dough} - T_{flour}$ (adapted from Cauvain, 2007). Directly after water addition, the mixing process was started with a scraper at 420 rpm with a wire whip for 8 min. For comparison of high speed beating with traditional processing, one dough (quinoa, 105% water) was mixed at 200 rpm with a spiral kneader for 8 min. Dough temperature after mixing was detected with a thermometer (TLC 730, Ebro Electronic GmbH, Ingolstadt, Germany). The dough density ρ_{dough} was determined by carefully filling two dough samples into shallow glass containers with a specified filling volume and by dividing their weight by their volume. Mixing trials were performed in triplicates.

2.3. Monitoring energy consumption during mixing

The motor-power P_{dough} was recorded during mixing with an external kilowatt-hour meter with a resolution of 0.1 W (Energy Logger 4000, Voltcraft, Wollerau, Switzerland). To be able to subtract the no-load power P_0, the mixer was run empty. Because the power was recorded in 1-min-intervals, the first and last values during mixing could correspond to the warm-up or cool-down phase. Since these phases lasted approximately 15 s, the values for $t = 0.25$ min and $t = 8$ min were extrapolated. Integrating the power curves over 8 min of mixing (t_0 to t_{end}) gives the consumed energy. The subtraction of the integrated no-load curve from the integrated curve with dough reveals the energy required to mix a particular dough formulation and reflects the torque of the mixing arm (Shehzad et al., 2012). The energy required for mixing $E_{mix.}$ was calculated according to Eq. (1).

$$E_{mix.} = \int_{t_0}^{t_{end}} P_{dough} - \int_{t_0}^{t_{end}} P_0 \quad (1)$$

2.4. Determination of the glucose concentration for standardized yeast activity

Preliminary trials determined the minimum glucose concentration to maximize the gas production rate during yeast fermentation. For this purpose, the corn starch recipe (No. 10, Table 1) was supplemented with 0–4% glucose (fwb), which is a preferred monosaccharide for *Saccharomyces cerevisiae*. Corn starch was chosen as a control with negligible mono- and disaccharide content. After homogenizing dry ingredients with the lowest speed for 1 min, dough was produced by kneading at 200 rpm for 8 min in a KitchenAid (5KSM150, St. Joseph, USA). To monitor the biological aeration during fermentation, two dough samples were filled into the bottom of previously cut glass cylinders with an inner diameter of 35.6 mm and were weighed to obtain the initial density. The cylinder bottoms had a filling volume of 27 ml. Subsequently, after the cylinder top was reapplied and fixed with parafilm, the cylinders were placed for 45 min into proofing chambers (30 °C, 80% relative humidity). Dough density development was monitored through measuring the dough height every 5 min with a precision caliper. Due to interactions with the cylinder wall, in most cases the dough surface became spherical during fermentation, which was factored into density calculations. The preliminary trial was performed once with two samples per glucose concentration.

Table 1
Gluten-free recipes.

No.	1	2	3	4	5	6	7	8	9	10
	Quinoa	Quinoa	Quinoa	Quinoa	Rice/corn (2:1)	Rice/corn (2:1)	Rice/corn (2:1)	Rice	Refined rice	Corn starch
Flour	75	75	75	75	75	75	75	75	75	0
Corn starch	25	25	25	25	25	25	25	25	25	100
Water	105	120	105	90	105	105	90	120	120	105
HPMC	2	2	0	0	2	0	0	2	2	2
NaCl	2	2	2	2	2	2	2	2	2	2
Dry yeast	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	0
α-D-glucose	2	2	2	2	2	2	2	2	2	0

The combination of flour and starch results in 100 parts to which the other ingredients are related. The quantities can thus be regarded as percentages related to the flour/starch weight base. Dry yeast and glucose were only used for baking trials, not for Rheometer or mixing experiments.

2.5. Baking trials with standardized biological aeration

This study aimed at identifying the influence of different material properties on mechanical aeration and bubble stability. However, the variation of recipes also leads to different temperature profiles and milieu conditions, which, in turn, influence the degree of yeast fermentation (biological aeration). To standardize the amount of gas included through yeast, each dough sample was baked after reaching a target volume increase during fermentation. With the knowledge of the different levels of gas input, when mixing without yeast ($=\rho_{dough}$, Section 2.2), it was possible to calculate a target density for each dough, always representing the same amount of gas through fermentation.

To define a target value for biological aeration, recipe No. 1 (quinoa dough, Table 1) was fermented for 30 min. Dough was filled into a cut glass cylinder ($r_{cylinder} = 19.28$ mm) with known filling volume and weighed to obtain m_{dough}. The cylinder top was reattached onto the bottom, enabling the dough to rise. After 30 min in a proofing chamber, the new dough Volume $V_{ferm.}$ was determined. The volume of gas introduced exclusively from yeast $V_{gas\ biol.}$ was calculated according to Eq. (2).

$$V_{gas\ biol.} = \left(V_{ferm.} - \frac{m_{dough}}{\rho_{dough}}\right) \times \frac{100\ g}{m_{dough}} \quad (2)$$

All other recipes were directly baked after reaching this same level of biological aeration of 66 ml gas per 100 g dough. This means that the fermentation time was variable to allow for a constant biological aeration. After mixing, dough was filled into baking tins and the glass cylinder. The target dough volume was calculated, taking the respective dough density after mixing without yeast into account. As soon as the respective target volume was reached in the glass cylinder, the baking tins were placed into a deck oven (Matador MD 120, Werner & Pfleiderer, Dinkelsbühl, Germany) and baked for 35 min at 220 °C. Initial steam increased the humidity by 3.6 mg water vapor/l air. Per recipe, three independent batches were baked with 210 g ± 0.5 g dough filled into each of three tins (n = 9). Bread loafs were analyzed 2.5 h after baking. To obtain the bread density, the weight of each loaf was divided by its volume, which was measured with a laser-based volumeter (BVM-L370, Perten Instruments, Hägersten, Sweden). The specific bread volume (ml/g) was calculated as reciprocal value of the density.

2.6. Simulation of processing conditions for gluten-free bread production in a rheometer

For rheological measurements, 100 g dough was produced in a Z-kneader similar to the 50-g-bowl of a DoughLAB (Perten Instruments, Germany). Prior to water addition, dry ingredients including shortening were mixed for 1 min. Mixing was performed at low

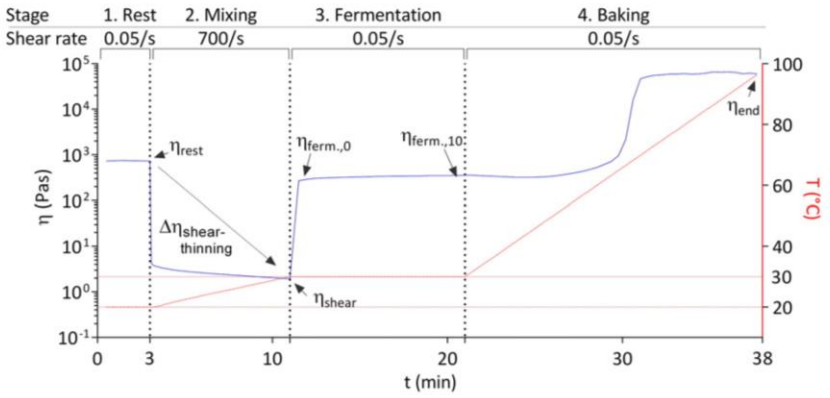

Fig. 1. Example for dough viscosity curves during simulated processing conditions in four stages. Dough viscosity η was measured over time t. Dough produced with minimum shearing and aeration was equilibrated for 1 min before measurements. Stage 1: 3 min of low-shearing at 20 °C. Stage 2: simulating mixing conditions for 8 min. Stage 3: simulating fermentation for 10 min. Stage 4: simulating baking with a heating ramp of 4 °C/min. Blue line: example of measured dough viscosity of quinoa dough with 105% water (fwb) (x-axis). Dashed red curve: Adjusted temperature (y-axis). Relevant viscosity data are marked with arrows. Shear thinning was calculated as percentage of viscosit decrease from η_{rest} to η_{shear}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

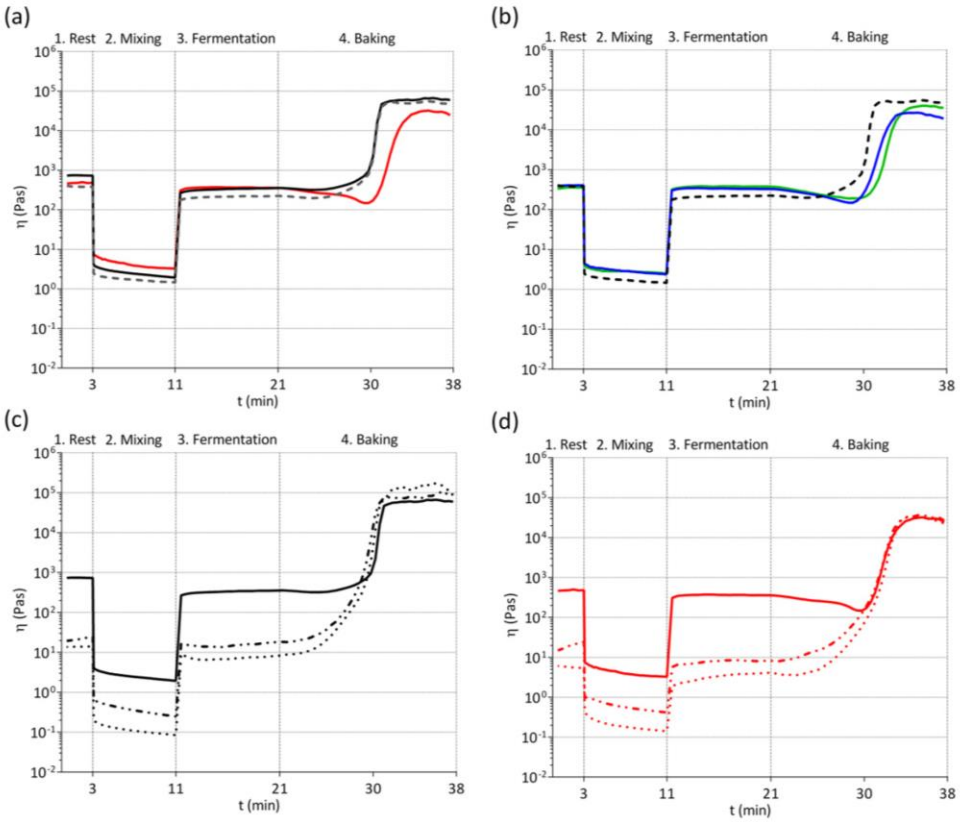

Fig. 2. Viscosity profiles of various gluten-free dough recipes during simulated processing. The viscosity η of dough, produced with minimum shearing and aeration, was measured over time t in 4 stages with shear rate/temperature conditions given in Fig. 1. (a) Quinoa dough with 105% water (fwb) — and 120% water - - - in comparison to rice/corn flour (2:1) dough with 105% water – –. (b) Recipes with 120% water: quinoa - - - in comparison to rice — and refined rice —. (c) Recipes with quinoa: 105% water, 2% HPMC —; 90% water, without HPMC - · · -; 105% water, without HPMC ···. (d) Recipes with rice/corn: 105% water, 2% HPMC — ; 190% water, without HPMC - · · -; 105% water, without HPMC ···. One line represents the mean of triplicates.

speed (63 rpm) for 3 min to guarantee homogenization while entrapping a minimum amount of gas (~ 6–9%). Water amount and temperature were adapted as mentioned in Section 2.2. Samples of 23 g dough were analyzed in a rheometer (AR-G2, TA instruments, New Castle, USA). A DIN aluminum conical concentric cylinder rotor, with a radius of 14 mm, 1 mm gap to the wall and 5.920 mm gap to the bottom, was connected to a smart-swap Peltier plate system to control the temperature. After 2 min of equilibration at 20 °C, the rotational measurement program started with four consecutive stages as presented in Fig. 1.

Before simulating processing conditions, the initial dough viscosity was measured in the first stage with minimum shearing (0.05 s^{-1}) at

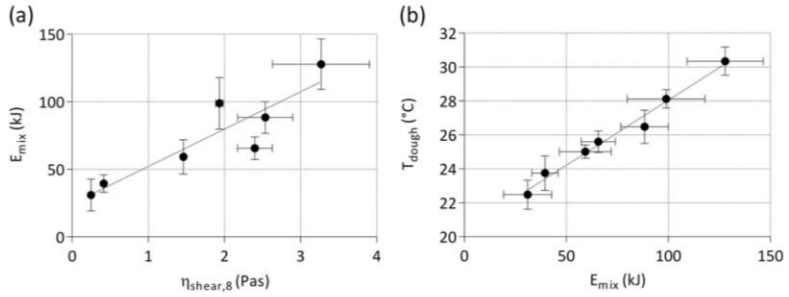

Fig. 3. Correlation between dough viscosity, energy input and dough temperature during mixing. (a) Influence of the dough viscosity $\eta_{shear,8}$ after 8 min of shearing at 700 s^{-1} on the required energy input during mixing. Dough viscosity was measured in a rheometer independent from mixing trials. The energy input was measured during mixing of 7 different gluten-free recipes in a planetary mixer with a wire whip at 420 rpm for 8 min. Dotted line: linear correlation with $R^{2\circ} = °0.8119$ and $p < 0.0001$. (b) Correlation between energy input and dough temperature after mixing. Dotted line: linear correlation with $R^{2\circ} = °0.9824$ and $p < 0.0001$.

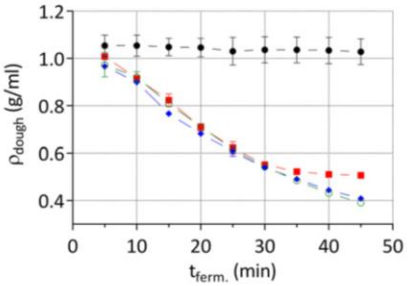

Fig. 4. Influence of glucose content on dough density during fermentation. Glucose was added to gluten-free dough based on corn starch and 80% water in the following concentrations (fwb): -•- 0.00%, -■- 1.00%, -♦- 2.00%, -○- 4.00% Density during fermentation was measured in a measuring cylinder. These preliminary measurements were performed in duplicates. Means are shown with standard deviation.

20 °C for 3 min. Mixing was simulated in a high-shear stage with a temperature ramp from 20 °C to 30 °C in 8 min. To provide conditions resembling the mixing process, the rotor speed in the rheometer approximated the one of the wire whisk in the mixer, resulting in a high shear rate of 700 s^{-1}. Consequently, to simulate fermentation during the third and fourth stage, low shear (0.05 s^{-1}) was applied at 30 °C for 10 min. In the following baking stage, dough was heated to 100 °C with a ramp of 4 °C/min. The resulting dough viscosity was monitored and the critical values, highlighted in the sample curve of Fig. 1, were compared for different recipes. The extent of shear thinning was calculated as percentage viscosity decrease from the last value of stage 1 to the last value of stage 2: $\Delta\eta_{|shear\text{-}thinning}$ shear-thinning $= (\eta_{|rest} - \eta_{|shear}) / \eta_{|rest}) \times 100$. Measurements were performed in triplicate.

2.7. Statistical analysis

Statistical analysis was performed with the aid of Prism 5 (Version 5.03, GraphPad Software, Inc.). The normality of data was evaluated for $n > 8$ via D'Agostino & Person omnibus normality test and for $n < 8$ via Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefors. Both tests were passed if $p > 0.05$. To detect significant differences between samples, variances were analyzed (ANOVA) with separation of means by the Tukey-Kramer test ($p < 0.05$). Correlation analysis served to quantify the degree of relation between two variables.

3. Results and discussion

3.1. Rheological characterization of gluten-free recipes

The viscosity of nine gluten-free dough recipes (Table 1) was analyzed during simulated processing conditions (see Fig. 1). The compositions were chosen to provide distinct differences regarding the rheological behavior by using different flours and varying the content of HPMC and water. For rheometer experiments, dough without yeast was produced with minimum gas and energy input. Viscosity curves resulting from the developed measurement program are presented in Fig. 2.

Already in the initial stage, the viscosities of some recipes varied considerably. In comparison to the viscosity of wheat dough, which was reported to be 160 kPa·s at 0.001 s^{-1}, gluten-free dough resistance to shearing was several magnitudes smaller (Stauffer, 2007). Without HPMC, the viscosities ranged between 6 and 19 Pa·s, while dough with 2% HPMC was elevated to 380–730 Pa·s, due to the high water binding capacity of this hydrocolloid. The pH-value, which is known to influence gel structures, did not vary significantly in the different doughs and ranged between 5.8 and 6.0 (data not shown).

In the second stage, the viscosity dropped immediately and continued to decrease because of the combined influence of heating and mechanical shearing, similar to the second stage in a Mixolab (Huang et al., 2010). The shear-thinning behavior of gluten-free dough is well-known (Demirkesen, Mert, Sumnu, & Sahin, 2010; Juszczak et al., 2012). The viscosity during the fermentation stage might be an indicator for the dough stability during bubble growth, which is evaluated in the following sections.

In the final heating stage, the viscosity is dominated by starch gelatinization. Although an elevation of the water content in quinoa dough from 105 to 120% reduced the initial dough viscosity, gelatinization was more pronounced when more water was available, which caused higher final viscosity. Similarly, differential scanning calorimetry (DSC) of wheat starch-water mixtures demonstrated that an increase of the water content from 40 to 70% strongly elevates the gelatinization enthalpy, while the transition temperature remains unaffected (Burt & Russell, 1983). HPMC delayed the onset of gelatinization because it reduces the amount of available water. However, during heating, the water becomes available and is responsible for the viscosity decrease of all curves containing this hydrocolloid. After gelatinization, the dough viscosities with HPMC resembled the ones without. The next goal was to evaluate how viscosity profiles influence the dough behavior during mixing.

Table 2
Comparison of the baking performance of various starch-based dough recipes produced with standardized biological aeration.

Recipe No.	1	2	4	1	5	7	8	9
Symbol (Fig. 2)	—	- - -	- · - ·	n. a.	—	- · - ·	—	—
Flour	Quinoa	Quinoa	Quinoa	Quinoa	Rice/corn	Rice/corn	Rice	Refined rice
Water	105%	120%	90%	105%	105%	90%	120%	120%
HPMC	2%	2%	0%	2%	2%	0%	2%	2%
Mixing	Beating	Beating	Beating	Kneading	Beating	Beating	Beating	Beating
ρ_{dough} (g/ml)	0.976 ± 0.036^{a}	0.968 ± 0.023^{a}	1.189 ± 0.018^{b}	1.075 ± 0.013^{c}	1.135 ± 0.016^{d}	1.222 ± 0.027^{b}	1.045 ± 0.018^{c}	0.988 ± 0.012^{a}
ρ_{bread} (g/ml)	0.440 ± 0.011$_{a}$	0.450 ± 0.016^{a}	0.579 ± 0.023^{b}	0.521 ± 0.011^{c}	0.445 ± 0.012^{a}	0.540 ± 0.012^{d}	0.491 ± 0.013^{e}	0.478 ± 0.009^{e}
$\tilde{x}(V_{pore})$ (mm³)	5.08 ± 0.45^{a}	5.45 ± 0.37^{a}	2.98 ± 0.18^{b}	2.61 ± 0.14^{c}	12.98 ± 1.67^{d}	6.74 ± 0.32^{e}	14.30 ± 1.56^{d}	8.51 ± 0.43^{f}

On the left of bread slice pictures, a scale is given, where one rectangle represents 1 cm bread height. Water and HPMC percentages are based on flour weight. $\tilde{x}(V_{pore})$: mean pore volume. Different letters denote significant differences between means in each row (One-way-ANOVA) $p < 0.05$.

Table 3
Comparison of the baking performance of various gluten-free recipes produced with standardized biological aeration.

			Dough rheology					
			η_{rest}	η_{shear}	$\Delta\eta_{shear\text{-}thinning}$	$\eta_{ferm,\,0}$	$\eta_{ferm,\,10}$	η_{end}
Dough and bread parameters	ρ_{dough}	R^2	0.5680	0.2437	**0.7383**			
		p	0.0504	0.2602	0.0132			
		r	−0.7536	−0.4937	−0.8592			
	ρ_{bread}	R^2				**0.6410**	**0.7163**	0.1631
		p				0.0305	0.0163	0.3689
		r				−0.8006	−0.8463	0.4038
	Median cell volume	R^2	0.0849	**0.5769**	0.0191	0.4247	0.3196	**0.6789**
		p	0.5261	0.0476	0.7678	0.1128	0.1860	0.0227
		r	0.2913	0.7596	−0.1381	0.6517	0.5653	−0.8239

Dough viscosity of 7 different gluten-free recipes was measured in a rheometer independent from mixing trials. Dough temperature and energy input were measured after mixing in a planetary mixer with a wire whip at 420 rpm for 8 min. Two-tailed correlation analysis was performed with Pearson correlation coefficients. Values for R^2 above 0.5 with $p < 0.05$ are in bold.

3.2. Influence of viscosity on energy input and dough temperature

In order to guarantee a specific rotational speed, the mixer has to compensate differences in resistance by adjusting the power input. The more viscous a material, the more energy is consumed during the mixing process. It can be expected that the power input during mixing should correlate with the viscosity measured in a rotating rheometer geometry, if the shear conditions are similar. Thus, the interrelations between dough viscosity, mixing energy and dough temperature were analyzed. For 3 kg of different gluten-free dough recipes the measured energy input varied from 30 kJ to 130 kJ, despite of constant mixing conditions. Fig. 3(a) shows that recipes producing higher viscosity during rheometer shearing at 700 s^{-1} also required more energy during mixing.

Fig. 3(b) reveals the strong correlation between the energy input and the dough temperature. Yeast activity depends on dough temperature, which is, in turn, a function of dough viscosity and energy input during mixing. Chiotellis and Campbell (2003) reported an increased gas production rate in wheat dough at higher dough temperatures due to higher yeast activity and less CO_2 solubility in the liquid dough phase. The range of recipes used in this study led to deviations regarding the substrate availability as well as the dough viscosity, so that different levels of biological aeration would result. Therefore, the amount of gas produced by yeast had to be standardized to study the interrelation between dough rheology and baking performance.

3.3. Baking performance after standardized biological aeration

In order to identify medium properties that are important for low bread density and small pores, baking trials were performed. A standardization of biological aeration was realized in two stages: (1) saturation of the glucose availability in all recipes and (2) individual fermentation times until a certain amount of gas was produced by yeast. For the first stage, Fig. 4 shows the development of the density of corn starch dough with different glucose concentrations. Without glucose addition, the dough density of this control dough did not decrease during 45 min fermentation. With the addition of 1–4% glucose (fwb) the dough density decreased significantly due to gas production of up to 130 ml/100 g dough. As a compromise between excessive addition and sufficient substrate availability, 2% glucose was added to all recipes in baking trials.

For the second stage, fermentation was stopped when reaching a predetermined dough volume increase in order to prevent that the CO_2 production depends on the dough temperature after mixing or other ingredient specific properties. To determine a target gas volume, quinoa dough (recipe 1) was used as a model system. Starting with

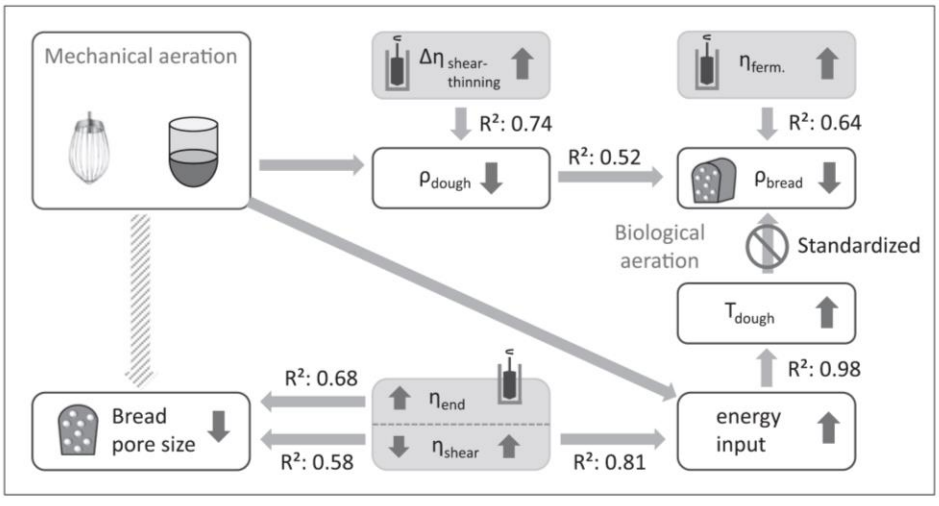

Fig. 5. Overview on variables affecting aeration and bubble stabilization of starch-based food foams. To avoid an influence of the dough temperature T_{dough} on the bread density ρ_{bread}, the biological aeration was standardized. In independent rheometer trials dough viscosities of 7 different recipes were monitored during different processing stages with η_{shear}: dough viscosity after 8 min shearing, $\eta_{ferm.}$: dough viscosity during simulated fermentation conditions, $\Delta\eta_{shear\text{-}thinning}$: percentage viscosity decrease after 8 min shearing and η_{end}: viscosity after simulated baking. ρ_{dough}: dough density after mixing without yeast. Correlation coefficients R^2 are given with $p < 0.05$.

the gas-free dough, mechanical aeration gave a gas volume fraction of 17%. Yeast produced 66 ml CO_2 per 100 g dough during fermentation, which increased the gas volume fraction to 50%. For all other recipes fermentation was also terminated after reaching 66 ml CO_2 per 100 g dough. Thus, density differences of varying recipes after mixing were maintained and only the biological gas input was standardized. The recipes without HPMC were too liquid for processing with 105% water (fwb) and were therefore excluded.

The results of the baking trials as well as the dough densities after mixing without yeast are displayed in Table 2. Similar to literature results for cake batter (Edoura-Gaena et al., 2007), the bread density did not properly correlate with the dough density. This indicates that bubbles stabilization and expansion during fermentation and baking can impair the success of mechanical aeration. Fig. 3 shows that the water content had no significant influence on the bread density after standardized biological aeration. A reduction of dough viscosity through the additional water did not destabilize the bubbles, since the pore distribution with 105% water was similar as with 120%.

A superior gas retention capacity of quinoa white flour was already shown in previous studies (Elgeti et al., 2014). Confirmative, fractionated quinoa and rice flour resulted in the highest aeration during mixing and bread with the lowest density. Whole grain rice flour gave less stability to bubbles, as shown by larger pores and less volume. Pruska-Kędzior et al. (2008) found that rice flour - presumably whole grain - had still better gas retention capacity than corn flour, buckwheat flour and corn starch. In accordance to the positive effect on loaf volume reported by Crockett, Ie, and Vodovotz (2011), 2% HPMC addition strongly reduced the bread density. HPMC probably facilitated gas entrainment during mixing because of its surface activity (BeMiller, 2009). Moreover, the pronounced shear-thinning with HPMC in Section 3.1, indicates the formation of a sensitive network.

Finally, the data suggest that high speed mixing is preferable for gluten-free dough than traditional wheat dough kneading, because it resulted in considerably higher bread volume. Since the bread density did not always depend on the dough density, relevant material properties are addressed in the following Section 3.4.

3.4. Correlation of rheological data with dough and bread parameters

The rheological data of the different dough formulations (Section 3.1) were correlated to their baking performance (Section 3.3) to elucidate the mechanisms relevant for bubble formation and stabilization. The large temperature variations after mixing would have influenced yeast activity extensively, but biological aeration was standardized in the conducted baking trials.

The results in Table 3 suggest that the extent of gas entrapment during mixing was not influenced by the absolute viscosity values during shearing but by the extent of shear thinning. The bigger the viscosity difference at low and high shearing, the more gas was entrained. For bread density, shear thinning was less important than bubble stabilization, since higher viscosity during resting elevated the bread volume. Higher dough viscosity reduces bubble movement caused by density differences, which would otherwise facilitate coalescence and buoyancy. Similarly, Ronda, Gómez, Blanco, and Caballero (2005) related lower viscosities in sugar-free batter to a loss in foam stability and consequent sponge cake volume reduction.

After shearing, the viscosity during the simulated fermentation stage correlated less with the bread density. In the rheometer, the shear rate and consequent structure damage might have been excessive because the rotational speed was adjusted to the mixer speed. Future trials might be improved by adapting a lower shear rate. Moreover, an introduction of biaxial tension in the fermentation stage might mimic the forces during bubble growth. Smaller pores were achieved with lower dough viscosity during mixing. In order to understand the mechanism of gas entrapment during mixing, further techniques, e.g. particle tracking and computational fluid dynamics, have to be employed. Pronounced viscosity increase by starch gelatinization correlated with smaller gas bubbles, which indicates better bubble stabilization during baking.

Chiotellis and Campbell (2003) suggested a strong relation between the bubble size distribution after mixing and the growth of bubbles during fermentation. It has been established that imaging via μCT is a valuable tool to monitor gas bubbles in dough (Babin et al., 2006; Bellido, Scanlon, Page, & Hallgrimsson, 2006; Demirkesen et al., 2014). In future trials, x-ray analysis might elucidate the effect of mixing parameters on the distribution of gas nuclei as well as bubble growth and coalescence during further processing stages. Finally, Fig. 5 visualizes the global findings of the present study. The graphic summarizes how mixing affects the aeration either directly through dough density reduction or indirectly through biological aeration. Moreover, it reveals the interrelation between rheological data and dough/bread parameters.

4. Conclusions

The presented results show that the rheology of starch-based dough systems influences the level of aeration during mixing and baking. The newly developed rheological procedure for simulating all processing conditions of bread production turned out to be a valuable tool to predict and understand the entrainment and stabilization of gas. Through the selected recipe variations, a wide range of viscosity profiles was achieved. As observed in numerous other studies, all starch-based dough compositions were shear thinning. More pronounced shear-thinning significantly reduced the gas input during mixing. This indicates that for gas entrapment during shearing a low viscosity is required, while the immediate stabilization of bubbles is facilitated by higher viscosities. Likewise, high bread volume depended on dough stability, since a negative correlation between bread density and dough viscosity was observed. On the other hand, the size distribution of pores in the bread crumb was mostly affected by the final viscosity after starch gelatinization. Destabilizing forces reach their maximum during baking, rendering gas retention especially important in this stage. Finally, the monitored energy input during mixing correlated with the viscosity during shearing in the rheometer and enabled a direct prediction of the dough temperature. Additionally, higher energy input reduced the bread density but the average pore size tended to grow. As primary industrial relevance, the aspired elevated volume in gluten-free bread can be obtained through low dough viscosity during mixing and high viscosity during fermentation and baking.

In general, the bread density of the different recipes was not directly related to the dough density. Consequently, high gas retention capacity is fundamental for the development of new aeration strategies, since bubble stabilization seems to be of special importance for gluten-free dough aeration. Particularly, the high pressure increase and extensional forces during baking have to be compensated. In this regard, fractionated quinoa or as a cheaper alternative, rice flour, are promising candidates for high quality gluten-free bread with low density and finely distributed pores. The water content was of little importance for mechanical aeration and gas stabilization when using a recipe based on quinoa flour. In contrast, the hydrocolloid HPMC strongly increased the dough viscosity, the extent of shear thinning, the gelatinization temperature and the resulting bread volume.

Notably, the implemented standardization of biological aeration through yeast enabled an independent assessment of mechanical gas input for different recipes. The evaluation of the bubble size distribution throughout processing, for example via x-ray, would probably provide further insights into stabilization mechanisms. For validating the presented findings, the list of ingredients should be extended to provide even more variations in the rheological behavior.

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3 Discussion, conclusions & outlook

For food manufacturers, the rising demand for gluten-free products offers an opportunity to expand the traditional range of ingredients and to develop new technologies. Moreover, studying gluten-free bread makes it possible to elucidate interactions of starch with other non-gluten ingredients in a model-like system. Gas entrapment and bubble stabilization are the most challenging factors for the production of gluten-free bread. Thus, this thesis initially reviewed different aeration methods. Consequently, the suitability and success of two of the identified strategies, namely yeast fermentation and high-speed beating, were tested in mixing and baking trials. In both cases, the underlying mechanisms were critically investigated, to understand the factors, which determine foam creation and stability in gluten-free systems. Based on the introductory hypothesis, the following points summarize the revelations obtained in the course of this thesis.

- ✓ Reviewing strategies for gas entrapment into food systems revealed that mechanical aeration is particularly promising for gluten-free dough.
- ✓ Quinoa provides high substrate availability for yeast fermentation and consequently increases the bread volume through biological aeration.
- ✓ Because of several rheological similarities of gluten-free dough and cake batter, the orientation towards batter processing, in contrast to the kneading process of traditional bread making, improves processability and gas input into gluten-free dough.
- ✓ Removal of bran from quinoa improves gas retention capacity and sensoric attributes.
- ✓ The development of a fundamental-rheological measurement procedure for gluten-free dough systems revealed that mechanical aeration benefits from pronounced shear-thinning, while foam stabilization is promoted by high viscosities during fermentation and baking.
- ✓ A combination of optimized aeration strategies yields a bread density similar to the one of wheat pan bread.

The biological gas input during fermentation with yeast depends on the suitability of the medium, particularly its substrates and physicochemical properties. The metabolism of *S. cerevisiae* is influenced by temperature, pH-value, osmotic pressure and nutrient concentration. In order to maximize the production of carbon dioxide, sucrose or malt are often added to bread dough, especially to gluten-free formulations. Unfortunately, the presence of sugar in the list of ingredient reduces the nutritional attractiveness of the product. As an alternative, amylolytic enzymes or modified starch can indirectly increase the availability of yeast substrates. Since these strategies do, however, require additional effort, the revelation of a 10-fold increased glucose content in quinoa milling products was of particular interest. It was shown that this was related to an exceptional activity of α-glucosidase, which converts long chain polysaccharides and maltose into glucose in the presence of water.

Thus, quinoa white flour amplified the biological aeration in comparison to rice and corn flour. Moreover, the removal of the bran fraction, whose negative impact on gluten-free foam stability has already been proven by Föste *et al*. (2014), significantly enhanced the gas retention capacity and led to high bread volume and small pores.

Unfortunately, the removal of bran is a loss for the nutritional value of bread. Quinoa white flour mainly consists of starch and its sum of proteins, lipids and fiber make only 15% (wet base), while the whole grain contains 32% of these components. Thus, the use of the white flour improves volume, texture and sensory attributes but with higher raw material costs and at the expense of the nutrient profile of the final product. Milling fractionation might, nevertheless, pay off since it makes it possible to omit the initial washing stage, which has the purpose of reducing the saponin content of grains. Particularly in warm and moist environments, this process presents a microbial and environmental hazard and further increases raw material costs. If some part of the bran would be redistributed into the white flour, a compromise between nutritional value and baking performance might be reached, with the additional advantage of a standardized product. This would satisfy the manufacturers' demand for reproducible and reliable product characteristics. Furthermore, Föste *et al.* (2015) already demonstrated that the remaining milling product, the bran fraction, provides a valuable substrate for the extraction of plant proteins with an excellent amino acid profile.

Another factor, which strongly influenced the degree of biological aeration in gluten-free dough was the temperature during or immediately after mixing. Different processing conditions and water levels directly influenced energy input and dough heating. Despite of a subsequent constant fermentation temperature, the final bread volume was directly proportional to dough temperature after mixing. The linear correlation had the formula $\rho_{bread} = -0.01546 * T$ which means that for each additional degree Celsius, 100 g of bread gained approximately 15 ml volume. This highlights the sensitivity of the yeast metabolism to external parameters. Chemical and enzymatic reactions typically accelerate with increasing temperature because the kinetic energy of the reactants rises. Together with specific genetic regulation mechanisms, this mainly accounts for an overall higher activity of some organisms in warmer conditions. *S. cerevisiae* is most active at 40-45 °C, which explains the increase in carbon dioxide production when raising the temperature.

Consequently, it was only possible to evaluate the influence of mechanical gas entrapment on bread volume and pore structure after standardizing the extent of biological aeration. For this purpose, glucose was added to saturate the yeast activity and the duration of fermentation was variably stopped after reaching a pre-defined volume increase. This facilitated the following evaluation of mechanical aeration and relevant medium properties.

Prior to fermentation, the processing step of mixing is crucial for the production of dough and bread because it is responsible for homogenization, hydration, structure formation, gas incorporation and bubble break-up. A vast variety of parameters has to be considered: material properties (η, ρ, γ), mixing speed and shear rate profile (Ω_R, Ω_r, v_D, $\dot{\gamma}_w$), bowl and agitator dimensions (R, r, d_W, x, n_W, xyz-position), headspace atmosphere (gas, p), temperature and scraping action. The planetary mixer used for this study is depicted in Figure 10 with the maximum radius of the agitator R = 70 mm, the radius of the agitator arm r = 47 mm and the maximum radius of the bowl r_v = 130 mm, all made of stainless steel. The single wires (n_W = 20) with a diameter d_W of 2.5 mm are equidistantly positioned in loops around the agitator axis.

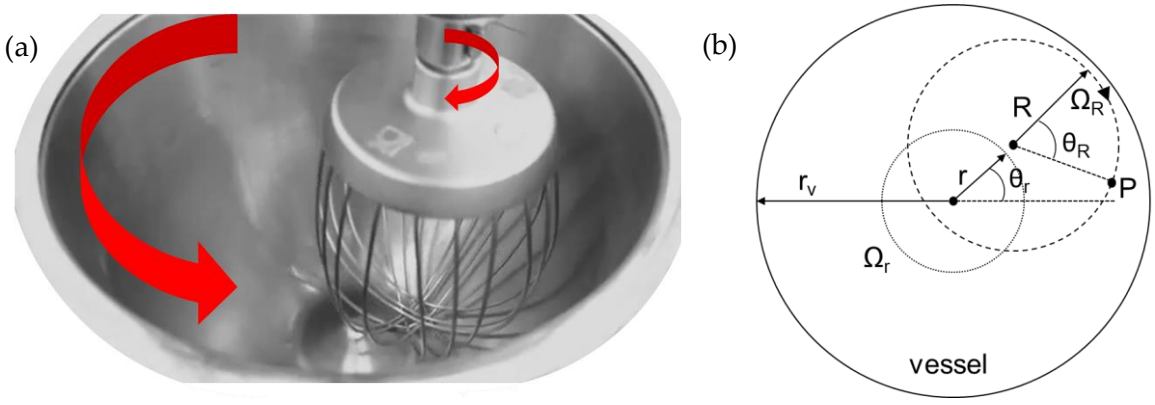

Figure 10. Visualization of the mixing motion in a planetary mixer. (a) The wire whip rotates clockwise around its axis while the mixing arm turns counter-clockwise in a planetary movement. (b) Plan view of the agitator motion. The bowl center is the orbital axis of the agitator arm motion. R: radius of agitator, r: radius of agitator arm, Ω_r: speed of agitator arm, Ω_R: speed of agitator. P: One point (single wire) of the agitator. Modified from (Chesterton *et al.* 2011b).

The planetary rotation of the wire whip is contrary to the orbital motion of the mixing arm, which creates a complex shear field. Chesterton *et al.* (2011b) and Auger *et al.* (2013) used Formula 4 to calculate the speed of a single point of the agitator v_D as it moves in the bowl (Hiseman 1995). Knowing the revolutions of the agitator and its shaft, enables the determination of the maximum speed, when the wire is closest to the wall and the minimum speed, when the wire is near the center. The mixer used in this study had a gearing ratio of 2.75, which means that the shaft rotated with Ω_r = 153 rpm in the opposite direction of the agitator at its maximum speed $v_{D,max}$ of Ω_R = 420 rpm. Applying Formula 4 for the angle θ_R of 0° and of 180°, corresponding speeds of 1.20 m/s and 2.48 m/s were calculated. In comparison, Chesterton et al (2011b) obtained a maximum speed of 2.18 m/s in a similar planetary mixer (Hobart).

$$v_D = 2\pi \sqrt{(\Omega_R - \Omega_r)^2 R^2 + \Omega_r^2 r^2 - 2\Omega_r (\Omega_R - \Omega_r) r R \cos(2\pi \Omega_R t)} \qquad \textbf{Formula 4}$$

Formula 5 enables an estimation of the shear rate $\dot{\gamma}_w$ occurring in the gap x between the wire and the bowl wall (Auger *et al.* 2013, Chesterton *et al.* 2011b).

With x = 2-10 mm, depending on the distance from the bowl bottom, the approximated shear rate varied from 250 s^{-1} to 1250 s^{-1} (using the calculated speed of 2.5 m/s). However, the temporal and special extent of this stress is constricted. For aeration in a Hobart mixer, a maximum shear rate of 500 s^{-1} has been reported (Chesterton *et al.* 2011b). Because of the complexity of the shear field, a realistic simulation of the stresses that the dough experiences in the mixer would require extensive computation and a reliable model (Chesterton *et al.* 2013).

$$\dot{\gamma_w} = \frac{v_D}{x}$$ **Formula 5**

Not only the processing parameters, but also the dough properties affect the success and effectiveness of mixing. As visualized in Figure 11, especially the choice of flour and the presence of the hydrocolloid HPMC have a very strong impact on the gas entrapment in the mixer. Despite of constant mixing conditions, the exchange of the rice/corn flour mixture by quinoa white flour increased the gas volume fractions from 7% to 21%. Without HPMC (and with less water), the quinoa dough entrapped less than a quarter of the gas volume. Interestingly, rice flour was just as effective for gas entrapment as quinoa white flour, but only in a refined version. Thus, refined rice flour presents a cheap alternative to quinoa not only during mixing but also for high bread volume (see Section 2.5).

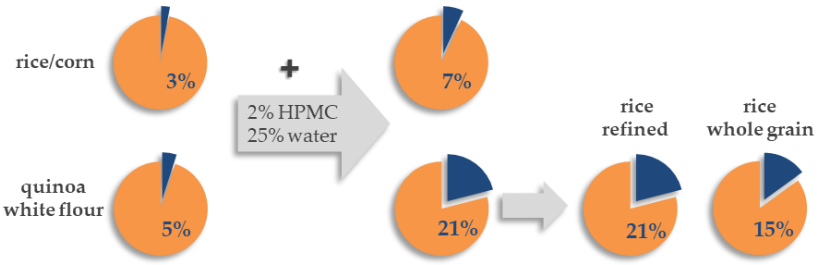

Figure 11. Influence of recipe components on the mechanical aeration of gluten-free dough. Dough density was directly measured after mixing (8 min, 420 rpm, wire whip). The gas volume fraction (blue cake fractions and percentages) were calculated with the gas-free dough density, which was determined via centrifugation. The recipe was either based on a mixture of rice and corn flour (2:1), fractionated quinoa white flour, refined rice flour or rice whole grain flour. Recipes on the left contain no HPMC and 90% water, while all recipes on the right contain 105% water and 2% HPMC (flour weight base).

The strong relation of the mixing effectiveness towards recipe alterations is connected to differences in dough rheology. Dough resistance, structure and flow behavior must be considered when choosing procedures for mixing, pumping, dosage, fermentation and thermal treatments. For gluten-free dough, no reliable measurements have previously been available to predict the performance of specific compositions. In the present thesis, the viscosities of various gluten-free dough recipes were analyzed in a novel rheometer protocol. Mixing, fermentation and baking were simulated consecutively with respective temperature and shear profiles.

The flow behavior of dough was monitored throughout the simulated processing stages. Different dough formulations were prepared with minimum gas entrapment before analysis.

In the present study, the power consumed by the mixer was directly related to the viscosity measured at high shear rates in the rheometer ($R^2 = 0.81$). Other authors have reported a similar relation for cake batter as well as for wheat dough in planetary mixers (Auger *et al.* 2013, Chesterton *et al.* 2013). Figure 12 enables the placement of the resulting viscosity ranges of gluten-free dough in relation to other materials.

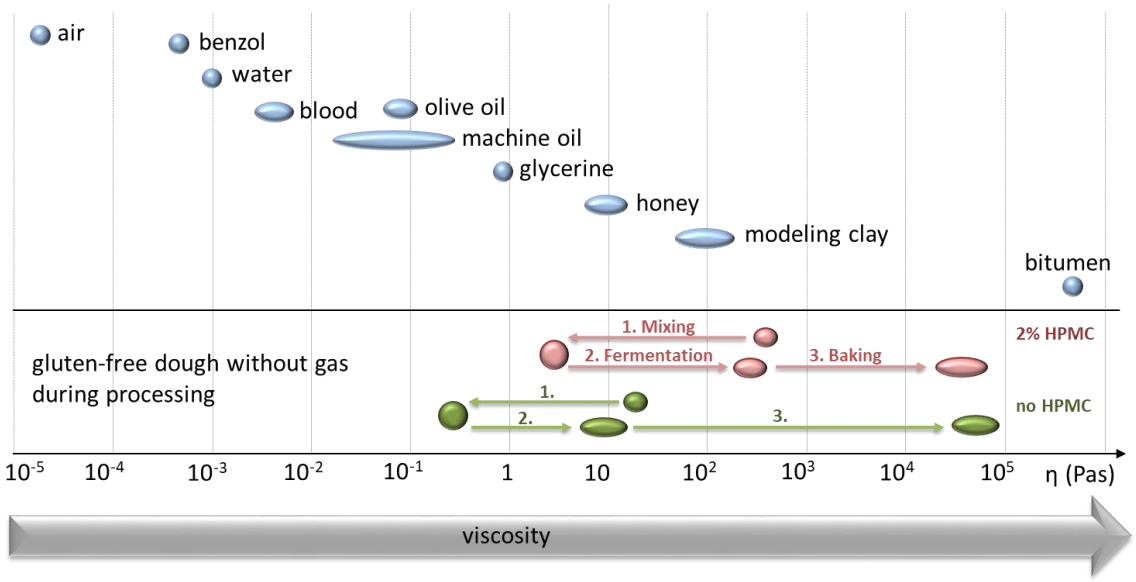

Figure 12. Viscosity of gluten-free dough during processing in comparison to other media. Display of the simplified viscosity ranges of comparative media (blue) at 20 °C and 1 bar on a logarithmic scale taken from (Sigloch 2014 and Mezger 2010). Undefined shear behavior must be taken into account. The viscosity of different gluten-free dough recipes was analyzed in a rheometer without gas, either with (red) or without HPMC (green). 1: reduction of the initial viscosity as a result of shearing in a rheometer at 700 s^{-1} to simulate mixing. The extent of shearing and viscosity reduction may be exaggerated. 2: partial regain of the viscosity during fermentation. 3: viscosity at the end of a heating stage to mimic baking.

The recipe variations determine the width of the green points. It becomes obvious that flour type and water content cause much less viscosity differences than the hydrocolloid HPMC, which is responsible for the deviation between green and red points, despite of its low concentration (2%). Although, it has often been stated that gluten-free dough is a shear-thinning material like cake batter and wheat dough, the displayed extent of this phenomenon is considerable and more pronounced with HPMC. Hereby, it has to be kept in mind, that the applied shear rate of 700 s^{-1} in the rheometer probably exceeded the one of the mixer. With the settings used in the present work, the dough underwent a transformation from a very thick, modeling-clay-like consistency to a thin, oil-like fluid.

While structure-loss during excessive wheat dough kneading is mostly irreversible, the gluten-free system regains almost all of its structure during fermentation. In the last stage, the fluid dough is transformed into solid bread through evaporation, gelatinization and denaturation.

All of these processes can be influenced by hydrocolloids, which would explain why HPMC addition reduced the extent of solidification. With the presented rheological program, also the gelatinization temperature T_g was detected. This makes it possible to renounce additional evaluations via differential scanning calorimetry, Amylograph or Rapid Visco Analyzer. The latter mostly require a dilution and, therefore, alienate the dough system. For a more accurate determination of T_g, prospectively, the resolution of measuring points should be enhanced during heating in the presented rheometer method. Generally, the observation of the gluten-free dough viscosity during simulated processing was an efficient tool for identifying structure-function relationships. Further trials with more recipe variations, comprising for example zein or xanthan, would strengthen the statistical independence of the correlations from single ingredients.

The simulation of the mixing process in the rheometer also expands the understanding of the complex flow behavior of starch-based dough in a stirred vessel. The Reynolds number *Re*, obtained by Formula 6, reveals the type of flow in the mixer depending on the viscosity η of the medium. Higher viscosities decrease *Re* and increase the tendency for laminar flow (Gabelle *et al.* 2013). Since all gluten-free recipes were strongly shear-thinning, *Re* was calculated twice – for η measured in the rheometer at low (0.05 s^{-1}) and at high shear (700 s^{-1}). Initially, the Reynolds number of the different dough compositions analyzed in this thesis approximated 50 at low shear (with an average $\rho_{gas\text{-}free}$ = 1.181 kg/l and η = 3.5 Pa s). This value ascended to 70 at the end of mixing (ρ_{bulk} = 0.930 kg/l, η = 1.8 Pa s). Rising *Re* numbers indicate decreasing flow consistency but complete turbulence only occurs above 10^4. Because of the great extent of shear-thinning, for gluten-free dough viscosity values below < 10 Pa s are possible during mixing. This increases the Reynolds number in gluten-free dough mixing in comparison to wheat dough kneading, where viscous forces dominate (Todd 2004).

The laminar region (*Re* < 10) would be exceeded if a shear rate of 700 s^{-1} was applied. However, the extent of shearing has probably been exaggerated in the rheometer test, and an actual prevalence of laminar flow in the mixer is likely. In order to further assess the influence of the flow field on the gluten-free dough behavior, the concentric cylinder was exchanged by a propeller geometry (data not shown). This creates a more complex flow field and prohibits a fundamental viscosity determination. Since the relative course of the resulting viscosity curve closely resembled the one of the same dough recipe measured in the concentric cylinder, it seems that the influence of the Reynolds number on the relative viscosity might be minor in the present range of intermediate flow behavior (neither laminar, nor turbulent). Thus, the concentric cylinder geometry can be maintained for future studies.

$$Re = \frac{(2R)^2 \, \rho \, n}{\eta}$$ **Formula 6**

In addition to the calculations, also visual observations were made to further elucidate the mechanical aeration with a wire whip. As visible in Figure 13a, the single strands of the geometry create elongated cavities as they move through the gluten-free dough. This complies with the principle of surface aeration described by Delaplace *et al.* (2012). They explained that the acceleration of the medium over the impeller surface can create a reduced pressure region, which encloses air, as outlined in Figure 13b. Thus, this hypothesis of surface aeration might also concern gluten-free starch-based dough systems.

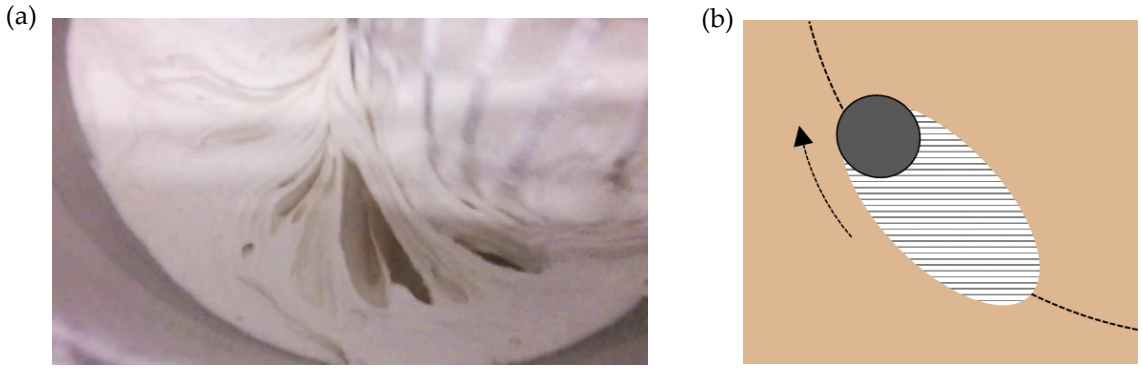

Figure 13. Wire whip moving through gluten-free dough. (a) Gluten-free quinoa dough with 105% water addition, mixed at 420 rpm. (b) Sketch (top-view) of a single wire moving through the dough and creating a reduced pressure region in its wake.

Mixing parameters also directly influenced the dough temperature and gas input. Figure 14 illustrates the effect of a step-wise adjustment of selected mixing parameters on the gas volume fraction of gluten-free dough. Here, the biggest impact results from elevating the mixing speed, which might have enhanced the above mentioned surface aeration. Elevated surface aeration through an exchange of the dough hook by the wire whip and mixing with higher speed lead to 60% (rel.) higher gas entrapment during mixing. In order to further assess the impact of mixing on bread volume it was necessary to become independent from the effect of dough heating on yeast activity. After standardizing the biological fermentation in Section 2.5, it was shown that beating instead of kneading also elevated the specific bread volume by 18%.

According to the manufacturer, the design of the wire whip mostly aimed at maximum stability and cleanability, to comply with customers' expectations in bakeries. Thicker and less numerous strands are favorable for cleaning and long-lasting usability, but experience teaches that a high number of thin wires improves the aeration performance. This makes it likely, that the aeration efficiency can be further optimized, especially with regard to gluten-free dough systems.

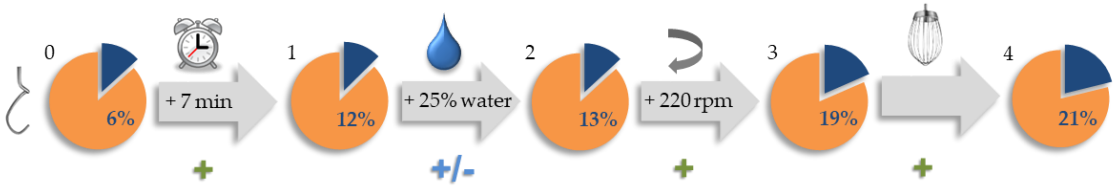

Figure 14. Influence of single parameters on the mechanical aeration of gluten-free dough. Four consecutive variations were performed during kneading/mixing of gluten-free dough without yeast. Dark blue area: dough gas volume fraction calculated with an average gas-free dough density of 1.18 g/ml. Beginning at step 0, for each new chart only one parameter was varied. 0: quinoa dough with 80% water addition, kneaded for 4 min at 200 rpm; 1: kneading duration elongated to 8 min; 2: water addition elevated to 105%; 3: mixing speed increased to 420 rpm; 4: the dough hook was exchanged by a wire whip.

The presented results (Section 2.5) revealed that the bread pore size decreases with increasing viscosity during baking. No other viscosity data obtained from the rheometer procedure correlated with the pore sizes. Ideally, it should be possible to adjust the mixing parameters in such a way that a specific pore size distribution is created in dough and bread for any given recipe. This would imply immediate monitoring of the bubble sizes during mixing. Moreover, the shear field and flow behavior would have to be predetermined. The presented thesis revealed a high correlation between the viscosity in rheometer measurements and the mixer power consumption as a function of viscous dissipation. Despite of notably different flow fields in the concentric cylinder rheometer gap and the planetary mixer, the shear-thinning, viscosity and temperature development can be directly estimated during mixing. Additional online monitoring of the bubble size distribution would complete the knowledge of the most important medium properties. A future intelligent mixing system might be able to adjust not only mixing speed but also shear gap width and angle to immediately respond to these data. This would allow a targeted adaption of shear rate and surface aeration to create the aspired bubble size distribution.

In the initial review of this work, methods to monitor the gas bubbles in dough were evaluated. X-ray analysis was identified as a popular non-invasive method that enables targeted imaging. With the help of computed tomography, two-dimensional x-ray images of different cross-sections can be combined into a three-dimensional visualization of the inside of a sample (Herman, 2009). This technique has already been used for wheat dough and bread (e.g., Besbes *et al.*, 2013; Demirkesen *et al.*, 2014; Van Dyck *et al.*, 2014). X-ray micro-tomography was also tested within the scope of this work at the Fraunhofer Development Center X-ray Technology in Fürth, Germany.

It was aspired to observe the bubble size distribution directly after mixing without yeast, in order to assess the effect of processing parameters and dough rheology on gas entrainment and dis-entrainment. Dough with different water content was analyzed directly after mixing with or without yeast.

Despite of comparatively short exposure times (34 s) and operating in high-power mode, the voxel sampling of 47x47x38 was not sufficient to display the total range of bubbles. As visible from Figure 15, the resulting resolution limit of 54 µm for single bubbles trimmed the frequency distribution and falsified the results. The number of bubbles per ml rose from 8,000 to 12,000 in the course of fermentation at 25 °C for 35 min (data not shown). Since it is known, that yeast is not able to produce new gas cells, the apparently increasing concentration probably results when small gas cells (below 54 µm) grow above the detection limit.

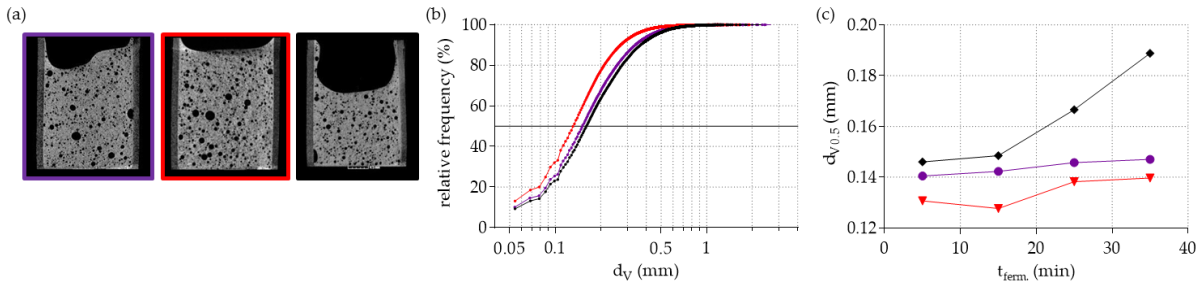

Figure 15. Preliminary x-ray micro-tomography results of gluten-free dough. Gluten-free quinoa dough was analyzed directly after mixing with 105% (purple, black) or 120% (red) water addition either with yeast (black) or without (purple, red). Measurements and calculations were conducted in duplicates at the Fraunhofer Development Center X-ray Technology. (a) Images of approximately 6 ml dough in vessels. (b) Initial frequency distribution of bubbles (5 min after mixing). (c) Median volume diameter $d_{V0.5}$ of bubbles measured repeatedly during 35 min of fermentation.

These preliminary trials did, however, prove that measurements of gluten-free dough can be performed without artefacts – even during yeast fermentation. An improvement of the resolution to the required range is assumed to be possible by (1) reducing the size of the region of interest, instead of measuring the total sample and (2) omission of data compression. Moreover, the prolonged size and location monitoring of single bubbles might reveal the significance of coalescence and Ostwald-ripening for the overall foam stability.

As indicated in the initial review of this thesis, another important parameter during mixing is the headspace atmosphere, comprising gas composition and pressure. The value of both variables has already been demonstrated for wheat bread (Chorleywood process) and cake batter (Chin and Campbell 2005, Massey *et al.* 2001). Higher pressure during mixing produces smaller bubbles that expand after mixing, when returning to normal pressure. A modification of the headspace atmosphere for gluten-free bread production might provide a more controlled aeration process.

Moreover, the rheology of the gluten-free system is less sensitive to oxidative reactions than wheat dough and might, therefore, facilitate the evaluation of underlying mechanisms. Preliminary experiments with reduced pressure were promising and revealed the significance of this variable (Figure 16). Furthermore, a comparison of the stability of bubbles filled with different gases might provide valuable new insights.

For example, the use of nitrogen with its low solubility makes it possible to neglect disentrainment of gas through disproportionation and diffusion.

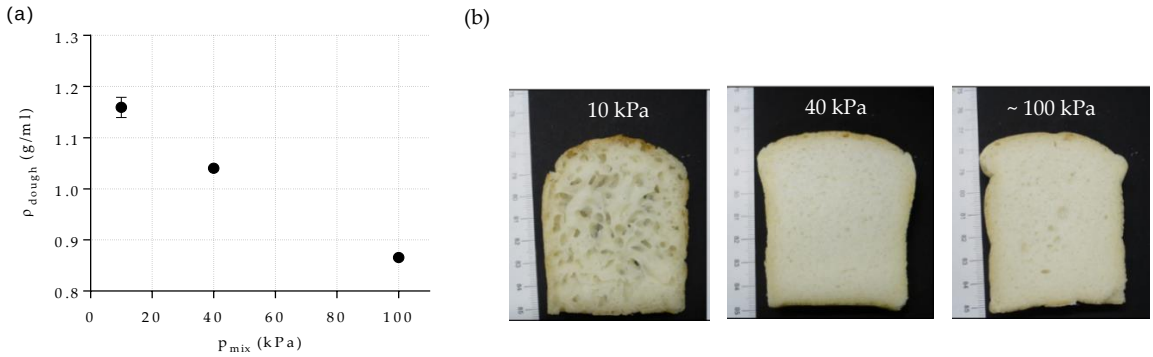

Figure 16. Influence of the mixing pressure on dough and bread density. The pressure was reduced to 40 kPa and 10 kPa bar during mixing at 750 rpm in a Stephan UMC 5 mixer. Gluten-free dough was based on refined rice flour with 120% water addition. (a) Dough density ρ_{dough} after mixing without yeast depending on the mixing pressure (p_{mix}). (b) Bread slices after mixing with yeast at different pressures.

In order to better evaluate the success of aeration based on the gas volume fraction, the gas-free density of the medium is required. In this work, the gas-free density of gluten-free dough was obtained by removing the gas in a centrifuge. The accuracy of this method can be validated, when consulting the results from vacuum mixing. As explained by Campbell *et al.* (1993) and discussed in the review of this thesis, an extrapolation of the measured pressure to zero gives the gas-free dough density. Neglecting the low number of data points, the linear regression of the density values of Figure 16 (a) intercepts the y-axis at 1.182 $\pm$ 0.016 g/ml, which is very close to the gas-free dough density obtained via centrifugation (1.181 g/ml). However, the stage of aeration in bread after baking is more difficult to assess. While the bread or crumb density can be used for an estimation, the actual gas volume fraction can only be calculated if the true density of the material is known. To date, methods have led to considerable deviations regarding the true density (1.3-15 g/ml) and consequently the gas volume fraction (38-97%) of wheat bread (Campbell and Mougeot 1999, Datta *et al.* 2007). It is not surprising that these values are even more confusing for gluten-free bread, where the degree of water absorption and composition are within broad ranges that further influence the density of the material. Thus, an efficient, accurate and reliable method for analyzing the true density of bread should be developed urgently.

The stability of gas bubbles does not only depend on the size distribution, or the medium density and viscosity, but also on the tension at the interface between dough and gas. Although it has been shown in this thesis, that the rheology plays a major role for retaining bubbles in gluten-free dough, future trials should also address the impact of surface activity.

It is possible that amphiphilic substances in quinoa white flour facilitate the mechanical aeration process and, thus, contribute to the superior gas volume fraction found in quinoa dough in comparison to other recipes. Ultra-centrifugation separates insoluble substances from the dough liquor (Primo-Martín *et al.* 2006, Salt *et al.* 2006). A pendant drop can be formed from the latter to enable analysis of the interfacial tension and rheology over time. The characterization of the dough liquor composition and selected enrichment of depletion of single substances might ultimately allow for a targeted improvement of the bubble stability.

In this regard, it might be helpful to exchange HPMC by a less reactive and amphiphilic hydrocolloid to avoid a simultaneous interference with dough rheology and surface activity. Another simplification would derive from the use of chemical leavening agents instead of yeast. A stoichiometric reaction makes it easier to predict the bubble growth kinetics and is less sensitive to medium variations. Moreover, the production of possibly functional secondary metabolites can be avoided with this approach.

In summary, successful methods to increase mechanical and biological aeration of gluten-free bread were developed. Particularly, high-speed beating in combination with a recipe based on fractionated quinoa flour significantly increased the bread volume. By applying a new rheological procedure, correlations between foam stabilization and dough viscosity during fermentation and baking were revealed.

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5 Appendix

The numbering of the following non-reviewed publications is continued from the peer-reviewed publications (page II).

5.1 Non-reviewed paper

8. Elgeti, D., Bernauer, S., Jekle, M., Becker, T.: Lipids in gluten-free bread. Baking+biscuit international 4 (2015), 88-92.
9. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Manufacture of gluten-free breads - a question of the substrate? Baking+biscuit 6 (2013), 46-49.
10. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Herstellung glutenfreier Brote – alles eine Frage des Substrats? Brot+Backwaren 5 (2013), 66-70.
11. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Innovation Award für fraktioniertes Quinoamehl als funktioneller RohstoffMühle + Mischfutter 153 (2016), 577-582.

5.2 Oral presentations with first authorship

12. Elgeti, D, Föste, M., Jekle, M., Becker, T.: Verbesserung der Qualität von glutenfreien Broten durch innovative Verfahren und Rohstoffe, DLG-Forum FoodTec: Lebensmittel für Zöliakie- und Glutenintoleranz-Betroffene, Frankfurt am Main, Germany, 2016-04-20.
13. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Glutenfreie Backwaren und Pseudocerealien - Seminar: Einfluss der Vermahlungstechnologie auf Backwaren, Bakery Innovation Center, Uzwil, Switzerland, 2015-07-24.
14. Elgeti, D., Jekle, M., Becker, T.: Identification of gluten-free dough properties relevant for aeration and foam stabilization, 7th International Symposium on Food Rheology and Structure, Zurich, Switzerland, 2015-06-07-11.
15. Elgeti, D., Stüttgen, A., Jekle, M., Becker, T.: Insights into aeration and foam stabilization mechanisms for gluten-free dough, 2014 AACC International Annual Meeting, Providence, U.S.A., 2014-10-5-8.
16. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Potential von Pseudocerealien zur Aufwertung gluten-freier Brote, Weinheimer Symposium 2014 - Backwaren für Besondere Zielgruppen, Weinheim, Germany, 2014-09-17.
17. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Glutenfreie Backwaren und deren Herstellungsweise - Seminar: Einfluss der Vermahlungstechnologie auf Backwaren, Bakery Innovation Center, Uzwil, Switzerland, 2014-07-18.
18. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Wertvolle Inhaltsstoffe, moderne Herstellung und Glutenfreiheit: Mit Quinoa und Amarant zur Aufwertung von Backwaren, 3.

Grenzübergreifendes Bürger- und Expertenforum: Lebensmittel von morgen, Kreuzlingen, Switzerland, 2014-07-02.

19. Elgeti, D., Yu, L., Jekle, M., Becker, T.: Entwicklung eines Aufschlagverfahrens für glutenfreie Brote, 3. Frühjahrstagung des Weihenstephaner Instituts für Getreideforschung, Freising, Germany, 2014-04-02.
20. Elgeti, D., Föste, M., Brunner, A.-K., Jekle, M., Becker, T.: Optimierung der Proteinextraktion aus Mahlfraktionen von Amarant für Lebens- und Nahrungsergänzungsmittel, Jahrestreffen der ProcessNet-Fachgruppen „Lebensmittelverfahrenstechnik“ und „Phytoextrakte“, Freising, Germany, 2014-02-27.
21. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Surface active components and fractionated pseudocereals as means to improve the foam stability of gluten-free bread, 3rd International Symposium on Gluten-Free Cereal Products and Beverages, Vienna, Austria, 2013-06-12/14.
22. Elgeti, D., Jekle, M., Becker, T.: Techno- and biofunctional impact of quinoa milling fractions and α-amylase treatment on gluten-free bread. 11th European Young Cereal Scientists and Technologists Workshop, Spain, Barcelona, 2012-05-10.
23. Elgeti D., Bernauer S., Jekle M., Becker T.: Einfluss fraktionierter Pseudocerealien und oberflächenaktiver Zusatzstoffe auf die Schaumstabilität und Qualität glutenfreier Brote. 63. Tagung für Getreidechemie, Detmold, Germany, 2012-06-20.
24. Elgeti, D., Jekle, M., Becker, T.: Beeinflussung der Qualität von glutenfreien Broten mit Pseudocerealien durch Amylase. 1. Frühjahrstagung des Weihenstephaner Instituts für Getreideforschung, Freising, Germany, 2012-03-29.

5.3 Poster presentations with first authorship

25. Elgeti, D., Jekle, M., Becker, T.: Optimierung von Prozessparametern und Rezeptur zur Maximierung des Gaseintrags in glutenfreie Teige, Tagung für Getreidechemie, Wien, Austria, 2015-10-01./02.
26. Elgeti, D., Peng, L., Stüttgen, A., Jekle, M., Becker, T.: Einfluss von Mixparametern auf den mechanischen Gaseintrag und die biologische Lockerung bei glutenfreien Teigen, 4. Frühjahrstagung des Weihenstephaner Instituts für Getreideforschung, Freising, Germany, 2015-04-21.
27. Elgeti, D., Jekle, M., Becker, T.: Improvement of gluten-free dough aeration through adapted processing conditions and ingredients, 13th European Young Cereal Scientists and Technologists Workshop, Freising, Germany, 2014-05-14.
28. Elgeti, D., Bernauer, S., Jekle, M., Becker, T.: Einfluss von Lipiden und Emulgatoren auf die Schaumstabilität und Qualität glutenfreier Brote, 2. Frühjahrstagung des Weihenstephaner Instituts für Getreideforschung, Freising, Germany, 2013-03-21/22

5.4 Curriculum Vitae

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EDUCATION

09/11-12/17	PhD Student, Technical University of Munich, Freising, DE Institute of Brewing and Beverage Technology Research Group Cereal Process Engineering
09/08-11/10	Student, Technical University of Munich, Freising, DE Food Technology and Biotechnology Degree: Master of Science
10/05-08/08	Student, Ludwig-Maximilians-Universität, Munich, DE Pharmaceutical Sciences Degree: Bachelor of Science
09/03-07/05	Student, Theresien Gymnasium München, Munich, DE General qualification for university entrance (Abitur)

EMPLOYMENT & INTERNSHIPS

Since 02/17	Manager Marketing Nutrition, Wacker Chemie, Munich, DE
03/11-09/11	Junior Research Assistant, Nestlé Research Center, Lausanne, SH Department of Food Science and Technology
01/11-03/11	Research Assistant, Technical University of Munich (TUM) Chair for Food Process Engineering and Dairy Technology, Freising, DE
01/11-01/11	Intern, BARDEHLE PAGENBERG Partnerschaft mbB, Munich, DE
08/09-10/09	Intern, Develey Senf & Feinkost GmbH, Unterhaching, DE

HONORS

- Best Master graduation of the class (WS 05/06)
- C&E Best Presentation Award, 11th WYCST Workshop, 2012
- DLG Innovation Award, 2015