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Structural investigations of yeasted wheat dough – the impact of CO_2 and glutathione

Christoph Verheyen

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1. Univ.-Prof. Dr. Thomas Becker
2. apl. Prof. Dr. Peter Köhler
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"A goal without a plan is just a wish"

~Antoine de Saint-Exupéry (1900-1944)~

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Preface

Christoph Verheyen

M.Sc.

The results and publications of this thesis were produced at the Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering from 2011 to 2015.

Publications

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

1. Verheyen, C., Albrecht, A., Becker, T., Jekle, M.: Destabilization of wheat dough: Interrelation between CO_2 and glutathione, Innovative Food Science and Emerging Technologies 34 (2016), 320-325, doi: 10.1016/j.ifset.2016.03.006

2. Verheyen, C., Albrecht, A., Elgeti, D., Jekle, M., Becker, T.: Impact of gas formation kinetics on dough development and bread quality, Food Research International 76 (2015), 860-866, doi:10.1016/j.foodres.2015.08.013

3. Verheyen, C., Albrecht, A., Herrmann, J., Strobl, M., Jekle, M., Becker, T.: The contribution of glutathione to the destabilizing effect of yeast on wheat dough, Food Chemistry 173 (2015), 243-249, doi:10.1016/j.foodchem.2014.10.021

4. Verheyen, C., Jekle, M., Becker, T.: Effects of *S. cerevisiae* on the structural kinetics of wheat dough, LWT - Food Science and Technology 58 (2014), 194-202, doi:10.1016/j.lwt.2014.02.050

Contents

Abbreviations

Abbreviation	Meaning
ADP	Adenosine diphosphate
ADY	Active dry yeast
Asc	Ascorbic acid
ATP	Adenosine triphosphate
C^*	Solute concentration of CO_2 at the interface
C_∞	Solute concentration of CO_2 in dough
cfu	Colony forming units
CY	Compressed yeast
Cys	Cysteine
D	Bubble diameter
db	Dry base
DH	Dehydrogenase
D_k	Coefficient of diffusivity
FDP	Fructose-1,6-diphosphate pathway
FU	Farino Units
G'	Elastic modulus
G*	Complex shear modulus
GDL	Glucono-delta-lactone
Glu	Glutamic acid
Gly	Glycine
GSH	Reduced glutathione, γ -glutamylcysteinylglycine
GSSG	Glutathione disulfide
h	Lamella thickness
H	Henry's law constant
HOG	High osmolality glycerol cascade
ICP	Intracellular pH
IDY	Instant dry yeast
N	Number of molecules
NAD+	Oxidized nicotinamide adenine dinucleotide

NADH	Reduced nicotine adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NV	Neutralization value
p	Pressure
P	Phosphate
P_∞	Solute pressure in dough
PSSG	Mixed disulfide of protein and glutathione
Q	Bubble growth rate
R	Universal gas constant
r	Bubble radius
ROR	Rate of reaction
ROS	Reactive oxygen species
SAPP	Sodium acid pyrophosphate
SD	Standard deviation
SH	Sulfhydryl group, thiol group
SS	Disulfide bond
T'_1	Time to reach the maximum gas formation rate
T'_2	Time to reach 90% of the maximum dough height
T_1	Time to reach the maximum dough height
T_2	Time to reach 90% of the maximum dough height (after the maximum)
tan δ	Loss modulus
TCA	Tricarboxylic acid cycle
T_x	Time of porosity (gas starts to escape the dough matrix)
V	volume
v_{CO2}	Maximum gas formation rate
ε	Gibbs coefficient of elasticity
γ	surface tension on the bubble surface
γ-GluCys	γ-glutamylcysteine
Δp	Laplace-pressure difference

Summary

Initiated by *Saccharomyces cerevisiae*, the transformation of saccharides into CO_2 serves as a fundamental biochemical process in the production of cereal-based foams. The intrinsic character of yeast cells defines the amount and rate of CO_2 production as well as the type and quantity of further metabolites. Although the sum of those metabolic products strongly affects the structural development of wheat dough and consequently the final product quality, the physiological state of yeast cells is usually not considered.

In this thesis, the application of fundamental rheological analyses revealed an asymptotic reduction of dough elasticity with increasing amounts of yeast. As the main gluten network destabilizers, both, the biaxial strain of growing gas bubbles, as well as the chemical disintegration by the yeast metabolite glutathione (GSH) were considered. The working hypothesis of the present work was that the interrelation between CO_2 and GSH destabilizes the integrity of dough and bread matrices.

The complexity resulting from the variety and quantity of metabolic products is already challenging when comparing fresh (CY) and dry yeast (IDY). While rising amounts of CY and the associated increase of the maximum gas formation rate v_{CO2} reduced the gas holding ability of dough, the CO_2-content in samples leavened with IDY remained almost constant. Although the application of IDY improved dough extensibility, the samples made with CY had a significantly higher loaf volume. The heat-induced gluten-cross-linking during baking appears to be impaired by IDY equivalent GSH levels. Therefore, the GSH content in 27 commercial fresh and dry yeasts was analyzed and studied further in chemically leavened matrices. It was shown that the native material properties of wheat dough allow for a linear increase of the CO_2 concentration and that particular GSH levels of dry yeasts improved the expansion of the matrix. In contrast, during the thermally induced transformation of dough to crumb, increasing levels of CO_2 and GSH provoked a collapse of the solidifying matrix.

The results confirm the working hypothesis and prove that controlling the type and quantity of metabolic products of yeast makes it possible to modify dough and bread texture. The application of chemical raising agents significantly reduces the leavening process, while offering the potential for a mechanistic investigation of foam structure defining yeast metabolites or baking agents.

Zusammenfassung

Die durch *Saccharomyces cerevisiae* initiierte Transformation von Sacchariden zu CO_2 dient als grundlegender biochemischer Prozess der Herstellung getreidebasierter Schäume. In diesem Zusammenhang bedingt der physiologische Zustand der Hefezelle die resultierende Menge und Rate des produzierten Gases, sowie Typ und Quantität freigesetzter Metaboliten. Obwohl die damit verbundene Veränderung der Matrixintegrität zu einer Beeinträchtigung der Produktqualität führen kann, bleibt der Zustand der Hefezellen bei der Herstellung von Backwaren in der Regel unberücksichtigt.

Die Applikation fundamental rheologischer Analysen ermöglichte die Detektion einer asymptotisch verlaufenden Reduktion der Teigelastizität mit steigender Hefemenge. Als maßgebliche Destabilisatoren wurden sowohl die mechanische Beeinträchtigung des Glutennetzwerks durch eine biaxiale Dehnung wachsender Gasblasen als auch die auf chemischer Ebene stattfindende Desintegration des Glutennetzwerks durch hefeeigenes Glutathion (GSH) in Betracht gezogen. Die in der vorliegenden Arbeit verfolgte Arbeitshypothese geht daher von einer destabilisierenden Interaktion zwischen CO_2 und GSH aus, welche die Integrität von Teig- und Brotmatrizen beeinträchtigt.

Die sich aus Variationen im metabolischen Profil von Hefezellen ergebende backtechnologische Komplexität zeigt sich deutlich bei Verwendung von Frisch- und Trockenhefen. Während die durch Erhöhung der Frischhefemenge erzielte Steigerung der maximalen Gasbildungsrate v_{CO_2} eine Reduktion der Gashaltefähigkeit bewirkte, blieb die im Teig zurückgehaltene Gasmenge trockenhefegelockerter Proben nahezu konstant. Obwohl Teige mit Trockenhefe also eine verbesserte Dehnbarkeit aufwiesen, besaßen die mit Frischhefe hergestellten Produkte ein signifikant höheres Endproduktvolumen. Die während des Backens induzierte Gluten-Quervernetzung scheint durch trockenhefeäquivalente GSH-Mengen beeinträchtigt zu werden. Daher wurde der GSH-Gehalt in 27 kommerziellen Frisch- und Trockenhefe analysiert und deren Einfluss in chemisch gelockerten Matrizen untersucht. Es konnte zunächst gezeigt werden, dass die nativen Materialeigenschaften von Weizenteigen eine lineare Steigerung des CO_2-Gehalts ermöglichen und dass insbesondere die in Trockenhefen vorkommenden GSH-Gehalte zu einer verbesserten Ausdehnung der Teigmatrix

beitragen. Dagegen bewirken steigende CO_2- und GSH-Gehalte während der thermisch induzierten Transformation von Teig zu Krume einen Kollaps der sich verfestigenden Matrix.

Die Ergebnisse bestätigen damit die Arbeitshypothese und zeigen am Beispiel von Glutathion, dass die Steuerung des metabolischen Profils von Hefen eine gezielte Texturierung von Teig- und Brotmatrizen generieren kann. Außerdem ermöglicht der Einsatz chemischer Triebmittel eine signifikante Reduktion des Fermentationsprozesses und bietet gleichzeitig das Potenzial einer mechanistischen Aufklärung strukturgebender Eigenschaften einzelner Hefemetaboliten bzw. anderer Ingredienzien in gelockerten Stärke-Protein-Matrizen.

1 Introduction

The standardization of biological processes is a major challenge in the food industry. For bread production the main part of the gas in dough as well as characterizing aroma components derive from the proving process with *Saccharomyces cerevisiae*. As a side effect, the metabolites can also influence the dough structure in positive or negative ways. Since the yeast metabolism is a very complex and sensitive system, it is beneficial to know the pathways that lead to techno-functional components. In the following, bread will be characterized as a food foam and different aeration methods will be discussed. Finally, the yeast metabolism and its most important structure affecting components will be explained, before presenting the thesis outline.

1.1 Formation and stability of cereal based food foams

A wide range of food systems with dispersed gas is available. When the gas proportion prevails, the dispersion of gas in a continuous liquid, semi-liquid or solid matrix is defined as a foam. In simple terms, a foam can be described as a multi-phase system based on gas bubbles separated by thin films. In the area of food processing two types of foams have to be considered (see Figure 1): bubbly foams with spherical bubbles and gas volume fractions between 50 and 80% (e.g. ice cream, dough, bread), and honeycombed structured polyhedral foams with gas contents above 85% (e.g. beer froth).

The incorporation of gas and the associated inflation of the food matrix can be obtained through different aeration strategies. Aside from the mechanical introduction of gas, chemical or biological strategies are equally applied for the production of food foams (Campbell and Mougeot 1999). From the customers' point of view the formation of a consumable product is the overall objective of the aeration and the intended reduction of the density.
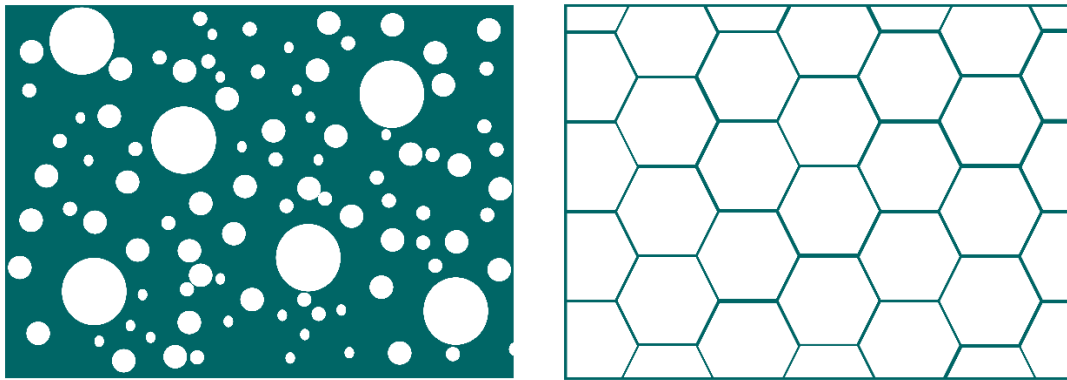
Figure 1: Classification of foams. Left: spherical foam (gas volume fractions between 50 and 80%), right: polyhedric foam (gas volume fractions above 85%).

Cereal based foams like bread play a particularly important role in the human diet. The control of the final foam architecture is subjected to complex processing operations comprising kneading, fermentation, and baking. During kneading, flour, water, baker's yeast (*Saccharomyces cerevisiae*), sodium chloride, and other specific ingredients are blended. Subsequently, the input of mechanical energy induces a continuous change of the dough consistency through the development of gluten network structures (Cauvain and Young 2003, Dobraszczyk and Morgenstern 2003). Moreover, depending on type, atmosphere and speed of the kneading geometry air nuclei, are incorporated into the dough matrix. The number and distribution of these inserted air filled cavities as well as the carbon dioxide production during proving mainly determine volume and pore structure of the final product (Crowley *et al.* 2000). Kneading provokes an increase of the gas volume fraction to 5.5–20%; the gas content of the matrix can rise to 80% in the course of proving (Shehzad *et al.* 2010). The resulting inflation of the dough matrix, therefore, derives from the sum of expansion of individual bubbles through the mass transfer of the CO_2 produced by yeast. In the course of proving, released CO_2 initially dissolves into the liquid dough phase and merges into incorporated air nuclei. Bubble growth requires no saturation of the liquid phase. According to calculations by Bloksma (1990), the saturation concentration of CO_2 in the liquid dough phase at 27 °C is 4.3×10^{-2} kmol/m^3. This means that based on the gas production rate of 2.5×10^{-5} kmol/s m^3 with 2% compressed yeast, the liquid phase would be saturated after 27 min (Bloksma 1990). Proving bread dough, however, evidently starts to inflate before this time. The following Equation 1 serves as a simplified description of bubble expansion through the mass transfer of CO_2 (Shah *et al.* 1998):

$$\frac{dD}{dt} = \frac{12\,RTD_K(C_\infty - C^*)}{3P_\infty D + 8\gamma}$$

Equation 1

where D is the bubble diameter, R the universal gas constant, T the temperature, D_K the coefficient of diffusivity, C_∞ and C^* the solute (CO_2) concentrations in the dough and at the interface, P_∞ the solute pressure in the dough and γ the surface tension on the bubble surface. The mathematical model does not consider rheological parameters, since the pressure in the bubbles during proving is predominantly affected by the surface tension. However, this approach is only valid for spherical bubbles and the assumption that C_∞, P_∞ and the Henry's law constant H do not change over time. Once the gluten network is stretched by rising CO_2 quantities, rheological properties have to be considered in the model. Thus Equation 1 is primarily valid to describe bubble growth during the early stages of proving, when the viscoelastic properties of the matrix are incidental (Shah *et al.* 1998). It should be pointed out that especially in the presence of gas, the generation of rheological values is quite complicated. This mainly derives from continuous changes of the foam structure, which reduce the reproducibility of fundamental rheological test methods.

Beside the generation of a sufficient CO_2-volume, the fixation of the gas is a key element in the production of aerated food stuff like bread. Thus, gas retention of the matrix is essential for high loaf volumes and soft crumb textures. In this context the **strain hardening** properties of gluten are one key factor to avoid the rupture of protein-based "cell walls" during proving and oven rise (Li *et al.* 2004, van Vliet 2008). Additionally, it has been shown that strain hardening may limit matrix destabilization by disproportionation during the first stage of proving (Vliet 1999).

Disproportionation (or Ostwald ripening or isothermal distillation) describes a successively growing inhomogeneity of the foam by mass transport of gas from small to large bubbles (see Figure 2 A)). Initially, due to the creation of gas bubbles with diameters varying from 10-100 µm, a concentration driving force for mass transfer exists, which allows CO_2 to diffuse from the liquid dough phase into gradual growing gas bubbles directly after mixing (Kokelaar and Prins 1995). In this context, the bubble growth rate Q can be described by Equation 2:

$$Q = \frac{dn}{dt} = f\left(\frac{dD_K}{dt}\right)$$

Equation 2

The rate of growth is affected by the number n of molecules of gas as well as by the classical diffusion theory with the diffusion coefficient D_K. Besides a low diffusion resistance, the self-accelerating disproportionation process is driven by the Laplace-pressure difference Δp between two bubbles, which results from their radius r and surface tension γ.

$$\Delta p = \frac{2\gamma}{r}$$
Equation 3

Based on Equation 3 it becomes evident that Δp rises, for smaller gas bubbles. Once two bubbles approach each other, the gas transfers from the smaller to the larger bubble resulting in a coarser foam structure. Related to the viscosity, the foam destabilization is based on the bubble size distribution, the gas solubility, the presence of surface-active dough ingredients, the rate of the mass transport, and the time of foam destabilization. As indicated in the formula (Equation 3), the stability of gas cells depends on the **surface properties** of the dough ingredients, which stabilize the liquid film between individual bubbles (Gan *et al.* 1995, Keller *et al.* 1997). However, the effectiveness of foam stabilization by these mechanisms is affected when different types of surfactants like proteins and lipids compete for the bubble interface. With the result that protein-protein interactions and high mobility of the lipids counteract and hence cause a quicker film rupture (Selmair 2009).

A further destabilizing mechanism, which limits the inflation of the dough matrix, is **coalescence**. Towards the end of the proofing process and during the first baking stage (oven rise) bubbles coalesce due to the temperature induced increase in CO_2 production as well as evaporating ethanol and water (Gan *et al.* 1995). Coalescence characterizes the fusion of two or more bubbles into a single droplet (see Figure 2 B)). Initiated by the formation of a small hole (100-200 nm) between the bulk phase of two bubbles, one larger gas filled cavity emerges. The fusion of small to large gas bubbles is supported by film thinning, separation of two or more bubbles and prolonged **drainage** during the leavening process. **Gravitational separation** (buoyancy) and drainage (see Figure 2 C)) as it is known from more liquid food foams like beer foam or cake batter is a minor factor at the beginning of proofing, but becomes more important as soon as the gas content rises (Mills *et al.* 2003).

Even when discontinuities are present in the dough matrix gas, they can be retained by a thin aqueous film, which prevents coalescence. In this context, two stabilizing mechanisms prevail in liquid films:

(1) Lamellae are stabilized by **adsorbed layers** consisting of proteins or polymers, which form a viscoelastic, two-dimensional network on the surface. Since the surface area of gas bubbles rises more than 10-fold in the course of proofing, highly surface-active gliadins as well as glutenins are eligible to form the physical barrier, which is responsible for foam stability (Örnebro *et al.* 1999, Örnebro *et al.* 2000, Örnebro *et al.* 2003). Moreover, to provide mechanical strength to the layer around the bubbles, proteins can form elastic networks by unfolding and reacting with adjacent proteins at the surface of the bubbles (Primo-Martín *et al.* 2006).

(2) The **Gibbs-Marangoni** effect describes an interfacial tension gradient of adsorbed surfactants, detergents, or emulsifiers in highly fluid layers. The decisive factor is the Gibbs coefficient of elasticity ε, which defines the resistance to surface deformation during thinning, whereby $d \ln h$ describes the relative change in lamella thickness:

$$\varepsilon = 2 \left(\frac{d\gamma}{d \ln A} \right) = 2 \left(\frac{d\gamma}{d \ln h} \right) \qquad \textbf{Equation 4}$$

The Gibbs coefficient depends on the concentration of surfactants and film thickness. The higher ε the more stable the film. In the case of bubble growth, the Marangoni effect tends to oppose any rapid displacement of the surface and provides a temporary restoring force to almost breaking films. Thus, to counteract layer deformation by growing gas bubbles, the flow of surfactants goes towards the thinner region to rebuild a homogeneous layer (Prins 1988).

In summary, highly viscous cereal foam structures like dough or bread depend on the number and size distribution of gas nuclei and the following bubble growth through mass, momentum and energy transfer between the gas bubble and the viscosity of the surrounding matrix (Advani and Arefmanesh 1993).
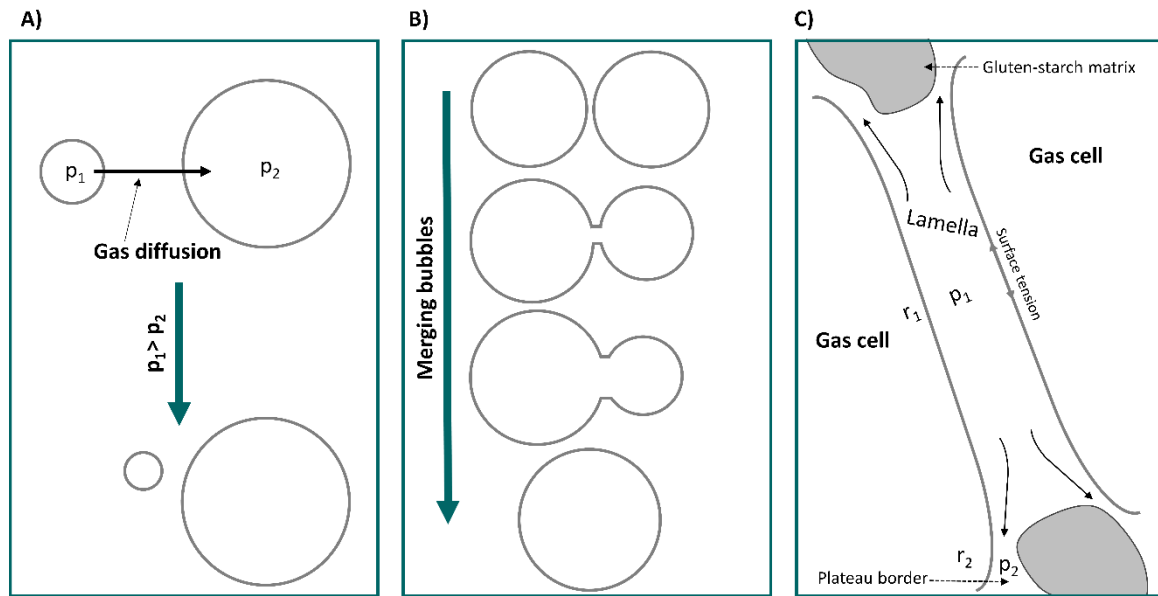
Figure 2: Schematic representation of destabilizing bubble mechanisms. A) Disproportionation describes the mass transport of gas from smaller into larger gas bubbles driven by the Laplace pressure Δp. The Laplace pressure in smaller bubbles (p_1) is greater than in larger bubbles (p_1); B) Coalescence defines the process by which two or more bubbles merge during contact to form a single daughter bubble; C) Drainage: Drainage mechanism from foam lamella. Surface tension forces increase pressure inside gas cell. Radius of curvature in plateau border (r_2) is smaller than in lamella (r_1). Hence the pressure in the border (p_2) is less than the pressure in lamella (p_1), resulting in a net flow of liquid from lamella into plateau border (arrows).

1.2 Aeration methods for the creation of cereal foam architectures

The construction of cereal-based foams by the incorporation of gas is directly linked to the leavening process. Through varying aeration strategies and the related volume increase of the matrix a wide range of product characteristics can be generated (Elgeti *et al.* 2015). Moreover, apart from the gas formation for all methods the overarching goal is the stabilization of gas-filled bubbles by the surrounding matrix. The associated leavening methods for cereal-based foams are typically divided into three classes, which are explained below (Campbell and Mougeot 1999):

(1) On the premise of low viscous fluids, the **physical aeration processes** are subdivided into (i) whipping/shaking, (ii) mixing, (iii) expansion extrusion, and (iv) air entrapment between layers.

(2) The aeration through **chemical raising agents** by the application of single or dual component baking powder.

(3) The gas entry into the continuous dough phase by yeast (e.g. *Saccharomyces cerevisiae*) and/or sourdough microflora during fermentation is described as **biological aeration**.

Physical aeration by whipping or mixing is applied for a wide range of low viscous cereal based products with higher fat contents and eggs (e.g. cake batter). Based on the mixing/whipping procedure two different mechanisms of air incorporation in cake batters are described in literature (Wilderjans *et al.* 2013). During multi-stage mixing incorporated air is immobilized by fat crystals, which are located in the surrounded aqueous matrix. The stepwise blending process starts with the production of a foam consisting of fat and sugar with incorporated air nuclei. Following the creaming step, eggs are added and the water-in-oil emulsion is transformed to an oil-in-water emulsion in which finally flour is admitted. During the baking stage, fat crystals melt and the growing bubbles become stabilized by egg proteins in the liquid phase. Contrary, all ingredients are mixed simultaneously using the single stage mixing procedure. First gas bubbles are stabilized by egg proteins and with proceeding mixing fat crystals from the margarine adsorb onto air bubbles and thus both prevent coalescence of gas bubbles. During thermal treatment the oil covers the inner surface of the bubble which stabilizes the gas cells during expansion (Shepherd and Yoell 1976).

The formation of aerated structures by further physical procedures like expansion extrusion, in which the superheated product under pressure emerges suddenly from an extruder, as well as the air entrapment between layers like in puff pastries or croissants is generated through the evaporation of water. The final aeration stages for most cereal products occur during baking, when water and ethanol evaporate and the existing gas bubbles expand according to the ideal gas law. For highly viscous cereal-based foams like wheat dough, the incorporation of air nuclei during kneading as well as the evaporation of ethanol and water are essential for aeration level of the final product. However, the incorporation of gas exclusively by physical methods is not sufficient since the maximum reachable gas volume fraction varies between 5.5 and 20% and is thus far below desirable gas proportions of 80% (Shehzad *et al.* 2010).

Alone or in combination with physical aeration methods, the application of **chemical raising agents** provides another possibility to inflate cereal based foam structures (Bellido *et al.* 2009). Beside cake batter and waffles, the application of baking powder is also common for the production of Irish soda bread, which is made from wheat flour with low gluten content (Leuschner *et al.* 1997). The gas release in chemically leavened cereal matrices mostly follows a stoichiometric neutralization reaction between an acid- and a CO_2-carrier. In this context, the "neutralization value" (*NV*)

describes the quantitative effectiveness of the acid carrier. The NV is a dimensionless variable, which reflects how much gram of the acid carrier is converted by 100 gram of the CO_2-carrier (see Equation 5):

$$NV = \frac{Quantity\ CO_2\text{-}carrier}{Quantity\ acid\text{-}carrier} \times 100$$

Equation 5

As CO_2-carrier, typically, sodium bicarbonate ($NaHCO_3$) is applied. Potassium hydrogen carbonate, sodium carbonate, potassium carbonate (potash), and amorphous calcium carbonate are also used. To control or reduce the release kinetics, the reacting particles can be coated with solid fat, which starts melting during thermal treatment. For acid carriers a wide range of fast-reacting substances exists, which are categorized according to their "rate of reaction" (ROR) with the CO_2-carrier. The higher the ROR the higher the pre-release of CO_2 during the first 8 min at 27 °C in a gas-tight kneading system. The ROR is calculated based on 100% CO_2 that theoretically could be released by $NaHCO_3$. Fast reacting acid carriers are organic acids like citric or tartaric acid (ROR = 60-70), while the ROR of different types of sodium acid pyrophosphate (SAPP) ranges between 10 and 40. The gas-release of glucono-delta-lactone (GDL) during the first 8 min of kneading is up to 30%. Through the continuous cleavage of the acidifying gluconic acid from lactone, the leavening process by GDL is comparable to the fermentation by yeast and is therefore applied in the production of cool stored pizza dough (Heidolph 1996, Kulkarni and Smith 2003). Since chemical raising agents are used in soda bread and pizza dough, it appears possible to inflate further highly viscous samples like bread dough.

The **biological leavening process** by yeast and/or sourdough cultures is typically used for semi-solid foams like wheat dough. The aeration is carried out with strains of the monocellular, facultative anaerobe organism *Saccharomyces cerevisiae* (baker's yeast). The process of propagation and growth of commercial yeast occurs by budding (mitotic cell cycle) from a mother's cell. The generation time of baker's yeast takes around 90 min and is comparatively short (Scholey *et al.* 2003). The circulatory system repeats itself until a great number of propagation cycles pass or unfavorable living conditions like nutrient deficiency occur. Hence, yeast cells are subjected to aging processes, which limit the lifespan and reduce the leavening performance (Powell *et al.* 2003). Filtered air is typically introduced into the fermenter to accelerate the reproduction of yeast cells during cell propagation. Subsequently, cells are harvested

and further processed to trading forms with different storage requirements, as dictated by their water content:

(1) Resulting from yeast propagation the **liquid yeast or yeast-milk** is particularly suitable for bakery products with a high automation level.

(2) Through a subsequent process step, **compressed yeast** is produced with the use of a vacuum-rotary filter. Because of the forced dehydration, different forms of compressed yeast (pound, bar, cubic, granules) with a dry matter content of 28-33% exist.

(3) Reducing the water content to less than 10% by a fluidized bed dryer enables the production of **dry yeast** with a shelf life of up to 2 years. Dry yeasts can be distinguished in active (ADY) and instant dry yeast (IDY). ADY has larger granules and needs to be pre-dissolved in the bulk water before using, while IDY has a finer particle size and can be mixed with the dough ingredients. Emulsifiers like citric ester of mono- and diglycerides of fatty acids or sorbitan monostearate are added to improve the dispersibility of IDY.

The manufacturing process and storage requirements determine the biological activity. In this context, especially the rough conditions during the manufacturing process of dry yeast are negative factors (Bayrock and Ingledew 1997, Shima and Takagi 2009). Thus, to create and control aerated dough structures, the knowledge about the viability and vitality (physiological state) of the microorganisms is worthwhile. The viability is the proportion of living to dead cells. Traditionally, the viability of yeast is measured via dye exclusion methods, the use of flow cytometry, or the determination of colony forming units (cfu) after dilution and plating cells on a growth medium (Pierce and For the Analysis 1970, Jones 1987, Lloyd and Hayes 1995, Attfield *et al.* 2000). The physiological state can be defined as the ability of the organism to survive, propagate, and tolerate stress while retaining the possibility to ferment. In this context several yeast metabolites like intracellular adenosine triphosphate (ATP), ergosterol, unsaturated fatty acids, and glycogen are related to the activity. Moreover, intracellular pH (ICP), acidification power test, oxygen uptake rate, and CO_2-release provide information about the vital force (Mochaba *et al.* 1997).

1.3 Synthesis of structure relevant yeast metabolites

The fermentative activity of yeast results in the production of components, which influence dough and bread structure as well as aromatic sensation of the end product. The synthesis of flavor precursors and aromatic substances makes the fermentation essential for the gustatory perception of cereal food foams (Martinez-Anaya *et al.* 1990, Czerny and Schieberle 2002, Poinot *et al.* 2007, Pico *et al.* 2015). Moreover, several metabolic pathways of *S. cerevisiae* cause accumulation and excretion of dough structure defining substances, which are subjected to fluctuations during yeast and bread production (Gabrielă and Daniela 2010, Jayaram *et al.* 2014). The uncertainties in the application of yeast as leavening agent are deriving from (1) the pre-impairment of the organisms during cell propagation and further processing in the course of commercial yeast production, (2) the rarely known physiological state of the cells, (3) the substrates provided by the dough recipe, and (4) the external plus internal fluctuations during proofing (Randez-Gil *et al.* 1999).

1.3.1 Yeast metabolites emerging from the carbon metabolism

During proving *S. cerevisiae* requires saccharides for its energy metabolism. The main carbon sources in wheat flour are glucose, fructose, sucrose, and maltose, which are metabolized in a certain order. This mechanism is called glucose control. Yeast cells consume glucose or fructose in preference to other mono-, di-, and trisaccharides, while sources that require oxidative phosphorylation, such as glycerol, ethanol, or acetate, are chosen last (Kotyk , Klein *et al.* 1996, Broach 2012). Different molecular transmembrane hexose-transporters catalyze the uptake of glucose inside yeast cells. To improve cell growth and metabolism, baker's yeast possesses a system of high-, middle-, and low affine hexose transporters to adapt the glucose uptake to prevailing conditions like the glucose quantity and the presence of other hexoses (Boles and Hollenberg 1997). Subsequent to glucose uptake, degradation is initiated and bio-available energy is produced in the form of adenosine triphosphate (ATP) (Young *et al.* 2014). The nucleotide ATP is involved in signaling, energy metabolism, and nucleic acid synthesis.

During **pyruvate** production one mol glucose results in two mol of ATP and the reduced form of nicotine adenine dinucleotide (NADH/H$^+$). The latter is an essential coenzyme for redox reactions. As represented in Figure 3, which gives a summary of the most

relevant dough-structure-affecting yeast metabolites, the synthesis of pyruvate from glucose plays a key role in the production of dough relevant metabolites like CO_2, ethanol, glycerol or salts of organic acids (Pronk *et al.* 1996). The metabolic degradation of glucose to pyruvate is described by the fructose-1,6-diphosphate pathway (FDP), also termed Embden-Meyerhof-Parnas pathway.
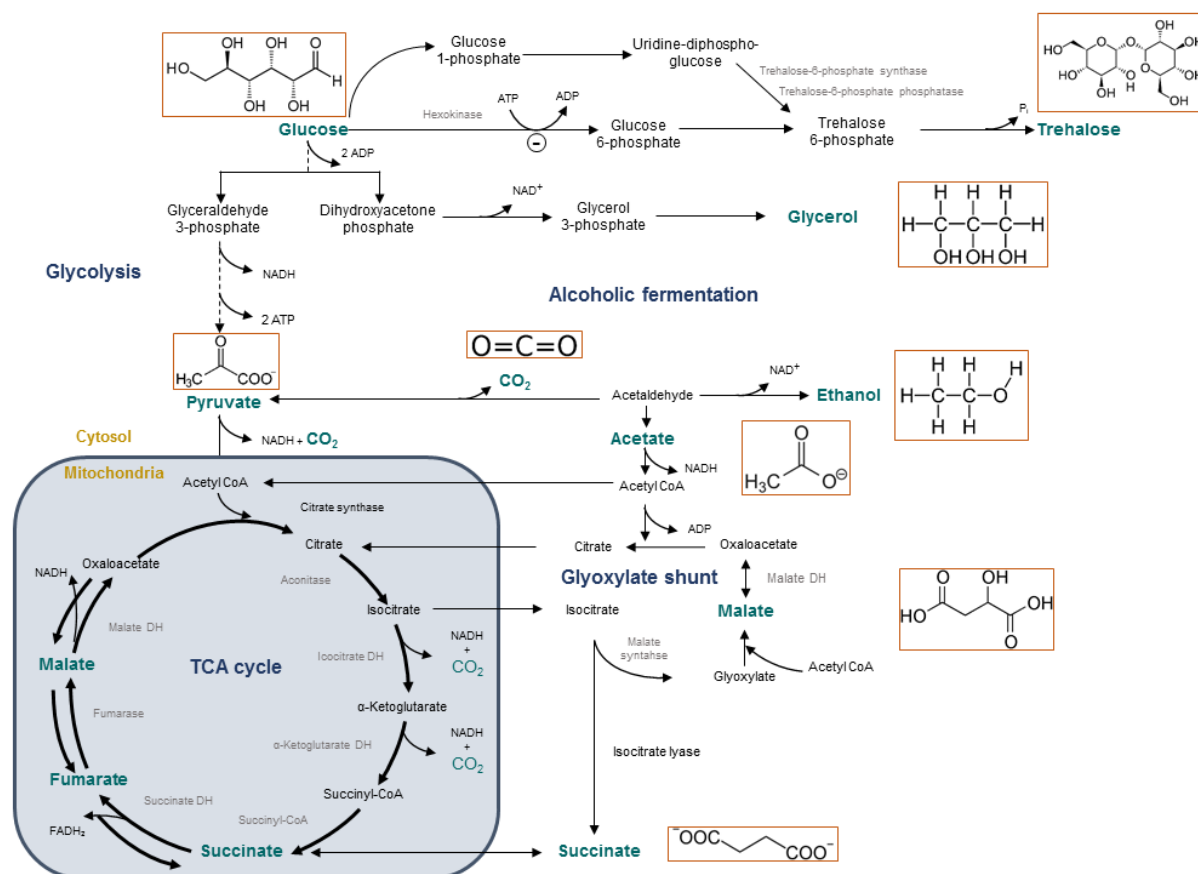

Figure 3: Synthesis pathways of relevant dough structure affecting yeast metabolites. During dough proving with yeast glycolysis, TCA cycle, trehalose biosynthesis, and glyoxylate shunt pathways occur. Not all intermediates' pathways are shown. DH: dehydrogenase; ADP: adenosine diphosphate; ATP: adenosine triphosphate; NAD$^+$: oxidized nicotinamide adenine dinucleotide; NADH: reduced nicotinamide adenine dinucleotide; P$_i$: Phosphate modified from (Gancedo and Flores 2004, Aslankoohi *et al.* 2015).

Controlled by the carbohydrate catabolism, the formation and emission of CO_2 plays a predominant role in the production of cereal-based foams. During the aerobic pathway, one glucose molecule is degraded to form six CO_2-molecules and 30 mol ATP, while the anaerobic metabolization of glucose produces two molecules of ethanol and CO_2 as well as 2 mol ATP respectively (see Equation 6):

Aerobic: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ 30 mol ATP
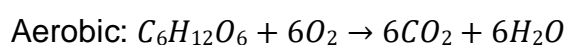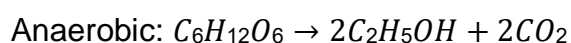
Anaerobic: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ 2 mol ATP

Equation 6

Although the respiration is energetically more favorable, the anaerobic energy release during fermentation is sufficient to maintain the yeast's cell cycle. The prerequisite, however, is a sufficient amount of nutrients like phosphates, amino acids, and minerals as well as the absence of inhibiting substances such as high quantities of ethanol (Jayaram *et al.* 2014) or pH decreasing organic acids (Graves *et al.* 2006).

In wheat dough, the oxygen incorporated during kneading is already consumed by yeast after 5 min (Joye *et al.* 2012). In the absence of oxygen, pyruvate is predominantly metabolized to CO_2 and ethanol. This also appears under aerobic conditions, when the glucose concentration exceeds a critical level of 100 mg per liter of culture medium (Crabtree-effect) (Postma *et al.* 1989). Because glucose concentration in 1 kg wheat flour is between 100 and 900 mg, the Crabtree-effect most likely occurs at the beginning of fermentation (Belitz *et al.* 2008). Thus, the anaerobic metabolism of *S. cerevisiae* prevails and equivalent amounts of **CO_2 and ethanol** are produced during the entire proofing process. However, based on carbon atoms present in different yeast metabolites after a fermentation cycle of 180 min, Jayaram *et al.* (2013) calculated the percentage of remaining ethanol as 45% and CO_2 as only 20.7% (Jayaram *et al.* 2013). The difference between ethanol and CO_2 derives from the loss of gaseous CO_2 during fermentation and preparation of the analytic. In the remainder of this study "fermentation" will be used to describe the general proofing process, since it reflects the anaerobic conditions that prevail almost continuously.

In addition, small amounts of so-called secondary yeast metabolites synthesized within the carbon metabolism and saccharides were analyzed in the dough: glycerol (11.8%), fructose (8.8%), glucose (5.6%), maltose (4.0%), succinic acid (2.3%), acetic acid (0.2%), and further "not classifiable" metabolites (0.7%). In accordance, Visser *et al.* (1995) described that in particular **organic acids** like acetate and succinate are produced when atmospheric oxygen is missing (Visser *et al.* 1995). Principally, the reductive formation of succinic acid is promoted under anaerobic conditions (Muratsubaki 1987, Raab and Lang 2011). Produced by the glyoxylate cycle or via the tricarboxylic acid cycle (TCA) and by the consumption of succinyl-coenzyme A (see Figure 3), succinic acid is primarily produced to regulate the cytoplasmic pH of yeast during alcoholic fermentation (Aslankoohi *et al.* 2015). The same applies for further salts of acids like malate, fumarate, and acetate.

Apart from Ethanol and CO_2, with 11.8% the **glycerol** levels produced by yeast in wheat dough were notably high (Jayaram *et al.* 2013). Glycerol originates from L-glycerole-3-phosphate when the phosphate group is severed within the glycerol pathway (see Figure 3). As an osmolyte of proliferating yeast cells, glycerol is synthesized by the high osmolality glycerol cascade (HOG) based on a transcriptional response to osmotic shock (Rep *et al.* 2000). The latter is typically provoked by high sugar (0.02 - 0.04 M; 3.6 – 7.2 g $C_6H_{12}O_6$ g per liter H_2O) or salt (0.4 M; 2.3 g NaCl per liter H_2O;) contents in the dough medium and leads to water loss and cell shrinking (Posas *et al.* 2000, Verstrepen *et al.* 2004, Hohmann 2009).

Furthermore, the non-reducing disaccharide **trehalose** is considered to play an important physiological function as a yeast protectant against cell death during environmental stress such as heat, freezing, dehydration and desiccation, and toxic chemicals. Glucose is transformed to trehalose in a two-step process involving the trehalose-6-phosphate-synthase-phosphatase enzyme complex (Lillie and Pringle 1980, Reinders *et al.* 1997). Consequently, trehalose accumulates as the cells enter the stationary phase to protect the organism against subsequent heat treatments. In *S. cerevisiae* trehalose levels, greater than 10% of cell dry weight were observed (Hottiger *et al.* 1987, Van Dijck *et al.* 1995). Although no direct impact of trehalose on the dough development has been proven, yeast survivability and CO_2 production are improved by the application of exogenous quantities of the storage saccharide (Huang *et al.* 2008).

1.3.2 Glutathione biosynthesis

Reduced **glutathione** (γ-glutamylcysteinylglycine, GSH) is not subjected to the carbon metabolism of *S. cerevisiae*, but can be considered as a further stress induced dough active yeast metabolite. As represented by Figure 4, the glutathione biosynthesis can be divided into two ATP-depending steps. First, γ-glutamylcysteine synthetase produces γ-glutamylcysteine (γ-GluCys) from the amino acids cysteine (Cys) and glutamic acid (Glu). In the second step, GSH is formed by GSH-synthetase, while glycine (Gly) is added to γ-GluCys (Grant 2001).
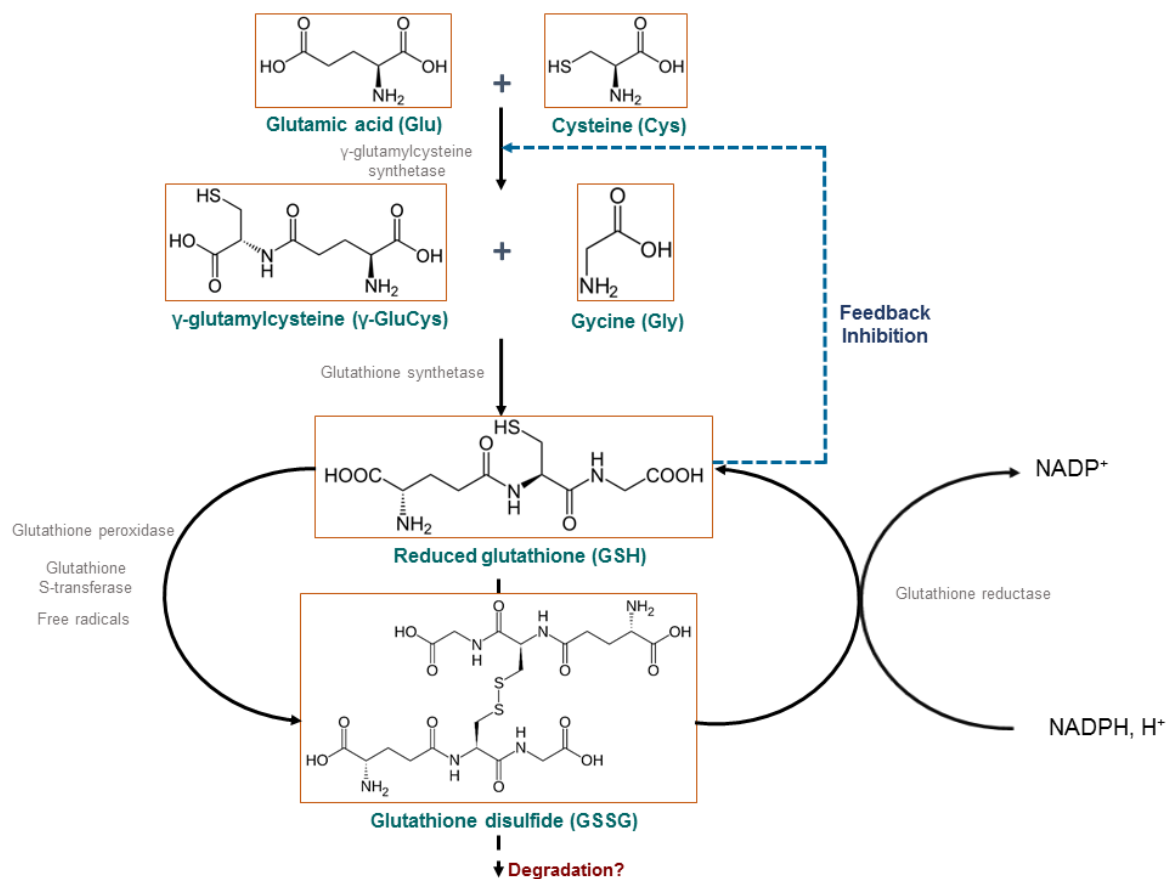
Figure 4: Biosynthesis and redox-reactions of glutathione (GSH). GSH is synthesized from the amino acids cysteine, glutamic acid, and glycine via two ATP-dependent formation steps. Initially, γ-glutamylcysteine synthetase catalyzes the formation of the dipeptide γ-glutamylcysteine (γ-GluCys) from glutamic acid and cysteine. In the second step, glutathione synthetase catalyzes the ligation of γ-GluCys with glycine (Gly). The first step in GSH synthesis is the rate-limiting step and is feedback inhibited by the end product GSH. GSH is oxidized to glutathione disulfide (GSSG) by reaction with free radicals or in reactions catalyzed by glutathione peroxidase or glutathione S-transferase. GSH is regenerated in an NADPH-dependent reaction catalyzed by glutathione reductase; modified from (Grant 2001).

Although the concentration of the tripeptide GSH in yeast cells is only 0.6-1.0%, its function as a redox system is essential to counteract xenobiotics, carcinogens, radiation or reactive oxygen species (ROS) (Grant *et al.* 1996). The antioxidant effect is premised by the reactive thiol group (-SH, sulfhydryl group) of Cys, which is able to form a disulfide bond with other thiol compounds. By the release of hydrogen atoms, free electrons become oxidized and the oxidative cell stress is diminished. A glutathione disulfide molecule (GSSG) is formed, which can be reduced into two GSH molecules with reducing equivalents from the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH) and the catalyzing enzyme glutathione reductase (Izawa *et al.* 1995). This raises the question if GSH can be referred to as a crucial indicator for rough process conditions during yeast and bread production (Autio and

Sinda 1992, Sakato and Tanaka 1992, Grant *et al.* 1996, Grant 2001). Worth mentioning here in particular are drying processes of yeast as well as freeze-thaw processes of dough.

1.4 Impact of yeast metabolites on dough structure

During dough production, the yeast metabolism is mainly anaerobic and apart from CO_2, numerous other structure relevant substances are formed. These can be categorized into stabilizing and destabilizing components. With 20-80 mmol per 100 g of flour, ethanol is the quantitatively dominating stabilizing or strengthening constituent, which exits yeast cells during bread production. It is well known that wheat grain proteins can be classified according to their solubility (Osborne 1907). In this context, ethanol (60% v/v) causes aggregation of the rather hydrophobic prolamine fraction and significantly increases the gluten yield during gluten starch separation by acting as washing liquid (Robertson *et al.* 2007). Moreover, as described by Cooper & Reed (1968), ethanol produced by yeast decreases the fermentation rate and lowers the interfacial tension of dough by partially swollen or dissolved proteins (Cooper and Reed 1968). Jayaram *et al.* analyzed the impact of yeast equivalent ethanol concentrations on descriptive rheological properties of non-leavened samples in 2014 (Jayaram *et al.* 2014). They were able to prove that ethanol does not affect the dough system in a Z-kneading system. Dough spreadability was reduced with increasing amounts of ethanol. This observation is explained by the counteraction of ethanol against proteolytic flour enzymes and is consistent with a reduced extensibility and a rise of the maximum force needed to extend dough analyzed with Kieffer-Rig. In conclusion, yeast equivalent ethanol decreased the dough extensibility and increased the stiffness. This effect was correlated with a greater tendency of the gluten particles to accumulate and was more pronounced in wheat flours with lower protein contents.

Similar to ethanol, but based on a different mechanism of action, **organic acids** like succinic, acetic, carbonic, fumaric, or malic acid enhance dough elasticity and stiffness (Clarke *et al.* 2002, Jayaram *et al.* 2014). The drop of the pH from 6.0 to 4.8 induced an increase in the positive net protein charge, which in turn increases the stiffness of the gluten structure (Beck *et al.* 2012). A stiffer and more fragile foam structure results from the swelling of gluten, more pronounced intermolecular electrostatic repulsive forces, and possibly more disulfide bridges. In contrast to acetic acid (0.15 mmol = 0.01 g per 100 g flour) and lactic acid (0.06 mmol = 0.005 g per 100 g flour), succinic

acid is, with up to 1.6 mmol, (= 1.9 g per 100 g flour) the predominant acid, which is produced during a fermentation cycle of 3h the presence of 5.3% compressed yeast (Wehrle *et al.* 1997, Jayaram *et al.* 2013, Jayaram *et al.* 2014, Rezaei *et al.* 2015). The drop of the pH is also triggered by lactic acid, which emerges however when yeast and/or flour are contaminated with lactic acid bacteria. Here too, the protein charge increases and protein folding in conjunction with a strong gluten network formation occurs (Galal *et al.* 1978). In consideration of the pH optimum of proteolytic (4.0) (Kawamura and Yonezawa 1982) and amylolytic enzymes (4.5-5.5) (Muralikrishna and Nirmala 2005) in wheat flour, the shift of the native dough pH from 6.0 to a minimum of 4.8 in the course of 3h-fermentation cycle promotes the degradation of the dough matrix by enzymatic cleavage (Jayaram *et al.* 2013).

As suggested by Liao *et al.* **H_2O_2** improves dough elasticity and increases the final loaf volume (Liao *et al.* 1998). Spread tests with 0.76 g yeast per 100 g flour demonstrated similar rheological effects compared to trials made with yeast equivalent H_2O_2 concentrations up to 2.3 µmol H_2O_2 per g flour. The dough strengthening mechanisms of H_2O_2 described by several research groups are based on oxidative cross-linking trough tyrosine residues in the gluten network (Tilley *et al.* 2001) or between ferulic acid residues in water-extractable arabinoxylans (Courtin and Delcour 2002). Bonet *et al.* (2006) also considered the reinforcement of the gluten network by the oxidation of thiol groups and formation of disulfide bonds (Bonet *et al.* 2006). Rezaei *et al.* (2015), however, negated the elasticity enhancing action of yeast equivalent H_2O_2 quantities on the dough matrix (Aslankoohi *et al.* 2015). Using the chemiluminescent HyPerBlu assay they were able show that no H_2O_2 was produced by yeast during fermentation either directly degraded by the presence of catalase in flour as well as by the fast reaction of H_2O_2 with gluten proteins. The previously reported high levels of H_2O_2 in fermenting dough as they were presented by Liao *et al.* (1998), Jayaram *et al.* (2013), and Rezaei *et al.* (2014) might have resulted from measuring inaccuracy by the applied potassium dichromate/acetic acid based method (Liao *et al.* 1998, Jayaram *et al.* 2013, Jayaram *et al.* 2014). While ethanol and organic acids are the most relevant dough stabilizing baking agents excreted by yeast into the dough, other metabolites produced during fermentation, however, cause a softening or destabilization of the matrix.

The osmolyte **glycerol**, which particularly limits dehydration of the cells during so called solid state fermentation processes (Myers *et al.* 1998), is formed in the early

phase of proofing. During a fermentation cycle of 180 min at 30 °C with 5.3 g compressed yeast per 100 g flour, 9.5 mmol (0,85 g) glycerol per 100 g flour was produced (Aslankoohi *et al.* 2015). Descriptive rheological tests made with Kieffer-Rig showed that glycerol increases the maximum dough extensibility and decreases the maximum force extension of non-leavened dough. These findings indicate a softening effect of glycerol on wheat dough and comply with the results obtained with the Rheofermentometer when exogenous glycerol was added to yeasted dough samples. Glycerol increases the gas holding capacity as well as dough height by reducing the extension force.

Carbon dioxide as gas is undoubtedly the main dough structure-affecting yeast metabolite. Before it can enter gas bubbles, it is dissolved in the water phase as carbonic acid where it acts like the above-mentioned organic acids. Through the inflation, the matrix is subjected to rheological changes, which can, in turn, influence the bubble stabilization (Gan *et al.* 1995, Campbell *et al.* 2001, Chiotellis and Campbell 2003, Chin *et al.* 2005). Only a few studies, however, were conducted to analyze the rheological properties of leavened bread dough (Casutt *et al.* 1984, Heitmann *et al.* 2015). The comparison of leavened and non-leavened dough samples illustrates that the resistance to extension decreases in the presence of baker's and brewer's yeast (Mann *et al.* 2005, Heitmann *et al.* 2015). The differences can be attributed to the biaxial elongation of individual gluten strands by the growth of gas bubbles (Sliwinski *et al.* 2004, Sroan *et al.* 2009). If the maximum expandability is exceeded, disproportionation and coalescence of gas bubbles cause a mechanical destabilization (Mills *et al.* 2003). The mechanical destabilization is represented by a loss of CO_2 and a decrease of the dough height during the fermentation process (Gobbetti *et al.* 1995). In this context, however, the impact of the gas release kinetics in conjunction with the total CO_2-volume were not considered in terms of affecting the matrix stability.

Different studies have shown that reducing agents like **glutathione** (γ-glutamylcysteinylglycine, GSH) destabilize the dough matrix by modifying the properties of the viscoelastic gluten network. The main effect on the cross-linked structure of the gluten network is the cleavage of individual disulfide bonds, which in the end leads to a weakened dough system (Tsen 1969, Li and Lee 1998, Wikström and Eliasson 1998, Lambert and Kokini 2001, Angioloni and Dalla Rosa 2007). The responsible is the reactive sulfhydryl-group of the cysteine side chain, which can

interact with another SH-group to form a disulfide bond (-SS-) or replacement with existing SS-groups. In theory, the coherence of gluten-proteins is based on the formation of disulfide bonds and to a minor extent on tyrosine interactions, which can be reduced by the free thiol group of GSH (Li and Lee 1998, Peña *et al.* 2006). In wheat flour and dough, respectively, a distinction is made between the free reduced molecule (GSH) and free oxidized form (GSSG) as well as the mixed disulfide of protein and glutathione (PSSG) (Kuninori and Matsumoto 1964, Schofield and Chen 1995, Chen and Schofield 1996). Mauritzen & Stewart (1963) have shown that most of the sulfhydryl-disulfide (SH-SS) interchange takes place during the first few minutes of kneading (Mauritzen and Stewart 1963). This is in accordance with Grosch & Wieser, who proved that the GSH content decreased rapidly during low-speed kneading while the content of GSSG and PSSG was elevated. This suggests that GSH prevents the formation of disulfide bridges between individual glutenin strands and cleaves already existing disulfide bonds. Hüttner and Wieser (2001) mixed wheat dough after the addition of ^{35}S-labelled reduced GSH to prove the specific and highly reactive cleavage of inter-protein disulfide bonds by glutathione (Hüttner and Wieser 2001). Due to the depolymerization of the gluten network, the dough structure is weakened (Lee and Lai 1969, Grosch and Wieser 1999). This weakening mechanism of GSH is especially known from its application as a baking agent for strong flours (Dong and Hoseney 1995). Similar to plasticizing effects of pure cysteine, increasing glutathione amounts showed a significant decrease of the elastic modulus G' and an increase of the loss factor tan δ immediately after kneading (Dong and Hoseney 1995). However, to what extent yeast equivalent GSH quantities effect the gluten network development has not yet been studied. Furthermore, it has to be taken into account that GSH reaches the dough medium exclusively via lysed yeast cells, since there is no active transport of the tripeptide into the liquid dough phase. In conclusion, reducing agents like glutathione diminish intermolecular disulfide bonds of flour protein aggregates like glutenin into small extractable units. Additionally, a reduced kneading time and faster dough development were observed (Tsen 1969).

The effect of yeast equivalent GSH quantities was not considered so far. Moreover, structure-affecting interrelations between GSH and CO_2 characteristics like volume and formation rate are not yet clarified. The same applies for all other yeast metabolites, whose structural impact is typically analyzed in presence of the yeast metabolism or in model dough without the presence of CO_2 (Dong and Hoseney 1995,

Nakamura and Kurata 1997). The necessary fundamental rheological data of yeasted dough samples are also missing due to the high sensitivity of the test method towards continuous changes of the dough matrix induced by the metabolism. (Newberry *et al.* 2002, Salvador *et al.* 2006). Thus, the impact of gas release characteristics, the addition of yeast equivalent amounts, as well as the interrelation between those factors are rarely taken into account.

1.5 Thesis outline

Since *Saccharomyces cerevisiae* is generally used as a tool to inflate the viscoelastic dough matrix, other intrinsic characteristics of the organism are typically not considered. However, pre-treatments as well as the resulting viability and vitality of the yeast cells modulate the biosynthesis and secretion of secondary metabolites (Randez-Gil *et al.* 1999, Gabrielă and Daniela 2010, Rezaei *et al.* 2014, Farrés *et al.* 2015). As represented by Figure 5, the trading form and possible additives, like ascorbic acid (Asc), of commercial yeast influence the descriptive dough rheology.
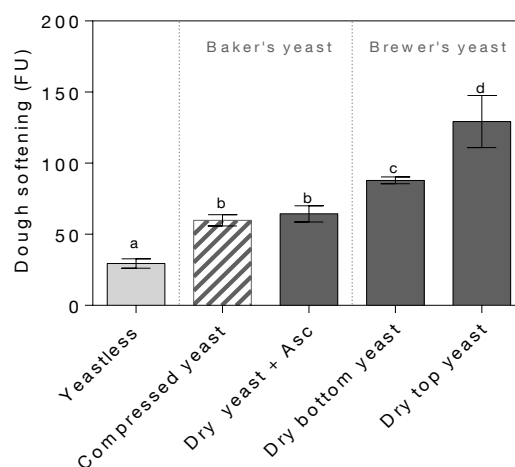

Figure 5: Impact of different trading forms of yeast on dough softening. Dough softening was measured in a Z-kneader in Farino Units (FU). All yeast types were added in a concentration of 1 g db yeast in 100 g flour. Light grey: yeastless model dough, crosshatched: compressed yeast, dark grey: different types of dry yeasts (dry yeast with ascorbic acid (Asc), dry bottom yeast, and dry top yeast). Means are shown with SD (n = 3). Different letters indicate significant differences (One-Way ANOVA, $p < 0.05$).

In comparison to compressed yeast, the application of dry yeast significantly softens the dough structure. Strengthening agents like ascorbic acid or sorbitan monostearate counteract the dough softening effect (Grosch and Wieser 1999, Gómez *et al.* 2004). The weakening effect of yeast was predominantly observed via descriptive rheological test methods (extensigraph, Kieffer-Rig, and torque-measuring Z-kneader). This,

however, does not allow for a description of the fundamental material properties, which can be gained in a rotational rheometer. However, all preceding attempts to acquire information with a controlled stress rheometer failed due to unsuitable and non-reproducible methods. (Newberry *et al.* 2002, Salvador *et al.* 2006). For this purpose, a fundamental rheological approach was developed in the present study to enable the investigation of structural changes provoked by the yeast metabolism.

Because the trading form of yeast apparently influences dough softening to a high extent, vital parameters as well as the related release of weakening yeast metabolites will be considered. Particularly, with regard to the enhanced dough softening by the application of dry yeast, glutathione (GSH) will be regarded as the main matrix disintegrating metabolite. GSH is formed as a response to extreme temperatures as they occur during dry yeast production or freeze-thaw processes of dough samples (Autio and Sinda 1992, Bayrock and Ingledew 1997) and enters the liquid dough phase exclusively via lysed yeast cells (Collins and Haley 1992). Although the weakening effect of glutathione is well known from the application as a baking agent, it is still unclear in which quantities GSH escapes from dead yeast cells into the matrix. The determination of yeast equivalent GSH quantities in compressed and dry yeast combined with subsequent structural investigations will permit the classification of GSH as a critical yeast metabolite for the breadmaking process. This initially assumes the application of GSH in non-leavened model doughs to gain general knowledge about the disintegrative effect of yeast equivalent quantities. Moreover, to provide a practical and realistic insight, the impact on leavened samples and the final loaf characteristics should be aspired. The major challenge therefore consists in analyzing the effect independently from the yeast metabolism to avoid interferences with further substances excreted by the organism.

The application of chemical raising agents presents a promising approach in light of the stoichiometric reaction between acid- and CO_2-carrier. The selection of an appropriate acid carrier, which is responsible for the release kinetics of the gas from the CO_2-carrier, will be used to inflate the viscoelastic dough matrix. This, in turn, will pose the potential to prove a dough weakening interrelation between GSH and CO_2, which is mentioned in conjunction with rough process conditions. In particular, the impairment of the gas retention capacity as well as consequences for the final loaf characteristics can be investigated in detail. In conjunction with the survey given in the

previous chapters, the dissertation will dedicate to clarify following working hypotheses:

- *Since yeast affects the material's performance during processing, it can be reasonably assumed that this is based on a shift in favor of the viscous modulus.*
- *The intrinsic character of S. cerevisiae varies depending on the trading form and accordingly the gas formation kinetics of compressed and dry yeast differ.*
- *Yeast-equivalent glutathione quantities are appropriate to depolymerize the gluten network and cause dough softening on the molecular level.*
- *There is a relevant dough structure affecting interrelation between GSH and CO_2.*

These hypotheses are closely linked to industrial problem instances, considering the production of yeast and frozen dough for "bake-off" products. The growing interest of the market in frozen bakery goods mainly derives from the economic advantage of a centralized production process and the baking-off of fresh goods adapted to the number and preferences of customers (Asghar *et al.* 2011). However, a loss of the gas retention capacity through GSH caused poor loaf volumes and strong alterations of structural properties in defrosted products (Autio and Sinda 1992, Inoue and Bushuk 1992).

Fresh yeast is predominantly applied for the production of baked goods, since its intrinsic characteristics do not substantially fluctuate. Despite the thermally induced fluctuations of the type and quantity of metabolic products, there are several benefits concerning the use of dry yeast. Trading forms with water contents below 10% enable long storage times over one year while retaining their original leavening quality. This makes dry yeast interesting for prolonged scientific studies dealing with cereal-based foams (Schirmer *et al.* 2011, Beck *et al.* 2012, Elgeti *et al.* 2014). Dry yeast, moreover, represents an alternative for the production of baked goods in warmer regions of the world, where the compliance of the cooling chain is difficult to exercise.

2 Results (Thesis Publications)

2.1 Summary of the results

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

Part I Section 2.2 Pages 29-37	Effects of *Saccharomyces cerevisiae* on the structural kinetics of wheat dough

The application of *Saccharomyces cerevisiae* presents an essential structuring process during breadmaking. However, especially the quantification of structural alterations during fermentation proves to be rather difficult. The continuous release of metabolites is considered as the main disturbing source reducing the accuracy of fundamental rheological analysis. Based on unsuccessfully performed oscillatory tests applied for leavened dough samples by other research groups, the measuring template for a rotational rheometer was adapted to fit the needs of yeasted dough. Thus, rheological data was determined during a fermentation cycle of 135 min and correlated with the pH, density, and elongation properties to deduce cause-relationships between dough structure and yeast metabolism. The adaption of the test method by using a crosshatched plate and resting phase of 10 min before measurement created the perquisites to identify the weakening impact of *S. cerevisiae* on the method independent material properties of wheat dough for the first time. By the variation of dry yeast concentration (0.0, 0.5, 1.0, 1.5, 2.0 g per 100 g of flour) it was verified that immediately after kneading, the loss factor tan δ increases asymptotically from 0.36 to 0.55. The non-linear reduction of the structural integrity indicates a limited impairment of the dough network by one or more yeast metabolites. The most likely candidates are CO_2 and glutathione whose weakening impact is based on two different mechanisms. While glutathione impairs the tertiary structure of the gluten network by preventing the formation of disulfide bridges, CO_2 possibly causes a mechanical destabilization provoked by an interaction of gas volume and release kinetics.

Part II	Impact of gas formation kinetics on dough development
Section 2.3	and bread quality
Pages 38-44	

This study aims to examine the impact of CO_2 formation kinetics and the gas volume fraction on the structural conditions of wheat dough and the resulting bread quality. By means of the Rheofermentometer, the gas release characteristics were evaluated for different colony forming units (cfu) of compressed (6.2×10^9-3.7×10^{10} cfu/100 g dough) and instant dry yeast (3.4×10^8-2.0×10^9 cfu/100 g dough). Through the integration of the gravity pressure curves resulting from fermentation cycles in the Rheofermentometer, the CO_2 volume at any fermentation time can be plotted. By fitting the sigmoidal shaped curves and determining the maximum slope of the tangent through the point of inflection, the maximum gas formation rate v_{CO2} of various yeast concentrations was observed. Despite a considerably lower number of colony forming units in instant dry yeast (IDY), the maximum formation rate of 1630 ml/h was higher compared with compressed yeast (CY: 1260 ml/h). However, the total CO_2 volume produced during the fermentation cycle of 180 min was identical for both types of *S. cerevisiae* (~1500 ml). Moreover, the time T_x when the dough matrix becomes gas permeable, was reached earlier with increasing formation rates of IDY and CY. Whereas for IDY the critical CO_2 content remained at an almost constant level between 538-564 ml, the permeability of dough samples leavened with CY was reduced to a minimum of 372 ml with rising gas formation rates. It seems that wheat dough leavened with IDY becomes more plastic and extensible so that the gas holding capacity is independent of the gas formation rate. A plausible explanation could be glutathione (GSH), which is typically associated with an increased number of dead cells in dried yeast samples. Contrary, the final bread volume of IDY leavened wheat dough was considerably lower (3.28 ml/g) than bread dough leavened with CY (3.88 ml/g). For this purpose, further investigations conduce to clarify the impact of yeast equivalent GSH quantities on the baking performance.

Part III	The contribution of glutathione to the destabilizing effect of
Section 2.4	yeast on wheat dough
Pages 45-50	

The presence of reducing agents like cysteine, reduced glutathione (GSH), and cysteine containing peptides crucially affect the wheat dough structure. To clarify the effect of yeast equivalent GSH quantities on the structural development of wheat dough, the glutathione content in 6 compressed (CY) and in 21 instant dry yeasts (IDY) was determined by a photometrical assay based on the Ellman's reagent. It was proven that the GSH content in CY ranges between 1.2 to 10.5 mg/g of yeast (dry base; db). In dried samples, however, the GSH concentration was significantly higher (5.4-81.2 mg/g yeast (db)). Based on the determined GSH content in commercial yeasts, subsequent structural analysis were performed with following quantities of the reducing agents: 0.0, 7.3, 27.8, 58.6, and 76.2 mg/g flour. Analysis in a Z-kneader showed that the dough development time as well as the stability was reduced by 67% respectively 89% by the application of yeast equivalent GSH quantities. Accordingly, the use of a rotational rheometer confirms the dough weakening effect of GSH represented by an asymptotic decrease of the complex shear modulus G* and an asymptotic increase of the loss factor tan δ, respectively. The limited weakening effect of GSH is due to the predetermined number of cleavable disulfide bonds of the glutenin chains. Finally, by the application of a chemical leavening system based on SAPP 10 (Sodium Acid Pyrophosphate) the effect of GSH on the gas holding properties was analyzed independently from the yeast metabolism. Based on the weakening coefficient it was proven that especially GSH quantities determined for dry yeast reduce the gas retention capacity up to 20.4%. However, the interrelation between GSH and different CO_2 formation rates and gas contents on the dough development and the end product quality were not considered in this study. Thus, succeeding analysis intend to clarify the interrelation of GSH and CO_2 on wheat flour based matrices.

Part IV	Destabilization of wheat dough: Interrelation between CO_2
Section 2.5	and glutathione
Pages 51-56	

Previous findings indicated a dough weakening interrelation between yeast equivalent CO_2 and glutathione (GSH) quantities. In order to exclude structural changes by the yeast metabolism, the rate and volume of CO_2 to inflate the dough matrix was applied by a chemical leavening system based on glucono-delta-lactone (GDL) and sodium bicarbonate ($NaHCO_3$). The GSH addition was based on the findings presented in part 3 (0.0, 7.3, 27.8, and 76.2 mg/g flour). Initially, the gas formation kinetics of varying concentrations of the chemical leavening system were investigated in a Rheofermentometer according to the approach in part 2. The selected concentration range of the chemical leavening system was suitable for simulating the v_{CO2} (279.0-2283.2 ml/h) reached with compressed and instant dry yeast (281.1-1632.1 ml/h). Moreover, the total gas volume in dough increased linearly with accelerated gas production. Thus, the ability of wheat dough to retain CO_2 exclusively depends on the velocity of CO_2 production and is not affected by the presence of GSH. Baking trials revealed that higher gas volume content in dough after resting also resulted in higher bread volume. Despite the positive effect of yeast equivalent GSH quantities for the aeration properties of wheat dough, the specific bread volume was impaired. The rapid bubble expansion during the first baking phase (oven rise) likely resulted in destabilization when the gluten network was weakened through GSH. The analysis confirm the weakening interrelation between yeast equivalent GSH quantities and CO_2. Especially during the transformation process from dough to crumb, the impairment of the native material properties of wheat dough became apparent.

2.2 Effects of *Saccharomyces cerevisiae* on the structural kinetics of wheat dough

Effects of *Saccharomyces cerevisiae* on the structural kinetics of wheat dough during fermentation

C. Verheyen, M. Jekle*, T. Becker

Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

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ABSTRACT

As shown in previous studies the application of yeast presents a challenge for rheological measurements. The continuously produced metabolites of this living organism strongly influence the resulting data. In the study on hand, the method for fundamental oscillatory tests was modified to fit the needs of the flow behavior of yeasted dough. The analysis of the pH-value offered a method to indirectly determine the carbon dioxide production, as was proven by a linear correlation with the density ($R^2 = 0.88$, $p < 0.001$). As evidenced by Rheofermentometer measurements, the gas holding capacity of wheat dough was not affected by the kinetics of CO_2-production, whereas the total amount of gas was decisive. Moreover, the method associated density correlated significantly ($p < 0.001$) with the resistance to extension, the extensibility and the complex shear modulus. Finally, different yeast concentrations provoked up to 57% higher loss factors immediately after kneading. In summary, it was proven that yeasted wheat dough was significantly more plastic and susceptible to pressure than the non-leavened models. In turn, this provides a practical and realistic insight into the structural changes of wheat dough as affected by the metabolism of *Saccharomyces cerevisiae*.

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1. Introduction

Wheat dough is a complex, viscoelastic mixture, whose flow and deformation behavior can be determined by fundamental and descriptive rheological methods. In this regard, both the interaction of the recipe components and the energy input during kneading are decisive for the flow behavior. The knowledge about the prevailing structural state of the dispersed system plays an important role regarding the dough's processability, the gas holding capacity in the course of fermentation and the baking performance (Janssen, van Vliet, & Vereijken, 1996).

In particular the occurring changes of gas retention forces of the dough matrix caused by the formation of CO_2 plays a crucial role during the bread making process. Thus, during the fermentation step the dough is exposed to subsequent dynamical stress factors like expansion, biaxial forces and rupture within the gas cell walls (Dobraszczyk & Roberts, 1994). Simulated by a biaxial extension test the excessive elongation of the dough network, due to the bubble growth, was confirmed (Sliwinski, Kolster, & van Vliet, 2004). Subsequently, derived by the Young–Laplace equation the

continuous CO_2-synthetization by yeast reduces the gas retention capacity of the respective dough formulation by coalescence of smaller to larger bubbles (Hilhorst et al. 1999). Consequently, the dough system becomes unstable towards external forces like pressure or vibrations. Demonstrated by (van Vliet, 1998) high strain hardening values of the gluten polymers ensure substantial resistance towards extension forces. In this context, the mechanical conditions of the dough matrix are of decisive importance.

For the analysis of the rheological and mechanical properties primarily model-dough is used. The typical formulation consists of flour, water and additives like sodium chloride without the application of active yeast (e.g. *Saccharomyces cerevisiae*) (Autio & Sinda, 1992). Despite the omission of the leavening agent, fundamental rheological values (e.g. tan δ, G' or $|G^*|$) of the model system are correlated with the loaf volume or the form ratio (H/W) of the baked goods (Autio, Flander, Kinnunen, & Heinonen, 2001; Tronsmo, Magnus, Færgestad, & Schofield, 2003; Uthayakumaran et al. 2002). Principally, the link between rheological characteristics of unleavened wheat dough and the aerated bread crumb enables a meaningful approximation of the transformation during the breadmaking process (Connelly & McIntier, 2008). Nevertheless, this approach does not enable a realistic description of the mechanical behavior of fermented dough, since important dependencies related to yeast metabolites like CO_2 are neglected.

* Corresponding author. Tel.: +49 8161713669.
  E-mail address: mjekle@wzw.tum.de (M. Jekle).

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Therefore, some fundamental rheological tests were performed on yeasted dough samples, to provide a more detailed structural analysis of wheat dough in correlation with the final product quality (Newberry, Phan-Thien, Larroque, Tanner, & Larsen, 2002; Salvador, Sanz, & Fiszman, 2006). Apparently, the sensitive measurement was disturbed by the yeast metabolism, rendering the level of reproducibility inadequate. This implies that the existing fundamental techniques have to be adapted to enable the structural investigation of leavened dough.

Therefore, in the cereal industry as well as in the scientific area a variety of descriptive test methods (e.g. farinograph, extensigraph, mixograph) are used instead of fundamental techniques. Thereby, the flow and deformation behavior can be investigated while yeast is present. As a result, a range of research projects reveal that rheological values gained with empirical methods are closely related to the breadmaking qualities of wheat flours. As an example, the rheological data obtained with the extensigraph or mixograph were positively correlated with the loaf volume (Jekle & Becker, 2011; Kenny, Wehrle, Dennehy, & Arendt, 1999; Khatkar, Bell, & Schofield, 1996; Nash et al. 2006). However, the particular disadvantages of descriptive techniques are insufficiently definable and destructive stress and strain states as well as an arbitrary selection of target figures (Dobraszczyk & Morgenstern, 2003). Summed up, these methods are unsatisfactory for an evaluation of the dynamical processes occurring through the fermentation of dough by *S. cerevisiae*.

On a limited basis the Rheofermentometer offers a solution to this challenge, since it enables dynamic monitoring. Besides the quantification of carbon dioxide and the sample volume, the gas holding capacity, respectively the collapse of the protein network in the process of fermentation, can be evaluated (Clarke, Schober, & Arendt, 2002; Gobbetti, Corsetti, & Rossi, 1995; Ktenioudaki, Butler, & Gallagher, 2010). Additionally, the knowledge of the current structural state by evaluating the density provides a useful tool to clarify the impact of evolving gas amounts on the resulting rheological data.

The primary objective of this work was the clarification of the current structural state of the dispersed dough-gas system by means of fundamental rheological measurements. Therefore, the basic prerequisite was the validation of a reproducible analysis technique. In preliminary baking tests, the amount of dry yeast was varied (0.0–2.0 g/100 g flour) to control the rate of metabolite formation in the course of fermentation. To confirm the mechanical effects of the yeast metabolites, uniaxial strain measurements were performed with SMS/Kieffer–Rig. Furthermore, the density was monitored to assess the proportion of gas during each measurement and its effect on the resulting structural data. This approach provides a substantially improved understanding about the link between the metabolism of *S. cerevisiae* and the mechanical conditions of wheat dough.

2. Experimental

2.1. Wheat dough composition

German commercial wheat flour Type 550 with 14.01 ± 0.06 g moisture/100 g flour, a protein content of 13.3 ± 0.2 g/100 g dry flour, 28.5 ± 0.4 g wet gluten/100 g flour, 0.58 ± 0.2 g ash/100 g dry flour, and 20 g ascorbic acid/100 kg flour was obtained from Rosenmühle (Landshut, Germany). As further dough components sodium chloride was selected from esco (Hannover, Germany) while dry yeast of the species *S. cerevisiae* was derived from Casteggio Liveti (Casteggio, Italy). For sample preparation demineralized water was applied.

2.2. Dough preparation

In accordance to AACC method 54-21.02 a torque measuring z-kneader (doughLAB; Perten Instruments, Germany) was used to determine the optimum water absorption and kneading time. In order to reach 500 Farino Units 60.3 g de-mineralized water/100 g flour and a kneading time of 195 s were required. Based on 50 g wheat flour (moisture content corrected to 14.00 g/100 g flour) 30.2 g de-mineralized water, 0.9 g sodium chloride and different dry yeast concentrations (0.0 g/100 g flour (model dough), 0.5, 1.0, 1.5, 2.0 g/100 g flour) were kneaded (195 s, 63 rpm, 30 °C). For the following analyses dough pieces were gently formed into smooth balls. The weight of the resulting balls was adapted to the respective method. To prevent dehydration during proofing, each sample was placed in a hermetically sealed beaker. Finally, to compare structural changes of non-leavened and yeasted dough during fermentation samples were kept in a drying chamber (Memmert UE 500; Schwabach, Germany) at 30 °C for proofing periods of $X = 0, 15, 30, 45, 60, 75, 105$, and 135 min.

2.3. Determination of the pH value of wheat dough

To analyze the impact of acidifying yeast metabolites on the flow and elongation behavior, the pH of 10 g dough balls was measured for each yeast concentration. Therefore, a testo-206-pH2 measuring instrument (Lenzkirch, Germany) was chosen, which specifically enables a rapid pH determination of viscoelastic samples. Following the previously mentioned storage times, the measurement of the pH value was performed by introducing the instrument's probe head into the dough sample. The measurements were performed as triplicates.

2.4. Elongation properties by SMS/Kieffer Extensibility Rig

Uniaxial elongation properties of yeasted and nonyeasted samples were analyzed following different proofing times using the SMS/Kieffer Extensibility Rig for the Ta.XT plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). For this purpose, each dough ball was placed into the Kieffer sample plate subsequent to the fermentation period. After 10 min equilibration time at 30 °C the distance at sample rupture (extensibility (E^K)) and the maximum peak force (resistance to extension (R^K_{max})) of five strands of each dough ball were recorded. The elongation properties are consistent with those obtained using the standard Brabender extensigraph (Grausgruber, Schöggl, & Ruckenbauer, 2002). The measurements were performed in triplicates.

2.5. Flow and deformation properties in a rotational rheometer

The fundamental rheological behavior of the dough samples were analyzed by a time-sweep oscillatory test. The according AR-G2 rheometer (TA instruments, New Castle, USA) was equipped with 4 cm parallel cross-hatched plates and a constant gap of 2 mm and connected to a smart swap peltier plate temperature system. The temperature was kept at 30 °C. The fermented dough sample was placed in the center of the stator plate. After setting the gap, excess dough edges were removed. To protect the outer dough surface against dehydration, paraffin oil was applied. Consequently, to ensure relaxation of the dough structure after handling, the rheological measurement started with an equilibration for 10 min. In the following oscillatory step a constant deformation of 0.1% was combined with a frequency of 1 Hz recording 3 measuring points. Preliminary measurements ensured a deformation in the linear viscoelastic range. The fundamental rheological properties of the dough samples were evaluated through the complex shear

modulus and the loss factor which are derived by the loss modulus G'' and the storage modulus G'. For each dough formulation the fundamental rheological measurement were carried out six times.

2.6. Gas production and dough density in Rheofermentometer

The Rheofermentometer F3 (RF) (Chopin, Villeneueve-La-Garenne Cedex, France) was employed to estimate the total CO_2-formation during fermentation (V_{CO_2} (mL)), the maximum height of the dough (H_m (mm)) and the time of porosity (T_x (min)). As illustrated in Fig. 1(A) a dough sample of 315 g (1) was placed into the fermentation basket (2) and covered with the optical sensor and an additional weight disc of 500 g. Finally, the proofing chamber was closed hermetically (3) to start the measuring series. The dough density (ρ_{RF}) was continuously deduced from the sample height without affecting its integrity. One proofing cycle lasted 135 min at 30 °C and the density was derived through Equation (1):

$$\rho_{RF(t)} = \frac{DW}{\frac{D^2}{4} \cdot \pi \cdot (H_{t=0} + H_{t=X})} \tag{1}$$

The time-depending dough density was calculated by dividing the dough weight (DW = 315 g) by the circular cylinder equation ($D = 120$ mm) with the height at beginning ($H_{t=0}$) and H_t at fixed fermentation times $t = X$. The measurements were performed in triplicates.

2.7. Determination of the method associated dough density

In addition to the analysis in the Rheofermentometer, the density of yeasted dough was analyzed during the course of the Kieffer–Rig and the rheometer measurements. Before measuring the elongation properties in the Kieffer–Rig, the density of each dough strand,

produced by the sample plate shown in Fig. 1(B) was calculated. With the aid of the top form the fermented dough ball was pressed into the troughs of the grooved base. Excessive dough was removed with a spatula, weighed and noted as "dough rest". Following an equilibration time of 10 min in the drying chamber, once again emerging dough residues as well as surplus dough strands were summed up to the previous "dough rest". According to Equation (2) the dough density in the Kieffer sample plate (ρ_{KR}) was calculated for the different proofing times ($t - X$) mentioned in chapter 3.2:

$$\rho_{KR(t)} = \frac{DW - DR_{t=X}}{H \cdot L \cdot W \cdot 5} \tag{2}$$

For one measurement dough with the initial dough weight (DW = 9.5 g) less the "dough rest" was divided into five strands which were placed into the five troughs of the bottom plate. The time-varying dough density was calculated by subtracting the DR from DW and dividing it by the total volume of the five troughs of the bottom plate ($L - 53$ mm, $W - 4$ mm, $H - 4$ mm).

In case of the rheometer analysis, the density of the dough was determined as illustrated in Fig. 1 (C). A fermented dough ball was placed between rotor and stator (1). Subsequently, the rotor-plate was lowered to a constant gap height, which guaranteed a gap volume completely filled by the sample (2). Finally, excessive wheat dough was separated by a plastic spatula, weighed and noted as "dough rest". According to Equation (3) the dough density (ρ_{RG}) in the rheometer gap was calculated for the different proofing times mentioned in chapter 3.2:

$$\rho_{RG(t)} = \frac{DW - DR_{t=X}}{\frac{D^2}{4} \cdot \pi \cdot H_{gap}} \tag{3}$$

The time-dependent dough volume was calculated by subtracting the "dough rest" (DR) from the dough weight (DW = 5.5 g)
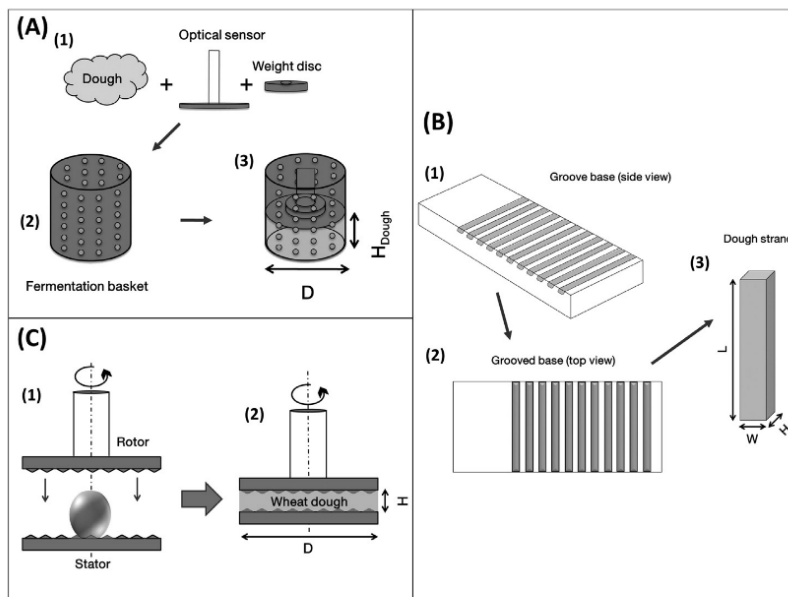

Fig. 1. Different methods to determine the density of dough. (A) Inside the Rheofermentometer. The sample (A1) was placed into the fermentation basket (A2) and covered by the optical sensor and one 500 g weight disc. The density was calculated through the continuous height alteration (A3) according to Equation (1) during the proofing cycle. (B) Inside the Kieffer sample plate (prior to an evaluation of elongation properties). Dough strands (B3) were formed in the grooved sample plate (B1-B2). The density was calculated by Equation (2) with H = height; L = length; W = width. (C) In a rheometer (prior to oscillatory tests). (C1) Rotor-stator system before positioning the gap for oscillatory tests. (C2) System after setting the gap (H = height of gap, D = diameter of rotor/stator plates) and excluding excessive dough. The density was calculated by Equation (3).

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and dividing it by the circular cylinder equation ($D = 40$ mm, $H = 2$ mm).

2.8. Statistical analysis

The statistical analysis was performed with the aid of Statgraphics Centurion (Statpoint Technologies Inc., Warrenton, Virginia). A simple linear regression analysis was used to investigate the relationship between the variables; in some cases the mathematical model was adapted. Consequently, correlations between process parameters and product values were analyzed, whereby the coefficient of determination R^2 represents the degree of correlation. To detect significant differences between dough samples a one-way ANOVA followed by Tukey-test was applied.

3. Results and discussion

The structural state of wheat dough as affected by varying amounts of yeast was analyzed by the use of empirical and fundamental rheological techniques. In addition, the pH-value, the dough density and the gas retention breakdown were monitored to support the validity of the structural conditions.

3.1. Dough pH as affected by yeast concentration and fermentation time

The dough matrix is strongly influenced by products of the yeast metabolism. In this context the yeast concentration as well as the fermentation time are crucial factors decreasing the pH through the production of acidic reagents. As illustrated in Fig. 2 the impact of acidifying yeast metabolites on dough pH was plotted for different yeast concentrations over a proofing period of 135 min. Highly significant differences between yeasted and nonyeasted dough were proven ($p < 0.001$, one-way ANOVA).

Immediately after kneading (fermentation time = 0 min) the pH of nonyeasted wheat dough was significantly higher ($p < 0.01$) than the one of yeasted samples. Moreover, increasing amounts of S. cerevisiae cause lower pH values of the dough. These findings suggest that the quantity of various acidifying metabolites diffusing into the dough medium increase with rising yeast cell amounts. The respiration products CO_2 in the form of carbonic acid (Campbell, Herrero-Sanchez, Payo-Rodriguez, & Merchan, 2001), as well as succinic acid and lactic acid lower the pH value in the liquid phase
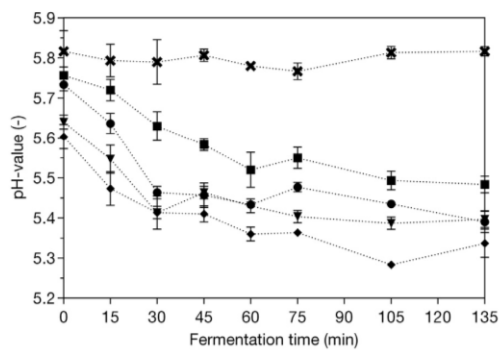
(Jayaram et al., 2013; Kati, Kaisa, & Karin, 2004; Wehrle, Grau, & Arendt, 1997). However, Jayaram et al. (2013) have proven, that lactic acid exclusively attains the dough medium through wheat flour or yeast preparations contaminated with lactic acid bacteria. In its further course the pH curve of the nonyeasted wheat dough remains at 5.80 ± 0.06 during the whole proofing period. On the contrary, dependent on the concentration, yeasted samples run asymptotically to a minimum of 5.33 ± 0.03. Hereby, increasing amounts of S. cerevisiae result in the lowest dough pH value ($R^2 = 0.88$, $p < 0.01$). As soon as the available oxygen is consumed, the pH reaches a plateau, since the organism switches to anaerobic respiration (Xu, 2001). Hence, derived from the energy metabolism of S. cerevisiae only one third of carbon dioxide is synthesized during the anaerobic fermentation. Moreover, due to the decreasing pH of the surrounding medium, organic acid production of S. cerevisiae is inhibited (Porro, Brambilla, Ranzi, Martegani, & Alberghina, 1995). A noticeable indication for the anaerobic pathway is the alcoholic smell of the dough samples with progressing fermentation duration.

3.2. Dough density as affected by the measuring device

During the course of three different dough characterization techniques, the density of yeasted dough was measured and the results were compared. While the density was monitored in the Rheofermentometer in a dynamic manner, the measurements in the Kieffer–Rig and the rheometer were invasive. In the Rheofermentometer the density development of wheat dough with different yeast concentrations during a proofing period of 135 min is illustrated in Fig. 3 (A). Highly significant differences between unleavened and yeasted samples were proven (one-way ANOVA, $p < 0.001$). As expected, the amount of gas in the yeastless dough-model, featuring a density of 1000 ± 15.2 kg m^{-3}, remained stable, except from a slight decrease between 0 and 15 min. After kneading, contracted gluten strands are able to relax, allowing the protein network to expand. The following increase to the origin density can be justified by the compression of the base plate on top of the dough sample.

The gas content of samples with yeast concentrations between 1.0 and 2.0 g/100 g flour increased asymptotically during proofing inducing a final density of 290–340 kg m^{-3}. In contrast, when using 0.5% dry yeast the plateau was not reached after 135 min. Different theories might be valid to explain the asymptotic curves. Firstly, as described for the pH-curves (Section 3.1) the metabolism alternates from aerobic to anaerobic, as soon as the free oxygen in the dough matrix is exhausted ($t = 30–45$ min). Additionally, the decreasing availability of mono- and disaccharides as substrates reduces the carbon dioxide production in the course of fermentation. The significant linear correlation between the pH value and the density in the Rheofermentometer ($R^2 = 0.88$, $p < 0.001$) confirm the assumption of the changing yeast metabolism and the associated reduced CO_2-production in the process of fermentation. Moreover, as shown in Table 1 the time of porosity T_x (0.5–2.0 g/100 g flour dry yeast: 142, 99, 60 and 49 min, respectively) emerged significantly earlier ($R^2 = 0.92$, $p < 0.01$) with ascending yeast concentrations. This point of breakage symbolizing the excess of the retention capacity was found at an average density of 311.5 ± 35.93 kg m^{-3} ($T_x = 142$: 350.52 ± 25.03 kg m^{-3}; $T_x = 99$: 318.04 ± 14.61 kg m^{-3}; $T_x = 60$: 310.55 ± 34.41 kg m^{-3}; $T_x = 49$: 284.79 ± 47.97 kg m^{-3}), with no significant differences between the samples (one-way ANOVA; $p < 0.05$). Furthermore, the retention capacity of the system is decisive for the plateau of the curves, since it limits the amount of gas which can be kept in the dough matrix. This assumption was confirmed by the negative linear correlation between yeast concentration and gas retention capacity ($R^2 = 0.92$,

Fig. 2. Effect of different dry yeast concentrations on the pH-development during a proofing period of 135 min at 30 °C. (×) 0.0 g dry yeast/100 g flour (■) 0.5 g dry yeast/100 g flour (●) 1.0 g dry yeast/100 g flour (▼) 1.5 dry yeast g/100 g flour (◆) 2.0 g dry yeast/100 g flour. Differences between yeasted and nonyeasted dough samples are highly significant (one-way ANOVA, $p < 0.001$). Data are shown as mean ± standard deviation ($n = 3$).
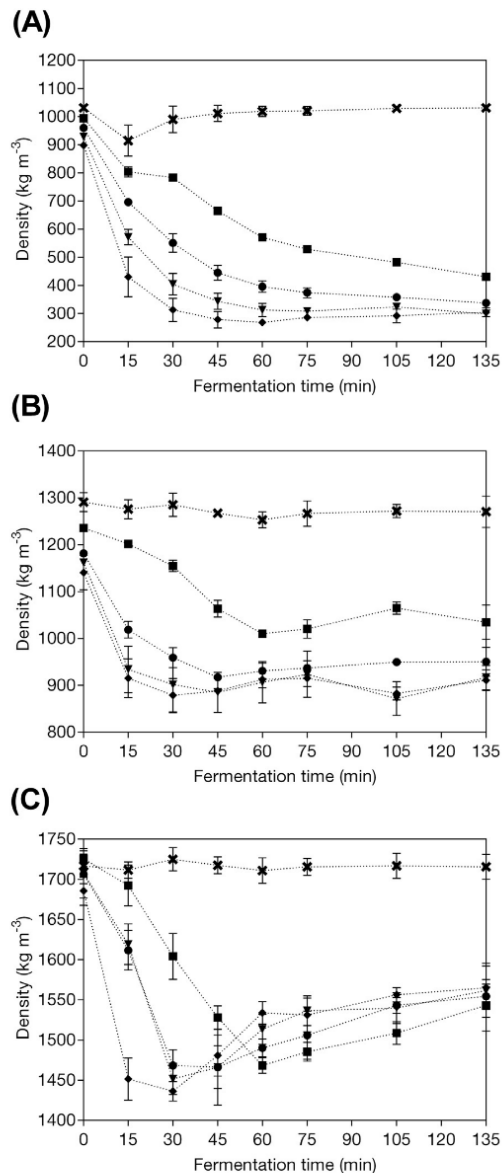
(A)

(B)

(C)

Fig. 3. Development of the dough density during proofing in different measuring systems with varying amounts of dry yeast. The proofing period lasted 135 min at 30 °C. (A) Rheofermentometer ($n = 2$) (B) Kieffer sample plate ($n = 3$) (C) Rheometer gap ($n = 6$) ($\times$) 0.0 g dry yeast/100 g flour ($\blacksquare$) 0.5 g dry yeast/100 g flour ($\bullet$) 1.0 g dry yeast/100 g flour ($\blacktriangledown$) 1.5 dry yeast g/100 g flour ($\blacklozenge$) 2.0 g dry yeast/100 g flour. Highly significant differences between yeasted and nonyeasted dough samples were proven (one-way ANOVA, $p < 0.001$). For improved resolution the range of the density scales were adapted for each method. Data are shown as means $\pm$ standard deviation.

$p < 0.01$). Consequently, the kinetics of CO_2-synthesis does not affect the gas retention capacity, which therefore is exclusively limited by the amount of gas and the stability of the dough matrix.

Prior to an evaluation of elongation properties the density was also determined inside the Kieffer sample plate during a fermentation of 135 min at 30 °C, which is shown in Fig. 3 (B). As proven for the density in the Rheofermentometer differences between the

model dough and the yeast raised samples are highly significant (one-way ANOVA $p < 0.001$) and the gas content of nonyeasted assays remained constant, provoking a dough density of 1273.3 ± 11.7 kg m^{-3}. Likewise, the curve shape of the yeasted dough samples resembles the ones of the Rheofermentometer. The density reaches a plateau of 895.2 ± 23.3 kg m^{-3} within 30–45 min with 1.0–2.0 g/100 g flour of yeast. With only 0.5 g/100 g flour yeast less gas is produced causing the minimum density to remain at a higher level of 1010.5 ± 3.6 kg m^{-3}. Here, the time to reach the plateau is elongated to 60 min. In comparison to the Rheofermentometer method, the density of the dough strands is elevated at all times because of the initial act of pressing the fermented dough into the sample troughs. Consequently, the mechanical stress causes the rupture of the gas-holding structure whereby the final density will be reached more quickly.

Prior to rheological measurements, the dough density was analyzed between the rotor and stator plate of the rheometer after fixing the dough sample. Highly significant ($p < 0.001$) differences between the model dough and the yeasted samples were found. While the density values in the Kieffer sample plate were generally higher than the ones of the Rheofermentometer, the effect is even stronger in the rheometer. Fixing the gap of the rheometer squeezes the air included through kneading as well as the carbon dioxide metabolized by *S. cerevisiae* sideways through the open area between rotor and stator. The density of nonyeasted dough remains at a constant level during fermentation, providing a density of 1714.7 ± 3.5 kg m^{-3}. This is in contrast to the findings of Chin, Martin, and Campbell (2005), who detected a density of approximately 1300 kg m^{-3} for degased dough. In addition to de-aeration through the compression, structural damage by positioning the rheometer gap might be a reason for the elevated density. The curve shapes of the yeasted models are similar to the previously described courses of the Rheofermentometer and Kieffer strands. However, the density decreases rapidly and reaches its minimum very early. For yeast concentrations of 1.0–2.0 g/100 g flour the minimum of 1436.1–1468.0 kg m^{-3} appears after 30 min of proofing and subsequently rises to a level of 1560.4 ± 25.2 kg m^{-3}. Wheat dough with 0.5 g/100 g flour dry yeast reaches the same value at the end of the fermentation, while the highest gas content causes a minimum of density after a resting time of 60 min. In comparison to the results of the Rheofermentometer and the Kieffer plates instead of forming a plateau, the density curves in the rheometer constantly rise after the distinct minimum. It seems that at a certain point of the fermentation the included carbon dioxide is steadily evading the dough matrix. The increasing gas volume during fermentation renders the dough ball more prone to external forces (Chin et al., 2005), thus the compression by fixing the measuring gap has a bigger influence on the density. The reduction of the gas holding capacity results from the altering number and size of integrated gas bubbles in the gluten network. At the beginning of the fermentation wheat dough contains numerous small air nuclei (Bellido, Scanlon, Page, & Hallgrimsson, 2006), which have a small volume compared to their circumference resulting in a high internal pressure (LAPLACE pressure). Subsequently, because of increasing CO_2 amount gas nuclei grow and merge into less numerous bigger gas filled cavities (Kokelaar & Prins, 1995). Finally, the expanded bubble size induces a biaxial elongation of the gluten strands and therefore the pressure-sensitivity of the sample increases (Sliwinski et al., 2004). Furthermore, the reasons for the limitation of gas inclusion explained before also apply for the rheometer method and contribute to the progress of the curves.

Although, the investigation of the dough density indicated method specific differences, a multiple regression analysis specified a linear relationship between the three methods ($R^2 = 0.88$,

Table 1

Linear correlation matrix of different dry yeast concentrations (0.5–2.0%) with the Rheofermentometer parameters at the time of the gas retention breakage (142, 99, 60 and 49 min). In all cases significant ($p < 0.01$) correlations (r) were found; $n = 3$.

	Dry yeast concentration	Time of porosity T_x	Density ρ_{RF}	Total CO_2-volume V_{CO_2}	CO_2-lost	Retention capacity
Dry yeast concentration		−0.96	−0.98	0.95	0.96	−0.94
Time of porosity T_x			0.98	−0.99	−0.98	0.98
Density ρ_{RF}				−0.97	−0.97	0.95
Total CO_2-volume					0.99	−0.94
CO_2-lost						−0.99

$p < 0.001$). The density ranges differ considerably when choosing techniques with varying types of applied forces. The dynamic determination in the Rheofermentometer was the least invasive method revealing the lowest density values between 1032.5 ± 0.0 and 279.3 ± 28.2 kg m^3. Similar density levels, and hence a validation of the applied method, were found by Campbell et al. (2001), who measured the dough density in xylene during a proofing time of nearly 25 min. The same xylene-method was used by Peighambardoust, Fallah, Hamer, and van der Goot (2010) to identify similar density values of up to 1210 kg m^3 for unleavened and 400 kg m^3 for yeasted wheat dough. When comparing three different methods (oil displacement, structured lightning and Rheofermentometer) for monitoring the dough density Ktenioudaki, Butler, Gonzales-Barron, Mc Carthy, and Gallagher (2009) found out that all methods produced nearly the same results. During a resting period of 45 min the density started at 1200 and ended at 400 kg m^3. As a conclusion, the most recent publications provided concordant results concerning the determination of the dynamic density.

In contrast, the density calculated for the nondynamic methods was considerably lower. The variations among the techniques can be associated to the method-linked structure deformation. In comparison, for both, the formation of dough strands and the adjustment of the rheometer gap the dough samples were deformed to fit into a standardized volume. Consequently, depending on the progress of fermentation, the measuring device was filled with alternating ratios of dough and gas. Finally, recognizable by the highest density values, the largest loss of carbon dioxide was induced by setting the gap of the rheometer. Summarized, the simultaneous acquisition of the density during a rheological measurement provides an opportunity to estimate structural conditions, thus enabling an improved interpretation and comparability of the rheological findings. In contrast the Rheofermentometer is suitable as an independent method for monitoring the changing dough-CO_2 content during fermentation.

3.3. Elongation properties of yeasted wheat dough

The resistance to extension (R_{max}^K) depending on the proofing time and the yeast concentration is shown in Fig. 4 (A). Statistical differences (one-way ANOVA, $p < 0.05$) were observed only between the curves with 0.0 g/100 g flour and 2.0 g/100 g flour yeast. After 45–75 min fermentation time R_{max}^K reaches a plateau for yeast concentrations of 1.0–2.0 g/100 g flour. However, the resistance to extension for nonyeasted samples and the lowest dry yeast concentration steadily decay throughout 135 min. Similar properties for wheat dough with 3 g/100 g flour fresh yeast during a fermentation time of 180 min were proven by Casutt, Preston, and Kilborn (1984). The authors suspected that the decreasing pH during fermentation promoted the activity of proteases and hence the weakening of the gluten structure. Likewise, the relationship between pH-value and R_{max}^K in the present case indicates pH-supported proteolytic degradation. The course of the pH value during proofing has been discussed in Section 3.1. Although the pH

of the nonyeasted sample remains at 5.8 throughout 135 min the gluten structure is weakened, since the incubation time of the proteases is elongated in the process of proofing. As described above (Section 3.2), bubble growth and the related biaxial elongation of the dough matrix are probable explanations for the reduced resistance against tensile forces of the hook. The alteration of the ratio of dough to CO_2 in favor of the gas amount (as proven by the density curve in Fig. 3 (B)) is an additional reason for the decreasing resistance.

The extensibility (E^K) of wheat dough with varying yeast concentrations and proofing durations is presented in Fig. 4 (B). During 135 min significant differences between yeast concentrations of 0.0–0.5 g/100 g flour and 1.0–2.0 g/100 g flour ($p < 0.01$, one-way ANOVA) were found. The comparison of single curves reveals an increase of the extensibility up to 45 min. Apart from the nonyeasted control sample where the extensibility increases throughout the graph each leavened variety has a maximum after which the extensibility wears off. Generally, during the resting time, the extensibility of nonyeasted dough increases and the final baking performance is improved (Anderssen, Bekes, Gras, Nikolov, & Wood, 2004). However, the CO_2-content and the corresponding internal pressure, which are subjected to the yeast amount and the storage time, soften the dough coherence. In addition, structural effects generated by pH-changes and enzymatic activities reduce the structure cohesion. Finally, the optimal extensibility depending on the yeast concentration and the proofing period as well as the transgression of the fermentation tolerance can be derived from the graph.

3.4. Fundamental rheological properties of yeasted wheat dough

The level of reproducibility of fundamental rheological measurements is reduced when S. cerevisae is present. In contrast to the techniques described in the literature, the method developed in this study enables highly reproducible results for the investigation of yeasted dough. Although previous measurements were also carried out with a controlled stress rheometer, several test settings differed from the current method. Newberry et al. (2002) reported insufficient reproducibility. They modified the measuring geometry by laminating the surface with sandpaper to prevent slipping of the sample. An equilibration time after setting the measuring gap was not considered. In the present study the standardized cross-hatched surface in combination with the equilibration time of 10 min might be responsible for higher reproducibility. Moreover, to reduce the normal force affecting the dough after setting the measuring gap, the distance between rotor and stator was set to 2 mm. Hence, the smaller gap of 1 mm applied by Salvador et al. (2006) might have reduced their measuring accuracy, in comparison.

As proven by (Petrofsky & Hoseney, 1995) and (Leroy, Pitura, Scanlon, & Page, 2010) the complex shear modulus ($|G^*|$) is highly suitable to describe the viscoelastic properties of wheat dough during oscillatory tests. Furthermore, (Jekle & Becker, 2012) demonstrated that $|G^*|$ is suitable to predict the volume of the of
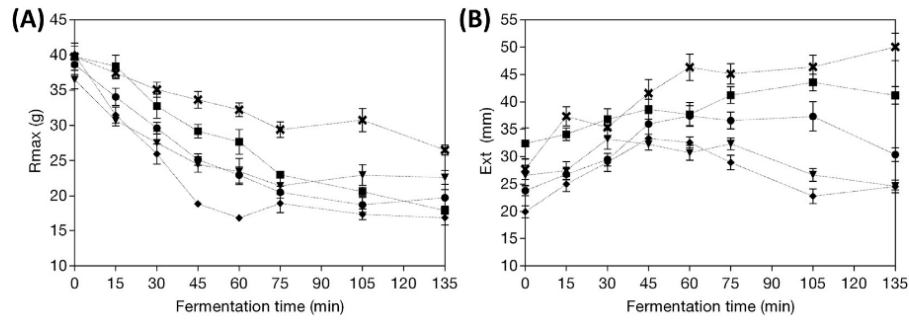
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Fig. 4. Uniaxial elongation properties of wheat dough samples with different dry yeast concentrations during proofing. Measurements were performed in a Kieffer Extensibility Rig during a proofing period of 135 min at 30 °C. (A) Maximum peak force (R^K_{max} (g)) (B) Distance at sample rupture (E^K (mm)) ($\times$) 0.0 g dry yeast/100 g flour ($\blacksquare$) 0.5 g dry yeast/100 g flour ($\bullet$) 1.0 g dry yeast/100 g flour ($\blacktriangledown$) 1.5 g dry yeast/100 g flour ($\blacklozenge$) 2.0 g dry yeast/100 g flour. For R^K_{max} significant differences between 0.0 g dry yeast/100 g flour and 2.0 g dry yeast/100 g flour were proven (one-way ANOVA, $p < 0.05$). For E^K significant differences between yeast concentrations of 0.0–0.5 g dry yeast/100 g flour and 1.0–2.0 g dry yeast/100 g flour were proven (one-way ANOVA, $p < 0.05$). Means of triplicates for 5 strands of each dough sample are shown with their standard deviation.

final baked goods. As illustrated in Fig. 5 (A) the complex shear modulus of nonyeasted models differs statistically significant from the yeasted samples ($p < 0.001$, one-way ANOVA). Besides, immediately after kneading (fermentation time $x = 0$ min) the stiffness of each sample was at its maximum. With progressing resting time each curve declined steeply during the first 30–45 min. Subsequently, in case of the yeasted samples, the complex shear modulus remained at 7–8 kPa throughout the remaining time. A plausible explanation for the increasing softness of the samples might be the cooperation of the pH-value and the gas proportion of the dough. As described by Kawamura and Yonezawa (1982), the pH-optimum of proteases is around 4.4. As shown in Fig. 2 the pH of yeasted

models approaches the pH optimum of proteolytic enzymes, though the optimum is not reached. Therefore, the pH-value as well as the incubation time are responsible for the continuously decreasing stiffness of dough through enzymatic cleavage of the gluten network (Wikström & Eliasson, 1998). The role of the gas production for the progression of the curves can be clarified by consulting the density graph in Fig. 3 (C). The plateau of the stiffness after 30 min (60 min for the lowest yeast concentration) is affected by the augmenting density between both rheometer plates. In summary, the production of carbon dioxide promotes the softening of wheat dough during the proofing period by inflating the dough matrix and decreasing the pH-value. As a result, the
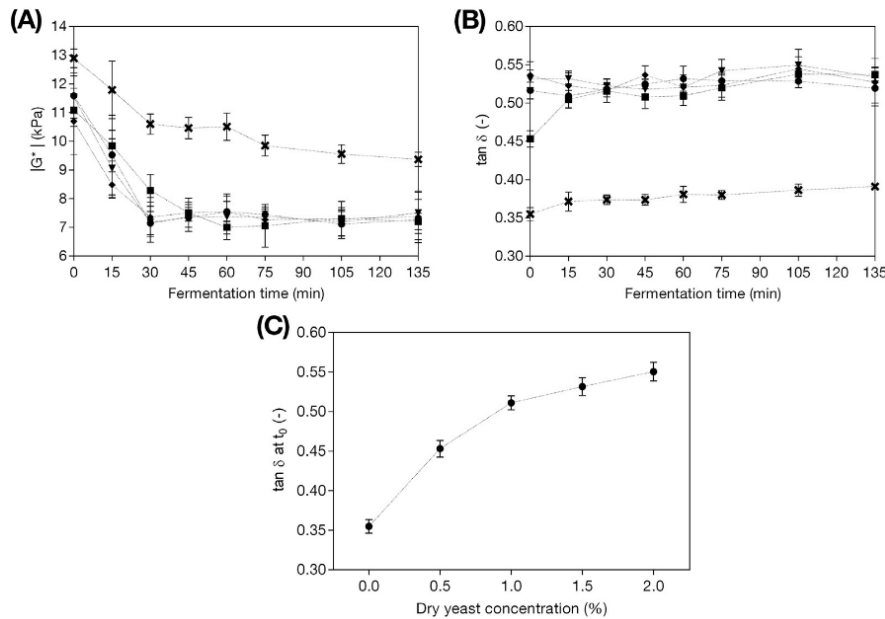

Fig. 5. Fundamental rheological properties of wheat dough samples with different dry yeast concentrations measured during proofing. Measurements at frequency of 1 Hz were performed in a rotational rheometer during a proofing period of 135 min at 30 °C. (A) Complex shear modulus G^* (Pa) (B) Loss factor tan δ (−); ($\times$) 0.0 g dry yeast/100 g flour ($\blacksquare$) 0.5 g dry yeast/100 g flour ($\bullet$) 1.0 g dry yeast/100 g flour ($\blacktriangledown$) 1.5 g dry yeast/100 g flour ($\blacklozenge$) 2.0 g dry yeast/100 g flour. Highly significant differences between yeasted and nonyeasted dough samples could be observed (one-way ANOVA, $p < 0.001$). (C) Loss factor tan δ for different yeast concentrations immediately after kneading ($t = 0$). The loss factor for yeast concentrations of 0.0–1.5 g dry yeast/100 g flour differ significantly (one-way ANOVA, $p < 0.05$). Means of each dough sample ($n = 6$) are shown with standard deviation.

application of *S. cerevisiae* reduced the complex shear modulus $|G^*|$ by 20–50%.

The loss factor tan δ has been investigated for different leavened wheat dough samples during a fermentation time of 135 min at 30 °C (Fig. 5 (B)). It was found that samples without *S. cerevisiae* differ significantly from wheat dough with different yeast concentrations ($p < 0.001$, one-way ANOVA). During the proofing period tan δ of nonyeasted dough increased by 11.0% (from 0.35 ± 0.01 to 0.39 ± 0.01) which means that due to the relaxation of the gluten network the dough structure becomes more plastic. As characterized by Dong (1992) the plasticization is caused by interchange reactions between sulfhydryl groups and disulfide bridges. As deducible from the graph, the effects of exchange reactions between cysteine residues of the protein chains apply until the end of the fermentation. Additionally, the enzymatic activity affecting $|G^*|$ of nonyeasted dough might also be valid to explain the course of tan δ. In comparison to the dough samples with yeast the loss factor of unleavened samples is approximately 50% lower throughout fermentation, while the curve of the lowest yeast concentration (0.5%) only reaches the height of the other yeast curves after 15 min. The assumption that a saturation of plasticizing metabolites occurs during the kneading process can be confirmed by Fig. 5 (C). The diagram proved significant differences ($p < 0.05$, one-way ANOVA) of the loss factor for each sample directly after kneading, except for the highest yeast concentrations 1.5 and 2.0 g/100 g flour. In this context, an agent disrupting disulfide bonds like glutathione might be the responsible impact factor (Autio & Sinda, 1992). As proven by Dong and Hoseney (1995) glutathione induces a sulfhydryl/disulfide bond interchange reaction described by a reduction of the storage modulus G'. However, with regard to the limited number of cleavable disulfide bonds of the gluten network increasing amounts of glutathione have no further effect (Grosch & Wieser, 1999). Elevated levels in glutathione would indicate a high proportion of dead cells from which the tripeptide diffuses into the dough medium. Indeed, scientific studies by Elizondo and Labuza (1974) and Bayrock and Ingledew (1997) have shown that the production process of dry yeast evokes a noticeable reduction of the cell viability. As a result, based on the drying treatment as well as the drying temperature, the viable cell concentration can be reduced by 60%.

4. Conclusion

The structure of wheat dough is decisively influenced by the leavening agent *S. cerevisiae* and its metabolic products such as carbon dioxide, organic acids and probably glutathione. Previously, the rheological characterization of yeasted wheat dough has presented a challenge. Therefore, the first goal of this work was the implementation of a reproducible rheological test to obtain the flow- and deformation behavior of leavened wheat dough. Based on this technique and improved by the combination of other dough characterizing methods the dough development during fermentation was clarified. It was proven that the acidification by yeast metabolites is related to the dough density in a linear manner and thus indirectly represents the CO_2 content ($R^2 = 0.88$, $p < 0.001$). Furthermore, the production rate of CO_2 does not affect the gas retention, which in turn is exclusively limited by the amount of gas and the stability of the dough matrix. However, due to the increasing gas amount the susceptibility to tensile and compression forces rises. This explains the discrepancy between the actual gas content produced during fermentation and the measured density in the devices. Therefore, only by investigating the method associated density, the rheological results can be interpreted correctly. Therefore, the rheological results only can be interpreted correctly by investigating the method associated density. Indeed, it was shown that the structural parameters R^K_{max}, E^K and $|G^*|$ were related to the device-depending dough density. Moreover, the use of different yeast concentrations provoked to 57% higher loss factors immediately after the brief kneading process. Most likely, the dough weakening agent glutathione, which is responsible for this finding. Furthermore it was proven that investigations carried out on yeastless models are not suitable to characterize the actual alteration of wheat dough during the proofing period. This is noticeable in the fact that the structure of yeasted wheat dough is significantly softer and susceptible to pressure than the typically analyzed non leavened models. That in turn provides a practical and realistic insight into the structural changes as affected by the metabolism of *S. cerevisiae*.

Acknowledgments

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2.3 Impact of gas formation kinetics on dough development and bread quality

Impact of gas formation kinetics on dough development and bread quality

C. Verheyen, A. Albrecht, D. Elgeti, M. Jekle *, T. Becker

Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

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ABSTRACT

The baking performance and gas retention capacity of wheat dough depends on production rate and total volume of CO_2 during fermentation. This study aims to examine the effect of the CO_2 formation kinetics and the gas volume fraction on the structural integrity of wheat dough and the resulting bread quality. The gas release characteristics were evaluated using a rheofermentometer with varied concentrations of compressed yeast and instant dry yeast (CY and IDY respectively). For this purpose, the maximum CO_2 formation rate was calculated through the derivation of hydrostatic pressure curves measured in the rheofermentometer. For CY, the time when gas escapes the dough matrix (Tx, time of porosity) as well as the corresponding gas volume depended on the gas formation rate, whereas the gas retention capacity of wheat dough leavened with IDY was not affected by the CO_2 formation rate. Although the dough leavened with IDY showed improved extensibility during fermentation, the specific bread volume was 21% below that of the samples leavened with CY. These results indicate that an increase in the maximum gas formation rate considerably affects the structural integrity of the dough matrix. Because of the reduction of the loaf volume, the effect is reinforced by the application of IDY.

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1. Introduction

As the connection between kneading and baking, the fermentation process is a decisive procedural step during breadmaking. Aeration of baked goods can generally be performed through three different approaches. Whereas physical procedures are sufficient for puff pastry, additional gas can be entrained into cake batter using chemical agents. In contrast, the production of wheat bread is typically based on a biological leavening process using baker's yeast (*Saccharomyces cerevisiae*). In this case, the substrate availability in the form of fermentable mono- and di-saccharides is essential for inflation of the dough matrix. For this purpose, CO_2 produced through the energy metabolism of the yeast initially dissolves in the liquid dough phase (Gugerli, Breguet, von Stockar & Marison, 2004). Chiotellis and Campbell (2003a,b) reported that a complete saturation of the aqueous dough phase is not mandatory for the diffusion of CO_2 into existing air nuclei during kneading. It was proved that the volume of dough samples increases before the liquid phase reaches CO_2 saturation. Thus, the inflation of the dough matrix during fermentation occurs through the growth of individual gas bubbles in the continuous gluten network.

The viscoelastic properties of wheat dough originating from the gluten proteins are critical for the expansion of gas bubbles and the retention of CO_2 in the matrix. The relation between elasticity and extensibility of the gluten network is crucial for preventing buoyancy, coalescence, and the disproportionation of gas bubbles. Gan, Ellis, and

Schofield (1995) reported that at the beginning of fermentation, gas bubbles are mainly surrounded by gluten strands. However, these strands rupture by expansion acceleration, and the bubble surface is consequently stabilized by a fine liquid film with surface active flour components such as gliadin and glutenin fractions, polar lipids, and pentosans (Gan et al., 1995).

In this context, Babin, Valle, Dendievel, Lassoued, and Salvo (2005) concluded that the gas retention capacity is less affected by the dough composition than by the present aeration status (Babin et al., 2005). In their study, computer-aided simulations of the final loaf volume revealed a linear correlation with the amount of gas in the dough samples. However, when the gas volume fraction exceeded 65%, the simulated volume of baked goods differed from that in the actual products. This result is associated with uncontrolled gas leakage when exceeding the critical CO_2 volume, which induces disruption of the membranes between individual gas bubbles. However, the influence of various gas release rates on the dough structure and the associated bread quality was not considered in their study. Furthermore, to determine the loss of structural integrity, it is necessary to clarify the proportion of the void fraction as well as yeast metabolites and flour enzymes.

In general, structural alterations can be examined by descriptive and fundamental rheological methods. Rheometer tests enable the exact determination of method independent structural dough properties (Dobraszczyk & Morgenstern, 2003). Several researchers (Newberry, Phan-Thien, Larroque, Tanner & Larsen, 2002; Salvador, Sanz & Fiszman, 2006) have referred to an inadequate reproducibility of fundamental rheological tests in the presence of yeast, although Verheyen, Jekle, and Becker (2014) were able to compensate for extensive deviations by adapting an analytical procedure. In particular, continuous CO_2

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

production by microorganisms was noted as the main source of disturbance. In contrast, the rheofermentometer enables descriptive statements regarding the structural stability and gas retention capacity of leavened wheat dough. Additional information is provided in the form of the total CO_2 volume produced during the fermentation cycle and the gas formation rate, indicated by changes in the hydrostatic pressure in millimeters. However, determination of pressure changes per unit of time does not provide a direct representation of CO_2 production in the course of fermentation.

The objectives of the present study are to monitor the evolution of CO_2 content and gas release kinetics in wheat dough, evaluate their interrelation with the structure, and analyze the changes during fermentation. The gas release curves determined using a rheofermentometer were therefore reprocessed through the derivation of direct gas release curves. By varying the yeast concentration, the impact of different CO_2-formation rates on the stability of the dough matrix and the related end product quality was studied in detail. By applying compressed yeast (CY) and instant dry yeast (IDY), the effects of yeast viability by colony forming units (cfu) and vitality (CO_2) on the fermentation characteristics and the impairment of the structural integrity of the dough matrix were determined. Finally, the dependency of bread density on the gas volume fraction in dough was evaluated. This approach substantially enhances the understanding of the CO_2-formation rate on the gas retention capacity of wheat dough and the resulting products.

2. Experimental

2.1. Determination of cell viability

For the determination of the cell viability via colony forming units (cfu), a 1 g of yeast sample was suspended in 10 ml of demineralized water. From this mixture, dilutions were prepared with Ringer solution, spread on wort-agar plates (Sigma-Aldrich, St Louis, Missouri, USA), and incubated at 28 °C for 72 h. Plates with 20–200 colonies were selected for yeast cell counts. Cell viability was calculated as the cfu per 1 g of dry yeast sample. The moisture content of all yeast samples was determined in advance by thermogravimetric analysis (Kern & Sohn, Balingen, Germany).

2.2. Wheat dough preparation

German commercial wheat flour type 550 with 14.08 ± 0.09 g of moisture, 12.23 ± 0.04 g of protein, and 0.62 ± 0.02 g of ash per 100 g of flour with a falling number of 446 ± 3.0 s was obtained from Rosenmühle (Landshut, Germany). In accordance with the American Association of Cereal Chemists (AACC) method 54-70.01, a torque measuring Z-kneader (doughLAB; Perten Instruments, Germany) was used to determine the optimum water absorption and kneading time. To reach 500 farino units, 58.6 g of demineralized water per 100 g of flour, with moisture content corrected to 14.00 g/100 g of flour, and a kneading time of 195 ± 10.4 s at 63 rpm and 30 °C were required. For baking trials, 5 kg dough was first kneaded at 100 rpm for 60 s and further 320 s at 200 rpm with a laboratory spiral kneader at 30 °C (Diosna Dierks & Söhne, Osnabrück, Germany). Additionally, different concentrations of compressed (CY) (Wieninger, Passau, Germany) or instant dry yeast (IDY) (CasteggioLieviti, Casteggio, Italy) of the species *S. cerevisiae* were applied. As explained in Section 2.1., the moisture content of both yeast types was determined to enable the dosage of equal amounts of dry matter. Thus, the following concentrations of CY and IDY are given as dry matter, and the additional amount of water provided by yeast was subtracted from the amount of water dictated by the aforementioned recipe. Thus, all dough samples contained the same amount of dry yeast and water. To 100 g of flour, 0.25–3.0 g (dry matter) CY at (6.2×10^9–3.7×10^{10} cfu/100 g of dough) or IDY at (3.4×10^8–2.0×10^9 cfu/100 dough) was applied for wheat dough preparation.

2.3. Determination of fermentation characteristics

A rheofermentometer F3 (Chopin, Villeneuve-La-Garenne Cedex, France) was applied to detect the gas release kinetics depending on the varied quantities of CY and IDY. Immediately after kneading, 315 g of a biologically leavened dough sample was placed into the fermentation basket and was covered with the optical sensor. The proofing chamber was closed hermetically to begin the measuring series at 30 °C for 180 min. Table 1 shows the obtained values.

2.4. Determination of the dough density after kneading

Campbell, Herrero-Sanchez, Payo-Rodriguez, and Merchan (2001) reported that dough density after kneading (ρ_K) was determined according to the Archimedean principle (Grant M. Campbell et al., 2001). Dough samples of (approximately 15 g) were weighed in air to 0.1 mg (resulting in m_{air}), immersed in silicone oil (VWR International, Radnor, USA) of known density ($\rho_I = 0.969$ g cm³ at 25 °C) and weighed again (m_{liquid}) using a spring balance (DIPSE SSR-Produkt, Osnabrück, Germany). On the basis of the differences in weight, the density was calculated using Eq. (1). The temperature of the liquid was controlled during the measurements to prevent density fluctuations.

$$\rho_K = \frac{m_{air}}{m_{air} - m_{liquid}} \rho_I \tag{1}$$

2.5. Determination of specific dough volume after fermentation

Alterations of the specific dough volume during fermentation were calculated by using a Rheofermentometer (Chopin, Villeneuve-La-Garenne Cedex, France). To determine the initial dough volume in the fermentation basket, dough height (h_0) after kneading was calculated by the density (ρ_K). According to the increased dough height (h_{RF}) at a given fermentation time the, specific dough volume was calculated using Eq. (2). Therefore, the sum of h_0 and h_{RF} was multiplied by the quotient of the cross-sectional area (A) of the fermentation basket and the dough weight (DW).

$$\text{Spec. dough volume} = (h_0 + h_{RF}) \frac{A}{DW} \tag{2}$$

2.6. Production and analysis of bread

After kneading 250 g of dough was placed into a loaf pan and stored in a proofing chamber at 30 °C with 80% relative humidity. The duration of the fermentation was varied for different CY and IDY concentrations according to the rheofermentometer results (Tv, T0, T1, T'1, T2, T'2, and Tx). After fermentation, baking was performed in a Matador 12.8

Table 1
Rheofermentometer values and their definitions.

Value (unit)	Definition
Tv (min)	Time to reach a defined CO_2 volume
T0 (min)	Time to reach 70% of the maximum dough height
T1 (min)	Time to reach the maximum dough height
T'1 (min)	Time to reach the maximum gas formation rate
T'2 (min)	Time to reach 90% of the maximum dough height
T2 (min)	Time to reach 90% of the maximum dough height (after the maximum)
Tx (min)	Time of porosity (gas starts to escape the dough matrix)
Hm (mm)	Maximum dough height
H'm (mm)	Maximum gas formation rate
H (mm)	Dough height at the end of measurement
Weakening coefficient (%)	$WC = \frac{Hm - H}{Hm} * 100$
V_{total} (ml)	Total CO_2 volume, which was produced during the measurement

oven (Werner & Pfleiderer Lebensmitteltechnik GmbH, Dinkelsbühl, Germany) at 230 °C with 0.5 l initial steam for 18 min. After cooling for 1 h at room temperature, the densities of four loaves per trial were determined using a laser volumeter (Volscan BVM-L 370) with a flat support plate rec. 30 × 50 (TexVol Instruments, Viken, Sweden). Baking trials were conducted as triplicates.

2.7. Statistical analysis

Statistical analysis was performed with the aid of GraphPad Prism 6 (Version 6.01, GraphPad Software Inc., La Jolla, USA) and Statgraphics Centurion (Statpoint Technologies Inc., Warrenton, Virginia). To detect significant differences between dough and bread samples, a one-way analysis of variance (ANOVA) was applied followed by the Tukey's test. A linear regression was used to investigate the relationship between dependent and independent variables. The correlations between processing parameters and product characteristics were then analyzed, whereby the coefficient of determination (R^2) represents the degree of correlation.

3. Results and discussion

3.1. Dough densities of various yeast concentrations after kneading

Relative evaluation of the gas entrapment directly after kneading was conducted for the various concentrations (0.5–3.0 g of yeast dry matter/100 g of dough) of compressed yeast (CY) and instant dry yeast (IDY). Dough density was used to assess the level of aeration, whereas the yeast concentration was calculated as colony forming units (cfu). As shown in Fig. 1, increasing the yeast amount resulted in a linear decrease of the dough density ($R^2_{CY} = 0.7975$; $R^2_{IDY} = 0.8912$) and a direct proportional relationship between cfu and dough density after kneading exists. Furthermore, it was proved that high cfu value implies a high CO_2 volume with a corresponding high gas formation rate. However, in addition to the amount of yeast, the type plays a huge role in dough density. Despite the fact that IDY has a lower cfu value than CY by approximately ten-fold, the dough density was not lower than that for CY, and the density decreased with a higher slope. Therefore, a higher yeast activity in IDY can be assumed. Campbell, Rielly, Fryer, and Sadd (1993) reported that the gas-free density of unleavened wheat dough begins at 1.25 g/ml (G. M. Campbell et al., 1993). Using this value, gas volume fractions of up to 23% for CY and up to 26% for IDY were incorporated during kneading. In comparison with non-yeasted samples in which the gas volume fraction was between 6% and 9%, the application of yeast significantly increased the gas formation during kneading.
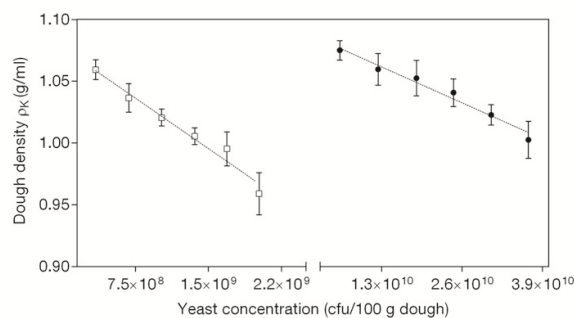

Fig. 1. Impact of compressed (●) and instant dry yeast (□) on wheat dough density after kneading. The x-axis is split at 2.5×10^9 to enable visualization of both curves at high resolution. Dotted lines represent linear regressions for compressed ($R^2 = 0.7975$) and dry yeast ($R^2 = 0.8912$). Means of measurements (n = 3) are shown with standard deviation.

3.2. Derivation of gas formation kinetics

In the Rheofermentometer the formation of gas elevates the pressure in the measurement chamber, which is expressed as hydrostatic pressure in millimeters. This measurement is repeated in 90 s intervals. The total CO_2 volume produced is given in milliliters. The proportion equation enabled the determination of the CO_2 volume in milliliters at each 90 s interval. Fig. 2A clearly illustrates the CO_2 production during the fermentation of wheat dough with CY (2.4×10^{10} cfu/100 g of flour).

To enable comparability for the various yeast cell counts, the gas formation curves were integrated as shown in Fig. 2B. Typically for biological growth processes, the result is a sigmoidal curve of the CO_2 volume (s-curve) (Qi et al., 2006). For the characterization of gas formation kinetics in wheat dough with varying yeast concentrations, the function was calculated by fitting the s-curve according to Eq. (3). The calculation of the CO_2 volume (V_{CO_2}) at the time of interest (t) is based on a function with three variables G, k and w. The maximum CO_2 volume is represented by the boundary value G, while k is the constant of proportionality, and w defines the location of the inflection point.

$$V_{CO_2}(t) = \frac{G}{1 + \exp(k*(w-t))} \qquad (3)$$

To evaluate the highest gas formation rate (v_{CO_2}) of the various yeast concentrations, the maximum slope of the tangent through the point of inflection was determined by using Eq. (4). Because of the linear increase of the s-curves at the reflection point, v_{CO_2} enables a meaningful assessment of the maximum CO_2 formation rate for varying yeast concentrations.

$$v_{CO_2} = \frac{G*k}{\exp(k(t-w)) + 2 + \exp(k(w-t))} \Rightarrow v_{CO_2} = \frac{G*k}{4} \qquad (4)$$

The CO_2 production during fermentation follows a sigmoidal function caused by growth retarding factors such as substrate availability and inhibitory substances (Chiotellis & Campbell, 2003a,b). Thus, following a lag-phase, the CO_2 formation rate increased exponentially, before running asymptotically toward zero when reaching the boundary value G. In particular, the slowdown of gas formation may be related to the limited availability of fermentable mono- and di-saccharides as well as amino acids (George, Larsson, Olsson & Enfors, 1998; Turkia, Sirén, Penttilä & Pitkänen, 2015). Although the production of CO_2 in the presence O_2 was three times higher than in an anaerobic area, limited amounts of O_2 did not contribute to the flattening of the s-curve. Jayaram et al. (2013) proved that the metabolic activity of S. cerevisiae in wheat dough is based on anaerobic conditions (Jayaram et al., 2013). This assessment was confirmed by Xu (2001) and Joye, Draganski, Delcour, and Ludescher (2012), who showed that the O_2 content in wheat dough is exhausted shortly after kneading (Joye et al., 2012; Xu, 2001).

In addition to metabolic restrictions, the presence of inhibiting substances such as ethanol can also affect cell growth (Bazua & Wilke, 1975). Maiorella, Blanch, and Wilke (1983) reported that during alcoholic fermentation, if the ethanol concentration of 70 g/l is exceeded, the cell mass is reduced by 80% (Maiorella et al., 1983). However, during 3 h fermentation only 32 g of ethanol per kg dough have been reported, which should not be responsible for cell destruction (Rezaei, Dornez, Verstrepen & Courtin, 2015). Yeast produces organic acids such as acetic, carbonic, and succinic acid which in the undissociated form diffuse into cells and reduces the intracellular pH value (Grant M. Campbell et al., 2001; Jayaram et al., 2013). Energy required for the maintenance of the intracellular pH is consequently not available for cell growth in such cases.
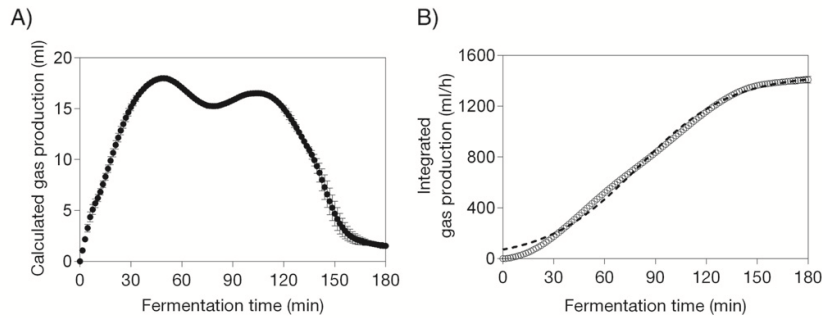
Fig. 2. Exemplary gas formation kinetics during fermentation of wheat dough. (A) Calculation of gas production in wheat dough using a Rheofermentometer with compressed yeast (2.4E+10 cfu/100 g of flour). Data are shown as means with standard deviation. (B) Integrated sigmoid function of the gas formation curve in (A) is shown by the dotted line through fitting.

3.3. Gas formation rates of various yeast concentrations

As shown in Fig. 2A and B, data of all CO_2 formation curves were reprocessed by integration and fitting. Subsequently, the maximum formation rate (v_{CO_2} in ml/h) was calculated for different cell numbers of *S. cerevisiae* as shown in Fig. 3A. It was proved that v_{CO_2} increases exponentially as the number of yeast cells increases ($R^2_{CY} = 0.9867$; $R^2_{IDY} = 0.9922$, and p < 0.001).

Confirming the results given in Section 3.1, the CO_2 formation rate correlated with only the cfu for one type of yeast sample. Considering the considerably lower amount of cfu in IDY, the formation rate was surprisingly high in comparison with CY, indicating higher yeast activity. Despite large variations regarding cfu, the yeast samples resulted in a similar total CO_2 volume (Table 2) expressed by an exponential curve progression ($R^2_{CY} = 0.9712$; $R^2_{IDY} = 0.9687$, and p < 0.001). In both CY and IDY, the maximum gas release rate ran asymptotically with increases in cell numbers. As previously mentioned in Section 3.2, yeast metabolism depends on an adequate number of substrates which is more quickly depleted with a higher number of yeast cells. Thus, acceleration of v_{CO_2} cannot be increased unrestrictedly and is limited by the substrate availability and inhibiting yeast metabolites.

The limitation of the yeast metabolism is also reflected by the asymptotic curve of the fermentation time t_{vCO_2} to reach v_{CO_2} (Fig. 3B). Although t_{vCO_2} decreased with an increase in cell numbers, a period of at least 45 min was required. Above a yeast concentration of 0.75 g of dry matter/100 g of flour, the CO_2 volume changed only slightly which is valid for both yeasts (CY = 9.29×10^9 cfu/100 g of flour respectively IDY = 5.13×10^8 cfu/100 g of flour). A higher CO_2 production rate

related to a shorter period required to reach t_{vCO_2}. Under the assumption of optimum fermentation conditions and nutrient saturation, t_{vCO_2} should depend on yeast activity and should be independent from its concentration; v_{CO_2} is always directly linked to both concentration and activity of the cells. However, influences such as inhibiting substances might influence yeast activity and cause similar CO_2 volumes despite the differences in production kinetics. Although a high level of gas production during fermentation is crucial for sufficient leavening of wheat pan bread, the loaf volume might still be impaired if the produced CO_2 cannot be stabilized throughout the production process.

3.4. Impact of gas formation rate on time and CO_2 volume at Tx

Although the previous sections have focused on the total CO_2 amount and its production rate, subsequent investigations consider the gas retention capacity of dough. Fig. 4A and B shows the impact of various gas production rates on the time of dough porosity Tx and the corresponding CO_2 volume. Above a concentration of 0.25 g of dry yeast matter per 100 g of flour, at some point during fermentation, more CO_2 was produced than the dough matrix was able to absorb. The resulting leakage of CO_2 out from the wheat dough matrix is described as dough porosity.

Because it was shown Section 3.3 that increasing cfu caused higher v_{CO_2}, it was expected that Tx would be reached earlier. As shown in Fig. 4A this was indeed the case, as Tx appeared earlier with an increase in the number of yeast cells and a corresponding higher CO_2 production rate. These results are consistent with earlier observations made by Verheyen et al. (2014), who found that the time required to reach Tx
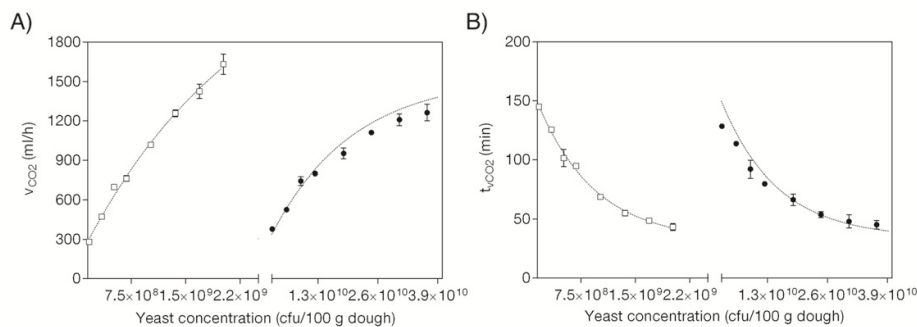

Fig. 3. Gas formation rate during fermentation depending on the yeast concentration. Wheat dough with varied concentrations of instant dry yeast(□) and compressed yeast (●). The colony forming units/100 g dough were fermented for 180 min using n a Rheofermentometer. (A) Maximum gas formation rate v_{CO_2} was determined as tangent through the point of inflection of integrated gas formation curves given by the Rheofermentometer, as shown in Figure 2. (B) Fermentation time required to reach v_{CO2} (min). Means (n = 3) are shown with standard deviation.

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Table 2
Total CO_2 production after 180 min fermentation depending on concentration, cfu and yeast type. cfu: colony forming units. CO_2 is given in ml per 315 g of dough samples, which were analyzed using a Rheofermentometer.

Type of yeast	Concentration (dry sample g/100 g of flour)							
	0.25	0.50	0.75	1.00	1.50	2.00	2.50	3.00
Colony forming units (cfu/100 g of flour)								
Compressed yeast	3.11×10^9	6.21×10^9	9.29×10^9	1.24×10^{10}	1.86×10^{10}	2.46×10^{10}	3.07×10^{10}	3.67×10^{10}
CO_2 (ml)	740 ± 30	1100 ± 10	1440 ± 11	1410 ± 30	1460 ± 0	1500 ± 10	1430 ± 10	1490 ± 40
Instant dry yeast	1.72×10^8	3.43×10^8	5.13×10^8	6.83×10^8	1.02×10^9	1.36×10^9	1.69×10^9	2.02×10^9
CO_2 (ml)	720 ± 10	1040 ± 10	1450 ± 10	1370 ± 61	1390 ± 10	1510 ± 30	1430 ± 50	1530 ± 80

was shortened with an increase in concentration (0.5–2.0 g/100 g of flour) of dry yeast (Verheyen et al., 2014).

As shown in Fig. 4B, the critical CO_2 volume for IDY remained at an almost constant level between 538 and 564 ml. This result is in accordance with that of Verheyen et al. (2014) who reported that the CO_2 volume and dough rheology, rather than the gas formation rate, are responsible for the gas retention capacity and Tx (Verheyen et al., 2014). Dough rheology depends primarily on the configuration and number of disulfide bridges (Jekle & Becker, 2015). However, glutathione (GSH) which diffuses out of dead yeast cells, splits disulfide bridges and contributes to a measurable depolymerization of the gluten network. Studies have shown that the addition of GSH reduces resistance and increases the extensibility of wheat dough. Thus, dough samples show an increased viscous behavior with loss of the elastic parts G′ (Dong & Hoseney, 1995; Hahn & Grosch, 1998). Verheyen et al. (2015) reported a significantly higher concentration of GSH in dry yeast (5.37–81.22 mg/g of yeast) in contrast with that in compressed yeast (1.16–10.51 mg/g of yeast) (Verheyen et al., 2015). Due to the fact that identical yeast samples were applied as in the study by Verheyen et al. (2015), it seems obvious that wheat dough produced with dry yeast becomes more plastical and extensible so that v_{CO_2} does not affect the gas holding capacity or Tx (Verheyen et al., 2015).

Because CY has GSH and thus less extensibility, its gas formation rate gains importance, and its effect on the critical gas volume becomes obvious (Fig. 4B). With a linear increase in the number of cfu, less CO_2 is required to increase Tx ($R^2 = 0.9134$, $p < 0.001$). A highly significant linear correlation between v_{CO_2} and the time required to reach Tx ($R^2 = 0.9653$, $p < 0.001$) was noted. Similarly, Rezaei et al. (2014) reported that the application of CY at various growth states results in a the loss of CO_2 depending on the gas formation rate (Rezaei et al., 2014). However, because of the differences in the growth states, the synthesis of further metabolites varied. In this context, the dough pH decreased down to 5.1 and the resulting reduction of the extensibility had a negative effect on the gas retention capacity (Jayaram, Cuyvers, Verstrepen, Delcour & Courtin, 2014). Furthermore, the weakening coefficient (WC) was determined using the rheofermentometer. For both CY and

IDY a highly significant linear correlation was found between WC and v_{CO_2} ($R^2_{CY} = 0.7246$; $R^2_{IDY} = 0.8179$, and $p < 0.01$).

3.5. Impact of the gas formation rate on specific bread volume

Dough was baked with different concentrations of CY and IDY after reaching characteristic Rheofermentometer times of T0, T1, T′1, T′2, T2, Tx, and Tv. The significance of the rheofermentometer data for the prediction of the baking performance was evaluated. Fig. 5A and B depicts the specific bread volume depending on the CO_2 volume after the respective fermentation duration. The highest CO_2 production during fermentation (1190 ml: density = 0.264 g/ml; 79.8% of gas) was reached with 2% of compressed yeast after 180 min, whereas the highest bread volume (3.9 ml/g; density: 0.257 g/ml) resulted from fermentation with 1.5% of yeast until reaching T1 (116 min). The specific bread volume tended to increase when more CO_2 was produced by CY during fermentation (Fig. 6A). For yeast concentrations above 1.0 g of dry matter per 100 g of flour, a critical CO_2 volume of approximately 1100 ml was noted. The additional gas likely rendered the dough matrix unstable due to disproportionation and coalescence. By progressive coalescence and the mechanical destabilization of the gluten network, CO_2 as well as evaporating water and ethanol escaped during baking and resulted in a reduction of the specific bread volume. This effect is strengthened when yeast concentration increased, however, the CO_2 volume produced with yeast amounts up to 0.5 g/100 g of flour did not appear to induce a collapse of the dough matrix.

Similarly, the specific bread volume for IDY increased with the CO_2 volume produced during fermentation until a critical destabilizing gas content of approximately 600 ml was reached. However, the impact of the dough density on the bread volume was lower. The maximum specific bread volume of IDY leavened products was approximately 3.3 ml/g, which is considerably lower than that produced with compressed yeast (3.9 ml/g). Considering that CY and IDY yielded similar ranges of CO_2 volume differences regarding the specific bread volume, this result was likely caused by the reduced gas retention capacity of dough with IDY, as discussed in Section 3.4. Although not shown in the graphs, the fact that
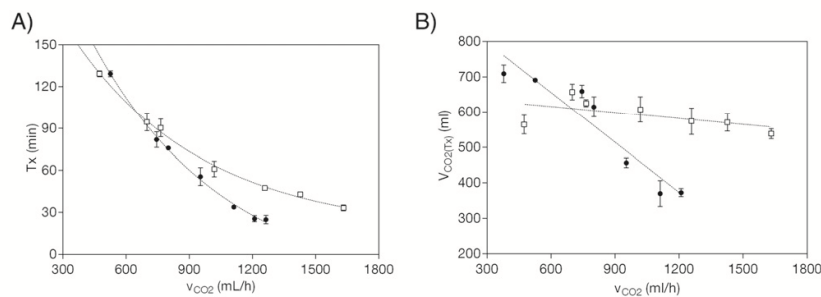

Fig. 4. Impact of gas formation rate on time of porosity (Tx) and the corresponding CO_2 volume during wheat dough fermentation. Wheat dough with various concentrations of instant dry yeast (□) and compressed yeast (●) (cfu/100 g dough) was fermented in a Rheofermentometer for 180 min. Various gas formation rates resulted from different yeast concentrations. (A) Time to reach dough porosity (Tx) depending on the gas formation rate v_{CO_2} for compressed ($R^2 = 0.9922$) and instant dry yeast ($R^2 = 0.9886$). For each graph, an exponential curve fitting (one phase decay) was applied. (B) Impact of CO_2 volume (ml) at Tx for various gas formation rates v_{CO_2} for compressed ($R^2 = 0.8977$) and instant dry yeast ($R^2 = 0.3383$). Means (n = 3) are shown with standard deviation.
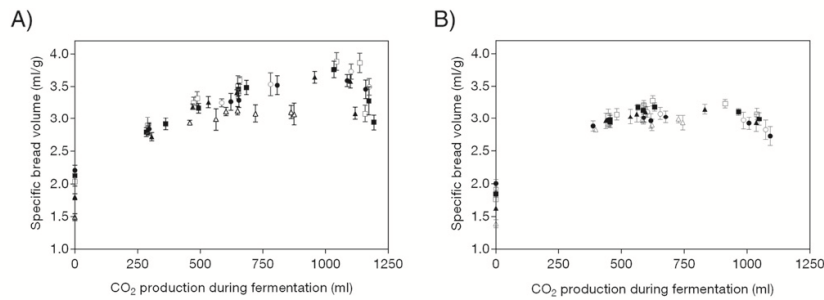
Fig. 5. Specific bread volume depending on CO_2 production during fermentation. (A) Compressed yeast (△ 6.2E09, ▲ 1.2E10, □ 1.9E10, ■ 2.5E10, ○ 3.1E10, and ● 3.7E10 cfu/100 g of flour). (B) Instant dry yeast (△ 3.4E08, ▲ 6.8E08, □ 1.0E09, ■ 1.48E09, ○ 1.7E09, and ● 2.0E09 cfu/100 g of dough). The CO_2 content of dough samples was determined using a Rheoefermentometer and was recalculated for dough samples of 250 g. The measuring points for each concentration and yeast sample correspond to the respective fermentations times resulting from the Rheofermentometer parameters (T0, T1, T'1, T2, T2, Tx, and Tv). Means (n = 3) are shown with standard deviation.

the Rheofermentometer data could not be used to predict the specific bread volume.

3.6. Impact of the specific dough volume on the oven rise

Although Section 3.5 evaluated the influence of the dough density on the specific bread volume, the respective oven rise is displayed in Fig. 6A and B. The oven rise of loaves was calculated by subtracting the specific dough volume from the specific bread volume. Due to a likely increase in the gas-free density of the surrounding matrix through water evaporation during baking, a negative oven rise does not necessarily mean that gas was lost. Again, different yeast types (IDY and CY) and concentrations were used, and fermentation was stopped after characteristic times (T0, T1, T'1, T'2, T2, Tx, and Tv). When using CY (Fig. 6A) an increase in initial dough volume connected to a decreasing final loaf volume. Furthermore, because of substrate limitation and inhibiting substances, the stimulation of the yeast metabolism by the temperature increase was lower when using higher concentrations. At the beginning of the baking process, it was expected that samples with cfu values contained more gas and higher loaf volumes because baking causes an expansion of CO_2 and an evaporation of water and ethanol. Thus, the dough weakening interrelation between mechanical destabilization and enzymatic activity might have counteracted the positive impact of a higher initial gas content of the dough samples. Moreover, it appears that at an identical initial dough volume, an increase in yeast concentration caused a higher volume growth during baking. A shortened fermentation time and an increased gas formation rate at beginning of the baking process appeared to improve the stability of the dough

matrix. Conversely, when using the lowest yeast concentrations of 0.5%, the fermentation period required to reach the same total gas volume was three times longer in comparison with concentrations of 2.5% or 3%, and the loaf volume was significantly lower at 3.1 ml/g rather than 3.5 ml/g. The extended fermentation might have intensified the enzymatic degradations, resulting in thinner lamellae between gas bubbles. This combined with the oven rise could have allowed more gas to escape during baking.

Similar to that shown for CY, the oven rise of IDY samples was reduced by increasing the initial specific dough volume (Fig. 6B). Furthermore, the dough weakening effects of IDY are reflected by lower values as well as negative values of oven rise. The high quantities of dead cells resulted in an increase of GSH, which reduced the gas holding capacity with an impaired specific loaf volume for all dough samples exceeding a specific volume of 3.0 g/ml. Moreover, because of the thermal pre-treatment during IDY production, oven rise depended on the pre-impairment as well as the availability of cell-protecting metabolites such as trehalose and chaperones (Verghese, Abrams, Wang & Morano, 2012). However, because of the reduced final specific volume, the production process of IDY showed a sustainable negative impact on the gas formation rate during baking.

4. Conclusions

The morphological structure of wheat dough is considerably affected by the volume and the production rate of CO_2 during fermentation. In the presented study, dough densities after kneading depend on the type of yeast and the number of colony forming units (cfu). A formula
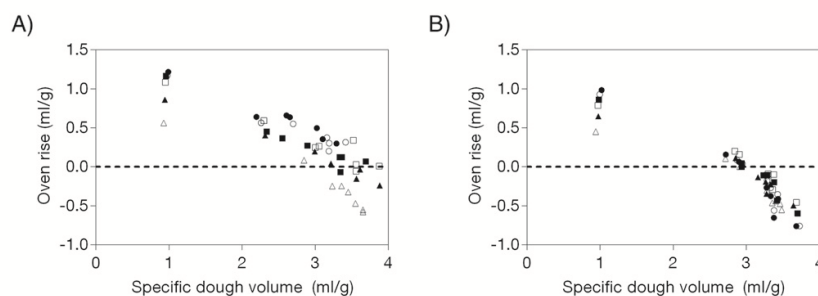

Fig. 6. Impact of the specific dough volume (ml/g) on the oven rise (ml/g). Dough samples were fermented with various concentrations of (A) compressed yeast (△ 6.2E09, ▲ 1.2E10, □ 1.9E10, ■ 2.5E10, ○ 3.1E10, ● 3.7E10 cfu/100 g of flour). (B) Instant dry yeast (△ 3.4E08, ▲ 6.8E08, □ 1.0E09, ■ 1.48E09, ○ 1.7E09, ● 2.0E09 cfu/100 g of dough). The specific dough volume was calculated using Eq. (2). The measuring points for each concentration and yeast sample correspond to the respective fermentations times resulting from the Rheofermentometer parameters (T0, T1, T'1, T'2, T2, Tx,and Tv).

was developed to determine the gas formation rate at any time of interest during fermentation. The resulting curve was sigmoidal, as typically found for biological growth processes. More colony forming units provoked higher CO_2 content and faster gas production, but at the same time the dough became more porous. However, above 0.75% of yeast saturation occurred.

After kneading, the gas volume fraction varied from 23% with compressed yeast to 26% with instant dry yeast. By fermenting, the gas level rose to 83% for both yeast types. Although great variations were noted in the concentrations of colony forming units between compressed and dry yeast were found, the fermentation behavior was surprisingly similar despite the fact that dry yeast appeared to be more vital. However, it appears that manufacturers have been able to standardize both products well, despite great variations in their production processes. Moreover, previous studies have indicated that IDY contains large amounts of GSH, which is known to impair dough structure. In fact, irrespective of yeast concentration or duration of fermentation, only small variations in the final bread volume were detected with IDY; the maximum value, 3.28 ml/g, was considerably lower than with CY at 3.88 ml/g. A promising approach to reveal further insight into the interrelation between yeast metabolites and baking performance might be achieved through substitution of yeast by a chemical raising agent. The stoichiometric reaction of leavening acids and sodium hydrogen carbonate enables a controlled production of CO_2 in which functional yeast metabolites can be analyzed independently.

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2.4 The contribution of glutathione to the destabilizing effect of yeast on wheat dough

The contribution of glutathione to the destabilizing effect of yeast on wheat dough

C. Verheyen, A. Albrecht, J. Herrmann, M. Strobl, M. Jekle [*], T. Becker

Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

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ABSTRACT

Any factor which impairs the development of the gluten network affects the gas retention capacity and the overall baking performance. This study aimed to examine why rising yeast concentrations (*Saccharomyces cerevisiae*) decrease the dough elasticity in an asymptotic manner. Since in 27 commercial fresh and dry yeasts up to 81 mg glutathione (GSH) per 1 g dry sample were found. Through the addition of reduced GSH in dough without yeast, the extent of dough weakening was analysed. Indeed rheological measurements confirmed that yeast-equivalent levels of GSH had a softening effect and during 3 h fermentation the weakening coefficient increased from 0.3% to 20.4% in a Rheofermentometer. The present results indicate that free –SH compounds, as represented by GSH, considerably contribute to the softening of dough through dead yeast cells.

1. Introduction

The production of leavened wheat dough requires the presence of a raising agent like yeast e.g. *Saccharomyces cerevisiae*. While the production of CO_2 is the main function of the raising agent, the microorganism additionally metabolizes several dough characterising organic compounds (acids, aroma precursors, reducing agents) which define the quality of the baked goods (Autio & Mattila-Sandholm, 1992; Hansen & Schieberle, 2005; Procopio, Krause, Hofmann, & Becker, 2013; Jayaram, Cuyvers, Verstrepen, Delcour, & Courtin, 2014). Yeast requires saccharides and amino acids as carbon and nitrogen sources as well as various minerals and vitamins (John, Nampoothiri, & Pandey, 2007; Krause, Birle, Hussein, & Becker, 2011).

In dough neither the activity nor the metabolism is a reliable constant. As a living organism yeast is able to adapt to varying environmental conditions. However, the composition and the processing parameters essentially influence the rate and kind of metabolic activity during the production of bread dough. Besides the dependency of the nutritional composition of the medium, the amount of cell stress which occurs during the yeast production and the breadmaking process is of a great importance. These forms of stress can be divided into four groups: critical temperature ranges, hyperosmolarity, oxidation and the type of proofing (interrupted and retarded fermentation) (Attfield, 1997). During the breadmaking process especially hyperosmolarity (freeze-thawing) as well as critical temperatures (baking) increase the level of cell stress.

In advance, also during the manufacturing of yeast products like liquid, compressed or dry yeast, different levels of thermal and oxidative stress take effect. While the liquid form originates from cell propagation, an additional step of dehydration in a rotary-filter is required to produce compressed yeast with a final water content around 70%. The loss of vitality and viability during storage (Ebbutt, 1961) as well as the necessity of a cooling chain (7–10 °C) are the motives for the production of long-life (>1 year) dry yeast forms. For this purpose the water content of the yeast-water solution can be concentrated by spray (100–150 °C) or fluidized-bed drying (25–110 °C) to a value below 10%. These drying processes are particularly suitable for the production of great yeast quantities, while the gentler freeze drying is less economic for the application in baked goods. An important quality factor of dry yeast is its ability to return into the vegetative state after rehydration.

Stressful conditions during processing result in an accumulation of cell protecting metabolites, such as trehalose, heat shock proteins (chaperones), polyamines, glutathione and glutathione reductase (Grant, MacIver, & Dawes, 1996; Attfield, 1997; Jamieson, 1998; Hong, Han, Snyder, & Choi, 1999; Tulha, Lima, Lucas, & Ferreira, 2010; Auesukaree et al., 2012; Mahmud, Hirasawa, Furusawa, Yoshikawa, & Shimizu, 2012). Especially spray drying can result in high thermal and oxidative stress levels.

* Corresponding author. Tel.: +49 8161 71 3669.
  E-mail address: mjekle@tum.de (M. Jekle).

Regardless of the amount of accumulated cell protectors, excessive stress can lead to cell death (Bayrock & Ingledew, 1997). Since the membrane of dead yeast becomes porous, all metabolites can exit the cell and enter the surrounding medium. Thus, substances such as glutathione, which are not able to pass intact membranes are now able to influence the rheology and development of dough.

The genetic background of the yeast may dictate its stress tolerance. Exemplarily, Stephen and Jamieson (1996) have shown, that two genetically deviating species of S. cerevisiae possess different adaptive responses when exposed to H$_2$O$_2$ and menadione. Intracellular glutathione (GSH) levels varied between 28.7–39.3 and 0.36–0.44 µmol per 10^6 cells, whereby the mortality rate was significantly higher for lower GSH quantities (Stephen & Jamieson, 1996). The same applies for further cellular protecting metabolites like trehalose and glycerol, whose concentration depends on the type and level of stress as well as the genome (Attfield, 1997).

Accordingly, different studies have shown that reducing agents like glutathione (γ-glutamylcysteinylglycine) affect the dough by modifying the properties of the viscoelastic gluten network (Kieffer, Kim, Walther, Laskawy, & Grosch, 1990; Chen & Schofield, 1996). As reported by Wieser (2007) the stability of the protein network decisively depends on the number disulphide bonds (Pr-SS-Pr), although only 2% of the amino acids in gluten proteins consists of sulfhydryl groups (Wieser, 2007). The main effect of GSH is the replacement of existing Pr-SS-Pr between gluten proteins by sulfhydryl–disulphide interchanges (Kuninori & Sullivan, 1968). Disulphide bonds are cleaved (PrSH) and the polymeric structure of the gluten network is affected which weakens the dough and finally impairs the baking performance (Li, Tsiami, Bollecker, & Schofield, 2004; Joye, Lagrain, & Delcour, 2009). In the course of the chemical reaction the GSH molecules are oxidised to glutathione-disulphide (GSSG) which has no effect on dough rheology. Thus commonly oxidants like ʟ-threo-dehydroascorbic acid are added to flour or dough in order to prevent the dough weakening effect (Sarwin, Laskawy, & Grosch, 1993). Although Verheyen, Jekle, and Becker (2014) used wheat flour supplemented with ascorbic acid, they reported an asymptotic plasticization of wheat dough when increasing the concentration of dry yeast (Verheyen et al., 2014). An association between the proportion of porous dead yeast cells and the concentration of glutathione in the flour-water mixture during kneading has been assumed. In this context Stewart and Mauritzen (1966) reported that, most of the sulfhydryl–disulphide interchange takes place during the first few minutes of kneading (Stewart & Mauritzen, 1966).

The primary objective of this study was the elucidation of the contribution of yeast equivalent glutathione (GSH) concentrations on the structural disintegration of wheat dough. Therefore, the content of glutathione in 27 commercial fresh and dry yeasts was analysed by a photometric analysis with Ellman's reagent and linked to the proportion of dead cells. Consequently, the rheological impact yeast equivalent quantities of these compounds was analysed by adding glutathione as their most important representative. The structural effects were analysed in a torque-recording Z-kneader and a rotational rheometer. Finally, chemically leavened model dough was used to monitor the impact on gas retention. This approach provides a substantially improved understanding about the contribution of glutathione to the softening of the wheat dough structure when adding S. cerevisiae.

2. Experimental

2.1. Quantitation of free glutathione in S. cerevisiae

The water soluble content of glutathione (GSH) was determined by a photometrical assay based on the reaction of free –SH compounds with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB) as published by Anderson (1985). The method eliminates free –SH in the form of cysteine containing polypeptides by protein precipitation. However, in addition to glutathione also small cysteine containing peptides as well as cysteine itself are influencing the result. Nevertheless, the result was expressed as glutathione, as was done by various other authors (Tietze, 1969; Alfafara et al., 1992; Dworeck, 2009; Mustafa et al., 2010). The resulting product is yellow and absorbs at a wavelength of 412 nm. The following six fresh yeast samples were analysed: DHW, Dr. Oetker, Rapunzel, Uniferm, Wonnemeyer, Wieninger (Germany). In the case of dry yeast the following 15 different products were chosen: Backfee, Baker's Corner (highly active), Baker's Corner (all natural), Belbake, Dr. Oetker, Engedura, Fermipan Red, Küchle, Lucullus, PUDA, Real Bio, Real Quality, RUF, Vitam, Werz. In addition, for evaluating variances within the same manufacturing conditions, samples with two different batch numbers were compared concerning the dry and fresh yeasts from Dr. Oetker, Fermipan Red, PUDA, Real Bio, Real Quality and Wonnemyer. In total 27 different samples were analysed. For being able to compare different yeast samples, the moisture content was analysed prior to the quantification GSH according to AACC-method 44-19.01. To 1 g dry matter of yeast 10 ml demineralised water were added and the mixture was shaken for 5 min. After centrifugation with a Hettich UNIVERSAL 320/320R Centrifuge (4000g, 4 °C, 30 min) the supernatants of two consecutive washing steps were united.

To precipitate proteins 500 µl of the supernatant was mixed with 250 µl 5% sulfosalicylic acid (5 g sulfosalicylic acid dihydrate (Carl Roth, Karlsruhe, DE) dissolved in 100 ml demin. water). After 5 min incubation at −18 °C proteins were spun down at 3000 g for 10 min at room temperature. Finally, 200 µl of the sample solution were transferred into a cuvette and supplemented with 1760 µl PBS buffer (0.1 M, pH 7.4, with 0.1 mM EDTA, Carl Roth, Karlsruhe, DE) and 40 µl Ellman's reagent (1.5 mg DTNB (Carl Roth, Karlsruhe, DE) per ml PBS buffer). After stirring and 30 s incubation, the extinction of the mixture at 412 nm was measured in a photometer against a blank (buffer + Ellman's reagent). To minimise oxidation preparations as well as measurements were carried out in sealed tubes with the lowest possible head space. Each measurement day a standard curve was prepared with 17 concentrations (5–700 mM) of commercial GSH extracted from S. cerevisiae (Carl Roth, Karlsruhe, DE). The content of free –SH groups was calculated with the resulting linear equation and all measurements were performed in triplicates.

2.2. Determination of yeast viability

To analyse the viability of yeast cells, the staining agent methylene blue (Carl Roth, Karlsruhe, Germany) was applied. Equal parts of a yeast suspension (1 g yeast/100 ml demineralised water) and a methylene blue solution (0.02 g methylene blue and 4 g tri-sodium citrate (both were obtained from Merck, Darmstadt, DE)/100 ml demineralised water) were pipetted into a Neubauer-improved counting-chamber. With the aid of a light microscope (40× magnification) a picture of the chamber was taken directly after 2 min incubation. The number of blue- (dead) and non-coloured (living) yeast cells was counted on the picture. Thus, the influence of the toxic staining agent on the viability of the yeast cells was kept to minimum. To calculate the proportion of dead yeast cells the sum of dead cells was divided by the total cell count and expressed as percentage. The measurements were performed in triplicates.

2.3. Wheat dough preparation

German commercial wheat flour Type 550 with 13.74 ± 0.14 g moisture, 10.32 ± 0.02 g protein, 65.0 ± 1.4 g starch and

0.56 ± 0.1 g ash per 100 g flour was obtained from Rosenmühle (Landshut, Germany). To 100 parts wheat flour (moisture content corrected to 14.00 g/100 g flour) 58.8 parts de-mineralised water and different concentrations of commercial reduced GSH, extracted from *S. cerevisiae* (Carl Roth, Karlsruhe, Germany) were added and 172 s kneading was performed in a Z-kneader (dough-LAB; Perten Instruments, Germany) at 63 rpm and 30 °C. The dosage of GSH was calculated to equal the concentration of free SH-compounds which were previously quantified in the respective yeast samples: to simulate 1 g of dry matter yeast per 100 g flour (moisture content corrected to 14.00 g/100 g flour) 7.3, 27.8, 58.6 or 76.2 mg GSH were used. No GSH was added to the model dough.

2.4. Dough water absorption and structural kinetics

In accordance to AACC method 54-21.02 a torque measuring Z-kneader (doughLAB; Perten Instruments, Germany) was used to determine the optimum water absorption and structural kinetics. The development time represents the duration to reach the maximum (torque) of the curve, while dough stability describes the period of time between exceeding the 500 FU-line and the first drop under 500 FU. Finally, the term "softening" is used to describe the decrease of the consistency measured 12 min after reaching the maximum of the kneading curve. In order to reach 500 Farino Units 58.8 g de-mineralised water and a kneading time of 172 s ± 18.2 s were required for 100 g flour. The measurements were performed in triplicates.

2.5. Determination of fermentation characteristics

A Rheofermentometer F3 (Chopin, Villeneuve-La-Garenne Cedex, France) was applied to detect the gas production and the gaseous release characteristics with and without yeast. Therefore, dough was prepared according to Chapter 3.3 with 300 g flour. For aeration either 1.5 g dry yeast or 9.9 g SAPP 10 (Sodium Acid Pyro Phosphate; kindly provided by Chemische Fabrik Budenheim, Budenheim, Germany) and 7.2 g NaHCO$_3$ were used. The amount of the chemical leavening agent was chosen to produce the same amount of gas as yeast (determined in preliminary trials). Bicarbonate was added after 67% of the kneading time to delay a premature release of CO$_2$. Immediately after kneading 315 g chemically leavened dough was placed into the fermentation basket and covered with the optical sensor. Finally, the proofing chamber was closed hermetically to start the measuring series at 30 °C for 3 h. The following values were obtained: H_m = maximum dough height; T_1 = time to reach H_m, H'_m = highest CO$_2$-formation kinetics, T'_1 = Time of H'_m, h = height at the end of the test; T_x = time of gas release; V_T = total gas production during measurement. Based on these values it is possible to calculate the weakening coefficient WC (Eq. (1)). The measurements were performed in triplicates:

$$WC = \frac{H_m - h}{H_m} * 100 \qquad (1)$$

2.6. Flow and deformation properties in a rotational rheometer

The fundamental rheological behaviour of the dough samples were analysed by a time-sweep oscillatory test. An AR-G2 rheometer (TA instruments, New Castle, USA) was equipped with 4.00 cm parallel cross-hatched plates with a constant gap of 2.00 mm and a smart swap peltier plate temperature system which kept the temperature at 30 °C during the measurements. A dough piece of approximately 5.5 g was gently formed into a smooth ball and placed onto the centre of the stator plate. After setting the gap, excess dough was removed. To protect the outer surface against dehydration, paraffin oil (Merck, Darmstadt, DE) was applied. The

rheological measurement started after 10 min of equilibration to ensure relaxation of the dough structure after handling. In the following oscillatory step a constant deformation of 0.1% was combined with a frequency of 1 Hz recording 3 measuring points. The deformation was within the linear viscoelastic range. The fundamental rheological properties of the dough samples were evaluated through the complex shear modulus G^* and the loss factor tanδ as derived by the loss modulus G'' and the storage modulus G'. For each dough formulation the fundamental rheological measurement were carried out three times.

2.7. Statistical analysis

The statistical analysis was performed with the aid of GraphPad Prism 6 (Version 6.01, GraphPad Software Inc., La Jolla, USA). To detect significant differences between dough samples a nonparametric *t*-test was applied.

3. Results and discussion

3.1. Relation between the proportion of dead cells and the extractable concentration of glutathione in yeast

In order to assess the impact of the yeast metabolite glutathione (GSH) on dough properties, first of all, the amount of water soluble (non-protein) free –SH compounds was determined in different commercial yeast products (6 fresh and 21 dry samples). Furthermore, the proportion of dead yeast cells was predicted to establish a link between the viability of the organism and the dough weakening agent GSH. As illustrated in Fig. 1 the correlation between the proportion of dead yeast cells and the GSH content might be linear ($R^2 = 0.8483$, $p < 0.001$). This confirms the results of Sugiyama, Kawamura, Izawa, and Inoue (2000) who found that the GSH content in cultivated *S. cerevisiae* depends on the number of dead cells (Sugiyama et al., 2000). The deviations from linearity are not surprising. Depending on the duration and extend of stress during manufacturing yeast produces more or less glutathione but only if the conditions result in cell death, the tripeptide passes the membrane and influences the dough. Therefore, only when the protective mechanisms do not suffice to withstand the external stress, the extractable glutathione content can be correlated with the proportion of dead cells. Moreover, differences between fresh and dry yeast are demonstrably present. The proportion of dead cells in fresh samples varies between 1.77% and 8.82%, while the
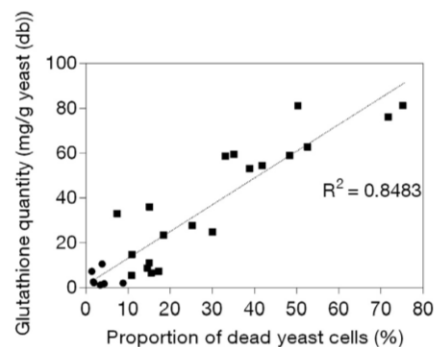

Fig. 1. Dependency of the extractable glutathione quantity (mg/g yeast db) of 27 commercial fresh (●) and dry yeasts (■) subjected to the proportion of dead yeast cells. By means of linear regressions analysis a significant correlation between the water soluble GSH content of yeast and the number of lysed cells could be observed ($R^2 = 0.8483$, $p < 0.001$).

quantity of GSH ranges from 1.16 to 10.51 mg/g of yeast (db). These GSH-contents are comparable to the findings of Rollini, Casiraghi, Pagani, and Manzoni (2007) who found 7.0 ± 2.0 mg GSH/g fresh yeast (Rollini et al., 2007). Dried samples, however, demonstrate a significantly higher concentration of dead cells (7.42–75.14%) and GSH (5.37–81.22 mg/g yeast (db)).

Interestingly, yeast products which were obtained from the same providers and only differed in regard to their production date considerably varied from each other. Although one would expect similar properties, for all of the six products that were tested, significant differences ($p < 0.001$, nonparametric t-test) in two independent batches were observed (data not shown). The relative deviation concerning the proportion of dead cells was up to 75% and up to 91% concerning the glutathione content. This suggests that the production process of fresh and dry yeast is subjected to fluctuations which strongly influence the quality of the final product. Summarised, the amount of dead cells is a good indicator for evaluating the impact of adverse conditions during processing. Especially through the application of dry yeast increased quantities of GSH can diffuse into the aqueous phase of wheat dough.

3.2. Yeast-equivalent glutathione quantities effect dough stability during kneading

For investigating the effect of yeast equivalent glutathione on the structural kinetics of wheat dough in comparison to a model dough, four more GSH quantities were chosen based on Fig. 1. The effect of yeast equivalent GSH quantities on the dynamic dough properties in the Z-kneader are represented in Fig. 2. The structural degradation by the addition of yeast-equivalent GSH amounts was established by monitoring the dough development (A), stability (B) and softening (C) during a kneading cycle of 20 min. It was proven that increasing GSH quantities significantly shorten the dough development time in an asymptotic manner from 7.4 ± 0.57 min (no GSH) to 2.5 ± 0.17 min (76.2 mg GSH/ 100 g flour). Consequently, the time to reach the maximum of the kneading curve was reduced by 67.0%, which is attributable to the disintegration of the gluten network (Tsen, 1969). However, the reduction of the processing time should not be assessed as positive, since GSH also impaired the dough texture. More specifically, increasing concentrations of GSH caused an asymptotic decrease to 1.8 ± 0.10 min (76.2 mg/100 g flour) concerning dough stability and an asymptotic rise regarding dough softening up to 282 ± 13.57 FU. Typically, the exceedance of the dough softening by more than 70 FU is connected with a high stickiness and indicates a limited extent of the processability. These findings are in accordance with Pyler and Gorton (1988) who describe that kneading time is generally reduced with the use of instant active dry yeasts, which they associate with the release of GSH (Pyler & Gorton, 1988). Moreover Kuninori and Sullivan (1968) showed that

30 mg GSH per 100 g wheat flour reduced the consistency of wheat dough rapidly after 3 min kneading (Kuninori & Sullivan, 1968). Furthermore, they describe that kneading energy enhances the weakening effect of GSH by stretching gluten strands and making the disulphide bonds more vulnerable to interchange by SH groups of the reducing agent.

As already hypothesised by Pyler and Gorton (1988) it was confirmed that dry yeast equivalent GSH quantities weaken the dough matrix, while no significant differences were observed when using a GSH concentration typical for fresh yeast (nonparametric t-test, $p < 0.05$). Furthermore, as proven by Grosch and Wieser (1999) and verified by the asymptotic course of the present curves the increase in the amount of glutathione has no further effect with regard to the limited number of cleavable disulphide bonds of glutenin chains in the gluten network (Grosch & Wieser, 1999). Subsequent investigations with a rotational rheometer were performed in order to confirm that yeast equivalent glutathione quantities also affect the method independent material properties of wheat dough.

3.3. Impairment of the dough elasticity by the addition of yeast-equivalent glutathione quantities

In addition to the effects of yeast equivalent GSH on the structural kinetics of wheat dough during kneading the method independent material properties were analysed in a rotational rheometer. The impact of yeast equivalent GSH quantities on the complex shear modulus G^* and the loss factor $\tan\delta$ is presented in Fig. 3. The pattern of both curves might indicate an asymptotic development of the dough softening. This suggests that GSH quantities diffusing from dry yeast into the dough medium sustainably weaken the gluten network. The stiffness of the sample represented by the complex shear modulus G^* decreases with the amount of GSH from 8170 ± 570 to 3550 ± 780 Pa. As expected, the loss factor $\tan\delta$ increases with rising GSH quantities from 0.39 ± 0.01 to 0.55 ± 0.03. This corresponds to the findings in the Z-kneader that GSH concentrations equally to the amount in fresh yeast sample do not significantly affect the rheological dough properties (nonparametric t-test, $p < 0.05$). This is roughly consistent with previous studies that GSH in concentrations from 5 to 15 mg/100 g flour reduce the elastic part of wheat dough (Berland & Launay, 1995; Dong & Hoseney, 1995). Moreover, reflected by the reduction of tenacity Kieffer et al. (1990) confirmed the elasticity weakening effect of GSH by measurements with the extensigraph (Kieffer et al., 1990). In these studies glutathione was added to evaluate its suitability as a baking agent. Moreover, the linear correlations between the descriptive and fundamental rheological values represented in Table 1 validates the respective results and they point out that both techniques are applicable for analysing the effect of yeast metabolites. Particu-
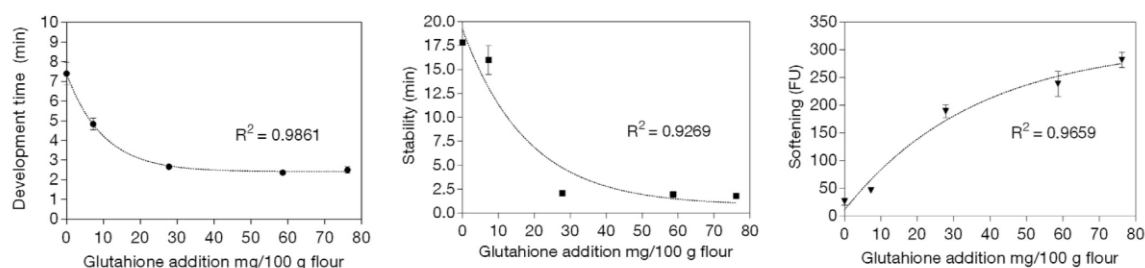

Fig. 2. Impact of yeast equivalent glutathione quantities (0.0, 7.3, 27.8, 58.6, 76.2 mg/100 g flour) on the (A) development time (●), (B) stability (■) and (C) softening (▼) of dough samples in a torque-measuring Z-kneader. For each graph an exponential curve fitting (one phase decay) was applied. For the development time ($R^2 = 0.9861$, $p < 0.001$) as well as the softening ($R^2 = 0.9659$, $p < 0.001$) an asymptotic rise could be proven. In contrast the dough stability decreases asymptotically with increasing glutathione quantities ($R^2 = 0.9269$, $p < 0.001$). Means of each dough sample ($n = 3$) are shown with standard deviation.
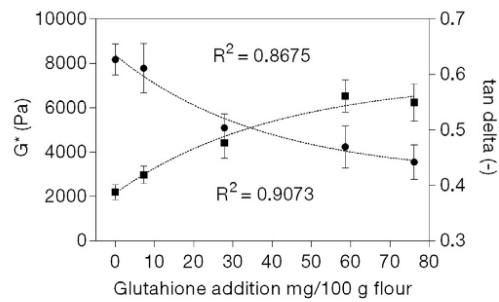
Fig. 3. Effect of glutathione on rheological properties of wheat dough without yeast. The complex shear modulus G^* (●) and the loss factor $\tan\delta$ (■) were measured in a rotational rheometer. The glutathione concentrations (7.3, 27.8, 58.6, 76.2 mg/100 g flour) equaled the detected amount in yeast samples. For each graph an exponential curve fitting was applied. For G^* the one phase decay had a coefficient of determination R^2 of 0.8675 ($p < 0.001$) and for $\tan\delta$ R^2 was 0.9073 ($p < 0.001$).

Table 1

Linear correlation matrix of descriptive and fundamental rheological values. Development time, stability and softening are descriptive values measured in a torque measuring Z-kneader. $\tan\delta$ and G^* are fundamental values measured in a rheometer. Dough with different GSH quantities (0.0–76.2 mg/100 g flour) was analysed. In all cases significant ($p < 0.001$) correlations (r) were found.

	Stability (min)	Softening (FU)	$\tan\delta$ (−)	G^* (Pa)
Development time (min)	0.9282	−0.8899	−0.8734	0.8353
Stability (min)		−0.9480	−0.8961	0.9188
Softening (FU)			0.9304	−0.9077
$\tan\delta$ (−)				−0.8713

larly, the connection between dough stability and stiffness G^* ($r = 0.9188$) as well as the relation between dough softening and $\tan\delta$ ($r = 0.9304$) support the conclusion that dough stability depends on the disulphide–sulfhydryl interchange between gluten subunits and reducing agents (Wieser, 2007).

3.4. Reduction of the gas retention capacity by the addition of yeast-equivalent glutathione quantities

By inflating the dough matrix with a chemical raising agent, the effect of yeast equivalent GSH quantities on the gas retention

Table 2

Sample	T_1 (min)	H_m (mm)	T'_1 (min)	H'_m (mm)
9.9 g SAPP 10 + 7.2 g NaHCO$_3$ + 0.0 mg GSH per 100 g flour	129.1 ±2.3	48.5 ±0.4	11.9 ±0.2	149.7 ±0.8
0.5 g Dry yeast per 100 g flour	178.5 ±1.5	42.4 ±1.1	176 0.8	40.9 0.1

capacity was analysed in a Rheofermentometer. The combination of sodium acid pyrophosphate 10 (SAPP 10) and sodium bicarbonate (NaHCO$_3$) was applied to analyse the effect of glutathione independently from the yeast metabolism. During a fermentation cycle of 180 min an average Volume V_t of 666 ± 30 ml of CO$_2$ was released by the chemical reaction of SAPP 10 and NaHCO$_3$ which is close to the typical CO$_2$ volume (700 ml) produced by 0.5 g dry yeast per 100 g flour (Verheyen et al., 2014). As a comparison Fig. 4A) represents the dough development (left y-axis) as well as the direct and indirect fermentation profile (right y-axis) of biologically (0.5 g dry yeast per 100 g flour) and chemically (9.9 g SAPP 10 + 7.2 g NaHCO$_3$ + 0.0 mg GSH per 100 g flour) leavened dough. Both curves reach a similar maximum dough height (see Table 2). However, the time T_1 until reaching the maximum dough height differs significantly. While it takes 129.1 ± 2.3 min for the chemically leavened product the yeasted sample requires 178.5 ± 1.5 min. This can be related to accelerated kinetics of the CO$_2$-formation of SAPP 10. Moreover, the chemical agent forms almost the total amount of gas within the first 45 min while the yeasted sample produces CO$_2$ continuously during the whole proofing period. In spite of the different gas formation rate, SAPP10 is suitable to analyse the effects of yeast equivalent quantities of GSH on the gas retention capacity, as represented by Fig. 4B). The higher the GSH concentration, the higher the destabilizing effect. Nevertheless, within the interval of 0.0–58.6 mg GSH per 100 g flour there was no gas release (T_x), although the WC increased from $0.25 \pm 0.07\%$ to $5.95 \pm 0.21\%$. The excess of the retention capacity exclusively eventuated at the highest GSH quantity of 76.2 mg/g yeast (db), when the WC reached a value of $20.4 \pm 1.56\%$. These findings are confirmed by the results obtained by Rezaei et al. (2014) who described that the impairment of gas holding properties is not exclusively affected by the total CO$_2$ volume (Rezaei et al., 2014). However, they suppose a connection between the amount of CO$_2$ and other metabolites diffusing from yeast into the dough medium. Based on the outcomes presented in this study glutathione is a very likely candidate.
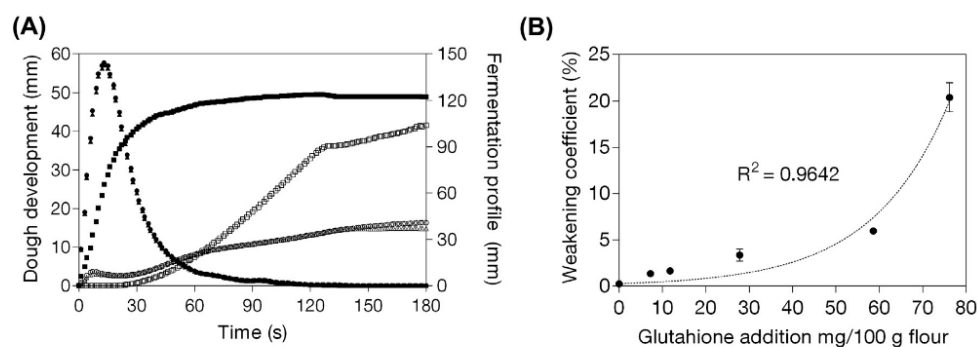

Fig. 4. Representation of the fermentation properties of chemical (sodium acid pyro phosphate) and biological leavened (dry yeast) wheat dough in the Rheofermentometer during a proofing period of 180 min at 30 °C. Exemplary presentation of the dough development (chemical (■); biological (□)) on the left y-axis as well as the direct (chemical (●); biological (○)) and indirect (chemical (▲); biological (Λ)) fermentation profile on the right y-axis. (B) The dotted curve represents the exponential growth ($R^2 = 0.9642$, $p < 0.001$) of the weakening coefficient WC.

4. Conclusion

The structure of wheat dough is crucially affected by the presence of reducing agents (cysteine, reduced glutathione (GSH) and cysteine containing peptides) which can diffuse from dead yeast cells into the sample. The content of free (non-protein) sulfhydryl groups in 21 dried samples ranged between 5.4 and 81.2 mg/g yeast (db) and in fresh yeast samples (6 products) 1.2–10.5 mg free –SH compounds/g (db) were detected. Since GSH protects the cell against reactive oxygen species (ROS) this suggests that there is a connection between the method of manufacturing and the level of stress acting on yeast as observable to the varying GSH-content. Thus, drying can cause a remarkable concentration of up to 8.4% in commercial products. This makes GSH an important ingredient rather than a minor accompanying substance. Furthermore in this study it was proven that the time to reach the maximum of the kneading curve (dough development time) as well as the dough stability was reduced by 67% respectively 89% by the addition of yeast-equivalent GSH quantities. Accordingly, the method independent dough properties represented by the complex shear modulus G^* and the loss factor $\tan\delta$ indicate a decrease of the elasticity. Subsequently, the destabilizing effect of GSH measured by descriptive as well as fundamental methods and the linear correlation of the obtained values confirmed the validity of the results. It was assumed that the asymptotic disintegration of the dough matrix was limited by the number of the cleavable disulphide bonds of the glutenin chains. In order to the use of a chemical raising agent in analyses turned out as a suitable method. Moreover, it was proven that investigations carried out on chemical leavened samples in the Rheofermentometer are suitable to determine the combined effect of the yeast metabolites CO_2 and GSH on the gas retention capacity without yeast. As expected, the weakening coefficient increased with the addition of GSH. However, there is a need to examine the extent of impairment of the gas retention capacity as well as the baking performance by the combined effect of the yeast metabolites GSH and CO_2 (formation rate and volume). Thus, it is assumed that GSH considerably contributes to the softening of wheat dough through dead yeast cells. Typically, yeast manufacturers focus on the carbon dioxide production when assessing their products but the present findings suggest, that also the exposition to stress as reflected in the proportion of dead yeast cells should be monitored in order to sufficiently estimate the baking performance.

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2.5 Destabilization of wheat dough: Interrelation between CO$_2$ and glutathione

Destabilization of wheat dough: Interrelation between CO$_2$ and glutathione

C. Verheyen, A. Albrecht, T. Becker, M. Jekle *

Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

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ABSTRACT

Minor variations in the metabolic profile of yeast strongly affect wheat dough structure, the resulting bread volume and its crumb texture. However, it is complex to analyze the impact of single yeast metabolites on the development of dough during processing. In this study, a chemical-leavening system based on glucono-delta-lactone and NaHCO$_3$ was applied to analyze aeration and gas retention in presence of glutathione independent from the yeast metabolism. The addition of glutathione-amplified gas release during kneading and reduced the time to reach the maximum gas formation rate. The results indicated that the inflation of dough is not limited by the amount of CO$_2$ nor its release kinetics; however, during baking further aeration is restricted. The oven rise was impaired by high initial CO$_2$ volumes, which is enhanced by the presence of glutathione.

Industrial relevance: This investigation addressed the effect of yeast equivalent glutathione on the properties of aerated dough and the resulting bread properties. Through the application of a chemical-leavening system the yeast metabolism can be avoided. GSH from lysed yeast cells had a weakening effect, which confirms observations of structure-loss after freeze–thaw processes of dough. Moreover, the use of chemical-leavening agents reduces bread production costs, since the fermentation process can be neglected.

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1. Introduction

During the breadmaking process, the fermentation step is fundamental for the final loaf characteristics, such as volume, pore structure, aroma, color, and texture. Typically, the aeration of bread is based on the biological leavening agent baker's yeast (*Saccharomyces cerevisiae*). The resulting CO$_2$ release depends on three major factors: (1) the type of yeast, (2) the growth state of yeast cells and (3) the availability of substrates (Rezaei et al., 2014). Thus, the application of commercial yeasts is problematic for a standardized analysis of yeast-induced changes of the dough matrix. Even though numerous dough-influencing yeast metabolites such as ethanol, succinic acid, hydrogen peroxide and glutathione are known, their individual contribution on the gas-holding properties of the dough matrix and the resulting loaf characteristics remains unclear (Jayaram et al., 2013; Rezaei, Aslankoohi, Verstrepen, & Courtin, 2015; Rezaei, Dornez, Verstrepen, & Courtin, 2015; Verheyen, Albrecht, Herrmann, et al., 2015). The results of Verheyen, Jekle, and Becker (2014) indicate that there is a critical retainable gas volume in yeasted dough which is neither influenced by the amount of yeast nor the production rate of CO$_2$ (Verheyen et al., 2014). However, higher dry yeast concentrations reduced the resistance to extensibility, which should have a negative impact on gas retention. This indicates that there are additional positive mechanisms through

which yeast counteracts the reduced extensibility. Thus, the effect of different yeast metabolites on the dough extensibility and other factors relevant for gas retention should be elucidated.

While yeast equivalent hydrogen peroxide quantities do not significantly affect non-leavened dough samples, succinic acid and ethanol strengthen the dough network up to 18% (Jayaram, Cuyvers, Verstrepen, Delcour, & Courtin, 2014; Rezaei, Aslankoohi, et al., 2015). In contrast, glutathione (GSH) is an antioxidant tripeptide with a free sulfhydryl group causing dough softening by preventing the formation of disulfide bridges in the gluten network (Dong & Hoseney, 1995). As a metabolic product of yeast, glutathione exclusively leaks out of lysed yeast cells into the dough medium. Studies made by Connelly and McIntier (2008) demonstrate that an inactivation of yeast reduces the resistance to extension of wheat dough due to an enhancement of lysed cells. This is in accordance with Verheyen et al. (2014) who postulated that the application of dry yeast, which commonly contains a higher proportion of lysed cells reduces the complex shear modulus G* during fermentation (Verheyen et al., 2014). Moreover, as indicated by a reduced resistance to extension the dough becomes more susceptible to pressure with rising dry yeast concentrations (Archer, 1972). This indicates a weakening interrelation between increasing quantities of GSH and CO$_2$.

In order to further elucidate the impact of yeast on the gas retention capacity of dough, one strategy is to analyze relevant yeast metabolites separately while provoking a dough inflation independent from yeast. For fundamental investigations concerning the impact of the gas formation kinetics on the structural integrity of wheat dough chemical-

* Corresponding author. Tel.: +49 8161 71 2683; fax: +49 8161 71 3883.
 E-mail address: christoph.verheyen@tum.de (C. Verheyen).

leavening systems are particularly suitable (Bellido, Scanlon, & Page, 2009). While the biological CO_2 production strongly depends on substrate availability, the performance of chemical raising agents is only related to the stoichiometric reaction of the leavening acid (acidulant) with a neutralization agent like sodium bicarbonate ($NaHCO_3$). The selection of an appropriate acidulant in combination with a monitored product temperature enables the regulation of CO_2 release and dough inflation (Bellido, Scanlon, Sapirstein, & Page, 2008). Through the neutralization reaction of the chemical-leavening system, the mechanical impact of the dynamic expansion of gas bubbles can be analyzed (Chin, Martin, & Campbell, 2005). Moreover, on the basis of almost constant material properties the gas retention can be studied in detail.

The goal of the present study was to identify the weakening interrelation of CO_2 and the reducing agent glutathione on the wheat dough structure. Varying glucono-delta-lactone concentrations were applied to analyze the impact of different CO_2-formation rates on dough stability and end product quality independently from the yeast metabolism and physiology. Concurrently, through the application of yeast equivalent glutathione concentrations, the structure-weakening effect of reducing agents on the material properties of inflated dough matrices and the derived products was determined. Gas release kinetics were determined in the Rheofermentometer and correlated with the final loaf volume to evaluate the transition properties from dough to crumb. This approach provides a substantially improved understanding about the baking performance of rapidly inflated dough in the presence of yeast equivalent glutathione quantities.

2. Experimental

2.1. Wheat dough preparation

German commercial wheat flour type 550 obtained from Rosenmühle (Landshut, Germany) was determined to be 14.08 ± 0.09 g moisture, 12.23 ± 0.04 g protein, and 0.62 ± 0.02 g ash per 100 g of sample. In accordance to American Association of Cereal Chemists (AACC) method 54–21.02 a torque measuring Z-kneader (doughLAB; Perten Instruments, Germany) was used to establish the optimum water absorption and kneading time. In order to reach 500 Farino Units, 58.6 g demineralized water per 100 g flour, with moisture content corrected to 14.00 g/100 g of flour, and a kneading time of 195 ± 10.4 s at 63 rpm and 30 °C were required. For baking trials, 5 kg dough was first kneaded at 100 rpm for 60 s and further 320 s at 200 rpm with a laboratory spiral kneader at 30 °C (Diosna Dierks & Söhne, Osnabrück, Germany). As raising agent, different concentrations of a chemical-leavening system consisting of glucono-delta-lactone (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) and $NaHCO_3$ (Solvay GmbH, Hannover, Germany) was applied. Based on the neutralization value (NV) of the acidulant glucono-delta-lactone (GDL), the sample weight was calculated for $NaHCO_3$ concentrations of 0.45, 0.9, 1.35, 1.8, 2.25, 2.7 and 3.87 per 100 g flour (see Eq. (1)).

$$Acidulant\ (g) = \frac{NaHCO_3}{NV} * 100 \tag{1}$$

The NV is defined as the amount of sodium bicarbonate (g) which will neutralize 100 g of the acidulant (GDL). The supplier provided a NV of 30 for glucono-delta-lactone. Furthermore yeast equivalent glutathione (Amresco SSL, Solon, USA) concentrations, as determined by Verheyen, Albrecht, Herrmann, et al. (2015), Verheyen, Albrecht, Elgeti, et al. (2015), were added. Equivalent to an addition of 1 g dry matter yeast per 100 g flour 7.3, 27.8 and 73.3 mg glutathione (GSH) were used. No GSH was applied for the preparation of the model dough.

2.2. Determination of fermentation characteristics

A Rheofermentometer F3 (Chopin, Villeneuve-La-Garenne Cedex, France) was applied to detect the gas release kinetics depending on the varied quantities of the chemical-leavening system. Immediately after kneading, 150 g of the leavened dough sample was placed into the fermentation basket and covered with the optical sensor. The recommended dough weight of 315 g was reduced to 150 g due to a pressure defect caused by an excessive gas evolution within the 90 s measuring intervals. The proofing chamber was closed hermetically before starting the measuring cycle at 30 °C for 180 min. The obtained variables were T_0 (Time to reach 70% of the maximum dough height), T_1 (Time to reach the maximum dough height), T_1 (Time to reach the maximum gas formation rate), T_2 (Time to reach 90% of the maximum dough height), T_x (Time of porosity (gas starts to escape the dough matrix)), V_{total} (Total CO_2 volume, which was produced during the measurement).

2.3. Determination of the dough density after kneading

Campbell, Herrero-Sanchez, Payo-Rodriguez, and Merchan (2001) reported that dough density after kneading (ρ_K) can be determined according to the Archimedean principle (Campbell et al., 2001). Dough samples of approximately 15 g were weighed in air to 0.1 mg (resulting in m_{air}), immersed in silicone oil (VWR International, Radnor, USA) of known density ($\rho_l = 0.969$ g cm^3 at 25 °C) and weighed again (m_{liquid}) using a spring balance (DIPSE SSR-Produkt, Osnabrück, Germany). On the basis of the differences in weight, the density was calculated using Eq. (2). In order to ensure comparability, the measurement was carried out exactly 2 min after kneading. The temperature of the liquid was controlled during the measurements to prevent density fluctuations.

$$\rho_K = \frac{m_{air}}{m_{air} - m_{liquid}} \rho_l \tag{2}$$

2.4. Determination of the specific dough volume after fermentation

Alterations of the specific dough volume during resting were calculated by using a Rheofermentometer (Chopin, Villeneuve-La-Garenne Cedex, France). To determine the initial dough volume in the fermentation basket, dough height (h_0) after kneading was calculated by the density (ρ_K). According to the increased dough height (h_{RF}) at a given fermentation time the, the specific dough volume was calculated using Eq. (3). Therefore, the sum of h_0 and h_{RF} was multiplied by the quotient of the cross-sectional area (A) of the fermentation basket and the dough weight (DW).

$$Spec.\ dough\ volume = (h_0 + h_{RF}) \frac{A}{DW} \tag{3}$$

2.5. Production and analysis of bread

After kneading in the 300 g-bowl of the doughLAB 250 g dough was placed into a loaf pan and stored in a proofing chamber at 30 °C with 80% relative humidity. The duration was varied according to the Rheofermentometer results and were between 0 and 117 min ($T0$, $T1$, $T1$, $T2$, and Tx). Subsequent to resting, baking was performed in a Matador 12.8 oven (Werner & Pfleiderer Lebensmitteltechnik GmbH, Dinkelsbühl, Germany) at 230 °C with 0.5 l initial steam for 18 min. After cooling for 1 h at room temperature, the densities of 4 loaves per trial were determined using a laser volumeter (Volscan BVM-L 370) with a Flat Support Plate Rec. 30 × 50. Baking trials were conducted as triplicates.

2.6. Statistical analysis

Statistical analysis was performed with the aid of GraphPad Prism 6 (Version 6.01, GraphPad Software Inc., La Jolla, USA) and Statgraphics Centurion (Statpoint Technologies Inc., Warrenton, Virginia). To detect significant differences between dough and bread samples, a one-way analysis of variance (ANOVA) was applied followed by the Tukey's-test. A linear regression was used to investigate the relationship between dependent and independent variables. The correlations between processing parameters and product characteristics were then analyzed, whereby the coefficient of determination (R^2) represents the degree of correlation.

3. Results and discussion

3.1. Effect of yeast equivalent glutathione and chemical aeration on dough density

A comparative evaluation of the gas entry during kneading depending on the concentration of chemical-leavening agents ($NaHCO_3$ + GDL) was performed. As shown in Fig. 1, the production of CO_2 reduces the dough density with rising $NaHCO_3$ amounts in a linear fashion. Affirmative, Verheyen, Albrecht, Herrmann, et al. (2015), Verheyen, Albrecht, Elgeti, et al. (2015) found that rising concentrations of dry yeast (0.5–3.0 g dry matter yeast /100 g flour) reduced the dough density after kneading from 1.08 to 0.96 g/ml. This proves that the amount of chemical-leavening agents provided a CO_2 production similar to yeast fermentation, but also higher gas volume fractions were achieved (density after kneading: 1.08–0.62 g/ml).

Additionally, a range of yeast equivalent glutathione (GSH) quantities (as previously detected by Verheyen, Albrecht, Herrmann, et al. (2015), Verheyen, Albrecht, Elgeti, et al. (2015) was applied to the dough samples. Moreover, the pH of non-leavened dough samples with varying GSH concentrations (0.0–76.2 mg/100 g flour) was analyzed to consider structural effects induced through possible pH differences. No statistically significant pH-difference (5.81 to 5.79) between the minimum and maximum GSH-concentration existed (see supplementary data). A structural impact by GSH-induced pH changes can be excluded. The addition of GSH causes a significant reduction of the dough density with lower concentrations of chemical-leavening agent, while starting from 1.35% $NaHCO_3$ the effect is no longer significant. GSH-induced cleavage of disulfide bridges or prevented their formation,
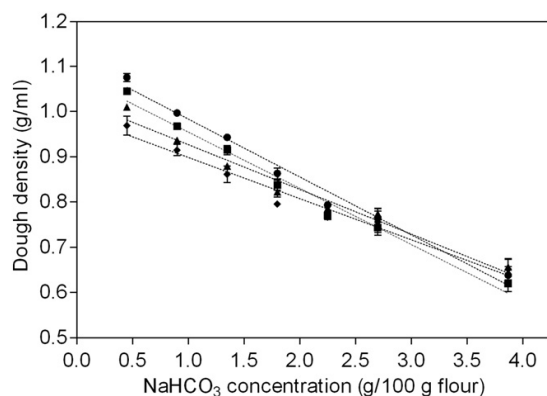
which is associated with reduced resistance of the gluten network to expansion (Hahn & Grosch, 1998). However, above 1.8% $NaHCO_3$, GSH had no significant impact on the dough density. Thus, the accelerated CO_2 formation rate as well as the higher gas volume superimposed the effect of GSH.

3.2. Effect of chemical aeration on the maximum gas formation rate in wheat dough

Analogous to the study of Verheyen, Albrecht, Herrmann, et al. (2015), Verheyen, Albrecht, Elgeti, et al. (2015) the gas formation rate t_{vCO2} was calculated by reprocessing the direct gas release curves measured in the Rheofermentometer. The gas formation curves were integrated and the resulting sigmoidal curve was fitted. In order to evaluate the highest gas formation rate (v_{CO2}) with varying concentrations of chemical-leavening agents, the maximum slope of the tangent through the point of inflection was determined. Due to the linear increase of the s-curves in the reflection point v_{CO2} enables a meaningful assessment of the maximum CO_2 formation rate for varying yeast concentrations. The CO_2 formation in yeast leavened dough followed a sigmoidal course. Comparable curves resulted from the chemically leavened dough samples (data not shown). Probably, the exponentially increasing release curves flatten due to the limited amount of acid and CO_2 carrier (Parks, Handleman, Abrnett, & Wright, 1960).

As presented by Fig. 2 (A), the maximum gas formation directly depends on the amount of $NaHCO_3$. With 0.45–3.87 g $NaHCO_3$ per 100 g flour, maximum CO_2 formation rates of 280–2160 ml per hour were achieved. An effect of GSH on v_{CO2} was not detected. This confirms the expectation that the maximum rate of CO_2 formation through the reaction of chemical-leavening agents is irrespective of the dough material properties. The maximum gas formation rate of chemically leavened dough samples is comparable with the v_{CO2} of yeast samples analyzed by Verheyen, Albrecht, Herrmann, et al. (2015), Verheyen, Albrecht, Elgeti, et al. (2015) (v_{CO2} ≈ 1200 ml/h with 3 g dry matter yeast per 100 g flour). However, exceeding 2.7 g $NaHCO_3$ per 100 g wheat flour causes an increase of v_{CO2} up to 2000 ml/h.

As represented by Fig. 2 (B), the time t_{vCO2} to reach the maximum gas formation rate v_{CO2} varied from 13 to 20 min. Surprisingly, an influence of GSH on t_{vCO2} was found, but only for small concentrations of $NaHCO_3$. GSH (starting with a content of 27.6 mg/100 g flour) seems to affect dough density as well as the activity of the chemical-leavening agents only in the case of a low CO_2 production rate. Improved dough extensibility through GSH might be beneficial for the aeration during kneading; however, elevated concentrations of $NaHCO_3$ provide non-reacted molecules, which raise the pH from 5.9 to 7.1. This might counteract the higher extensibility through GSH, because alkaline substances are known for their strengthening effect on dough (Jekle & Becker, 2012). As represented by Takano and Kondou (2002) unreacted sodium gluconate has the same stabilizing effect, since dough extensibility increases and the resistance to extension decreases. The dough handling properties, however, were not affected by the replacement of 100% sodium chloride with sodium gluconate. In contrast to yeasted dough samples, where t_{vCO2} varied between 45 and 150 min (Verheyen, Albrecht, Herrmann, et al., 2015; Verheyen, Albrecht, Elgeti, et al., 2015), chemical-leavening reduced the time to reach the maximum CO_2 production rate by a factor of 11.8. The resulting time savings during fermentation through the application of chemical-leavening agents offer the possibility for a significant reduction of the bread production period. It must, however, be noted that $NaHCO_3$ as well as some acid carriers like sodium acid pyrophosphate (SAPP) cause an alkaline or metallic aftertaste. In cake batter the unwanted taste is typically concealed by sugar and/or other flavoring compounds. In bread production, the application of malt extract offers a promising opportunity to cover the astringent, metallic taste. Moreover, malt can compensate the absence aroma compounds from yeast (Maloney & Foy, 2003).

Fig. 1. Impact of yeast equivalent glutathione on wheat dough density after kneading depending on $NaHCO_3$ concentration. $NaHCO_3$ was added to produce CO_2 by reacting with glucono-delta-lactone. Glutathione concentrations per 100 g flour: 0.0 mg (●), 7.3 mg (■), 27.6 mg (▲) and 76.2 mg (◆).Dotted lines represent linear regressions: R^2 = 0.9798 for (●), R^2 = 0.9797 for (■), R^2 = 0.9678 for (▲) and R^2 = 0.9551 for (◆). Means of measurements (n = 3) are shown with standard deviation.
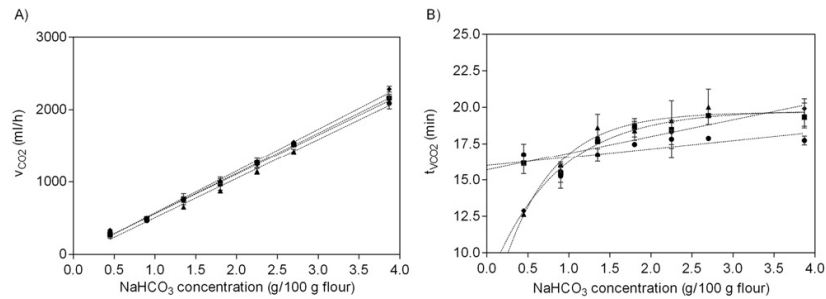
Fig. 2. Impact of yeast equivalent glutathione on the gas formation rate during resting depending on NaHCO₃ concentration. NaHCO₃ was added to produce CO_2 by reacting with glucono-delta-lactone. Glutathione concentrations per 100 g flour: 0.0 mg (●), 7.3 mg (■), 27.6 mg (▲) and 76.2 mg (◆). Dough samples were analyzed using a Rheofermentometer during 180 min. (A) Maximum gas formation rate v_{CO2} determined as tangent through the point of inflection of integrated gas formation curves (Verheyen, Albrecht, Herrmann, et al., 2015). Dotted lines represent linear regressions: $R^2 = 0.9958$ for (●), $R^2 = 0.9950$ for (■), $R^2 = 0.9906$ for (▲) and $R^2 = 0.9936$ for (◆). (B) Resting time t_{vCO2} (min) to reach v_{CO2}. Linear regressions: $R^2 = 0.4259$ for (●), $R^2 = 0.6264$ for (■) and exponential fitting with $R^2 = 0.9342$ for (▲) and $R^2 = 0.9459$ for (◆). Means (n = 3) are shown with standard deviation.

3.3. Impact of the gas formation rate on dough porosity

While previous sections focused on the total CO_2 volume and its formation rate, subsequent investigations considered the dough porosity. In the Rheofermentometer, Tx represents the time, when CO_2 starts to exit the dough matrix, indicating dough porosity. Fig. 3 (A) illustrates the impact of different gas production rates v_{CO2} on Tx. The step-wise increase of Tx with rising v_{CO2} might derive from the 90 s measuring interval of the Rheofermentometer. Furthermore, as mentioned in Section 3.2, the pH-increase with rising NaHCO₃ concentrations caused a stabilization of the dough matrix. Thus, despite accelerated gas formation, the leakage of CO_2 occurs with a delay. The variations of Tx are within a narrow range (30–34 min) and low in comparison to biological leavening (24–129 min) (Ktenioudaki, Butler, & Gallagher, 2011; Rosell & Collar, 2009; Verheyen, Albrecht, Elgeti, et al., 2015).

With 3.87% NaHCO₃ the aeration during kneading tended to be higher than with yeast (see Section 3.1). Consequently, Tx is reached earlier in the case of chemical-leavening. The positive effect of the alkaline chemical on dough stability (see Section 3.2) apparently counteracts the weakening of glutathione. Thus, Tx does not depend on GSH, but rather on v_{CO2}.

As depicted in Fig. 3 (B), the CO_2 volume to reach Tx, $V_{CO2}(Tx)$, increases linearly ($R^2 = 0.9876$) with accelerated gas formation. This is in contrast to wheat dough leavened with yeast, as proved by Verheyen, Albrecht, Herrmann, et al. (2015), Verheyen, Albrecht, Elgeti, et al. (2015). With compressed yeast, more gas can be stabilized when the aeration process is slower. It can be assumed that the

structure improvement by non-reacted sodium bicarbonate is responsible for the opposite effect of chemical and the biological leavening systems. GSH had no significant impact on the analyzed dough porosity characteristics.

The following Table 1 presents a summarized overview of further Rheofermentometer parameters, which inform about the influence of chemical-leavening on the gas retention characteristics.

As might be expected, rising amounts of the chemical-leavening system increase of total CO_2 volume in 150-g-dough samples. Whereas, for the lowest NaHCO₃ concentration of 0.45 g per 100 g flour 57.8 ± 10.1 ml of CO_2 was released, the gas volume of samples with 3.87 g NaHCO₃ per 100 g flour increased to 599.8 ± 60.3 ml. However, as represented by the retention coefficient, the gas fixation by the dough matrix improves with rising amounts of the chemical-leavening system. As mentioned before and proven by Jekle and Becker (2012), the pH defines dough stiffness with a local minimum at pH 5.6. In the present study, the pH after resting was 5.9 with 0.45 g NaHCO₃ per 100 g flour and 6.6 with 3.87 g NaHCO₃ per 100 g flour. Thus, the strengthening effect of the pH seems causal for the improved gas retention capacity despite the 10-fold amount of CO_2 in the dough matrix.

3.4. Impact of the gas formation rate on the volume of chemically leavened bread

Dough samples with different NaHCO₃ concentrations were baked after resting for respective Rheofermentometer times (T0, T1, T1, T2, and Tx). This enabled an assessment of the informative value of
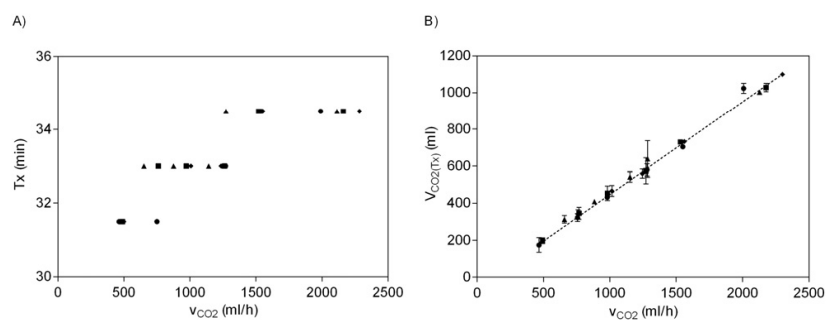

Fig. 3. Impact of yeast equivalent glutathione quantities on dough porosity depending on the NaHCO₃ concentration. To produce CO_2, 0.45–3.87 g NaHCO₃ per 100 g flour was added to react with glucono-delta-lactone. Glutathione concentrations per 100 g flour: 0.0 mg (●), 7.3 mg (■), 27.6 mg (▲) and 76.2 mg (◆). Dough samples were analyzed using a Rheofermentometer during 180 min. v_{CO2}: Maximum gas formation rate; Tx: Time of porosity. $V_{CO2}(Tx)$: produced CO_2 volume (ml) at Tx. (A) Time to reach dough porosity (Tx) depending on the gas formation rate v_{CO2} and GSH content. (B) Impact of CO_2 volume (ml) on Tx for different gas formation rates v_{CO2}. The dotted line represents a linear correlation ($R^2 = 0.9876$) between v_{CO2} and $V_{CO2}(Tx)$. Means (n = 3) are shown with standard deviation.

Table 1
Gas retention charactristics of chemically leavened wheat dough.

Rheofermentometer variables	NaHCO$_3$ quantity (g/100 g flour)						
	0.45	0.9	1.35	1.8	2.25	2.7	3.87
Total CO$_2$ volume (ml)	57.8 ± 10.1	108.0 ± 14.6	195.8 ± 13.7	262.1 ± 21.6	334.5 ± 27.7	419.8 ± 23.7	599.8 ± 60.3
Volume of CO$_2$ lost (ml)	5.4 ± 0.9	7.1 ± 0.4	9.0 ± 0.8	10.4 ± 0.8	12.5 ± 1.1	14.4 ± 1.4	23.9 ± 2.5
Volume of retention (ml)	52.3 ± 9.8	101.1 ± 14.6	187.0 ± 13.4	251.7 ± 20.8	322.3 ± 27.7	405.3 ± 22.9	575.8 ± 60.7
Retention coefficient (%)	90.5 ± 1.4	93.4 ± 1.2	95.4 ± 0.3	96.0 ± 0.2	96.3 ± 0.4	96.6 ± 0.2	96.6 ± 0.7

Rheofermentometer data for predicting the baking performance. Fig. 4 (A) presents the correlation between specific bread volume and CO$_2$ volume in dough. In order to reach the highest dough aeration (739 ml) and bread volume (3.3 ml/g) a maximum amount of NaHCO$_3$ (3.87 g/100 g flour) was required. Similarly, through biological leavening with yeast, 750 ml initial CO$_2$ volume in dough resulted in 3.3 ml/g bread (Verheyen, Albrecht, Elgeti, et al., 2015). This proves that the chemical system used in this study was well suited to simulate the aeration behavior of yeast. Moreover, the linear increase of the specific volume indicates no destabilization within the observed aeration level.

The oven rise was calculated by subtracting the specific dough volume from the specific bread volume (Fig. 4 B). To exclude alterations of the loaf characteristics based on water evaporation during baking, the specific bread volume, therefore, was calculated on the basis of the dough weight after resting. The volume development during baking shows a negative linear relationship ($R^2 = 0.9767$) with the initial specific dough volume after resting. During baking the rapid expansion appeared to destabilize dough with higher initial gas content, resulting in less oven rise for higher NaHCO$_3$ concentrations. The associated enhanced bubble expansion with rising CO$_2$ volumes causes mechanical stress and a partially rupture of individual gluten strands (Fan, Mitchell, & Blanshard, 1999). During crumb formation, heat increases the internal bubble pressure, which provokes a collapse of the gluten network. Subsequently, to prevent product quality impairment, a critical specific dough volume of 2.5 ml/g wheat dough can be derived. Although, the aeration level of wheat dough can be enhanced with increasing CO$_2$ formation rates, the transformation process from dough to crumb during thermal treatment provokes an impairment of the final matrix. Therefore, monitoring of the CO$_2$ release and the gas bubble distribution during baking should be addressed in future studies.

3.5. Impact of yeast equivalent glutathione on the volume of chemically leavened bread

The impact of yeast equivalent GSH (0.0–76.2 mg/100 g flour) and varying maximum CO$_2$ formation rates (280–2160 ml per hour) on the specific bread volume is illustrated in Fig. 5. In accordance with Ewart (1988) who added different amounts (0.0–30.7 g/100 g flour) of GSH to yeasted dough, the specific bread volume of chemically leavened samples is significantly reduced by the application of GSH (Ewart, 1988). Without GSH a maximum bread volume of 3.8 ml/g was detected, while 76.2 mg GSH/100 g flour reduced this value by 24% to 2.9 ml/g. Thus, contrary to the linear enhancement of the CO$_2$ volume in wheat dough with accelerated v_{CO2} (see Fig. 3 (B)), the increase of the loaf volume is limited. These results prove the assumption of several research groups who reported an elevated impairment of the baking performance through GSH introduced by lysed yeast cells (Autio & Sinda, 1992; Bayrock & Ingledew, 1997; Meziani et al., 2012; Wolt & D'Appolonia, 1984). This applies for both, the insertion of dead cells by yeast as a formulation ingredient as well as a subsequent provoked cell death by freeze–thaw processes of dough.

4. Conclusions

For chemically leavened wheat dough, the impact of the maximum CO$_2$ formation rate and yeast equivalent GSH on bread volume was evaluated. The replacement of the biological yeast aeration by a chemical system enabled independence from the yeast metabolism. The concentration of the chemical system was adapted to simulate gas incorporation during kneading. Addition of yeast equivalent GSH improved the aeration during kneading. However, high concentrations of the chemical-leavening system counteracted the effect of GSH, since increasing pH strengthens the dough. The simulation of CO$_2$ formation through yeast by chemical aeration revealed that the total gas volume in dough increased linearly with accelerated gas production. Thus, the ability of wheat dough to retain CO$_2$ exclusively depends on the rate of CO$_2$ production and is not affected by the presence of glutathione. In addition to the positive effect on the aeration during kneading, the maximum gas formation rate during resting was reached earlier with GSH.

In baking trials, higher gas volume content in dough after resting also resulted in higher bread volume. Despite the positive effect of
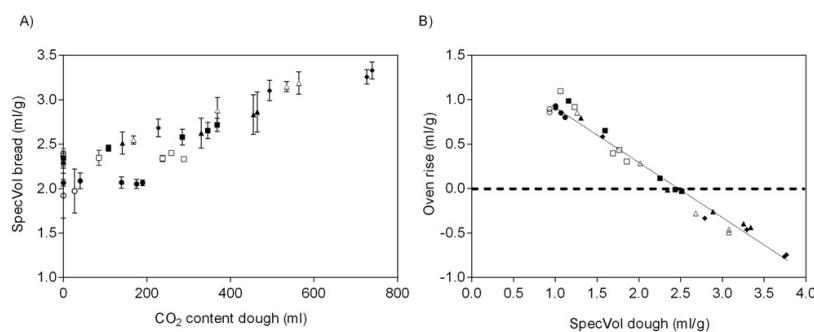

Fig. 4. Impact of different NaHCO$_3$ concentrations (and varying fermentation times) on bread volume and oven rise. To produce CO$_2$, the following NaHCO$_3$ concentrations per 100 g flour were added to react with glucono-delta-lactone: 0.45 g (○), 0.9 g (●), 1.35 g (□), 1.8 g (■), 2.25 g (▲), 2.7 g (△), 3.87 g (◆). For each NaHCO$_3$ concentration the fermentation times were varied according to *T0, T1, T1, T2,* and *Tx* using a Rheofermentometer. (A) Specific bread volume depending on the CO$_2$ content after resting using a Rheomfermentometer (CO$_2$ content for 150 g dough in Rheofermentometer was adapted to 250 g dough for baking). B) Oven rise (ml/g) — representing the specific volume increase during baking — over specific dough volume after resting using a Rheofermentometer(see Eq. (3)). Means (n = 3) are shown with standard deviation.
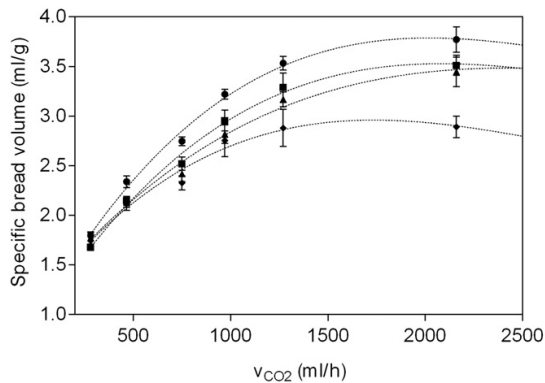
Fig. 5. Specific bread volume depending on glutathione and maximum CO_2 formation rate. Wheat dough was produced with yeast equivalent glutathione concentrations: 0.0 mg (●), 7.3 mg (■), 27.6 mg (▲), and 76.2 mg (◆) per 100 g flour. Different CO_2 formation rates resulted from varying $NaHCO_3$ concentrations (0.45–3.87 g per 100 g flour), as shown in Fig. 2. Gas formation rate was calculated from Rheofermentometer data. Dotted lines: cubic fit of data: ($R^2_{0.0\ mg\ GSH} = 0.9149$; $R^2_{7.3\ mg\ GSH} = 0.9725$, $R^2_{27.6\ mg\ GSH} = 0.9773$, $R^2_{76.2\ mg\ GSH} = 0.9865$). Means of triplicate baking trials (4 bread samples per trial) are shown with standard deviation.

GSH during kneading and resting, the specific bread volume was impaired. The rapid bubble expansion during the first baking phase likely resulted in destabilization when the gluten network was weakened through GSH. Similarly, more than 650 ml CO_2 per 250 g dough after resting produced a negative oven rise indicating an exceeded gas retention capacity.

In summary, the application of chemical-leavening systems revealed that the native material properties of wheat dough enabled linearly increasing aeration within the analyzed range. In addition to GSH, also other yeast metabolites might affect bubble stability in dough. Thus, the separate evaluation of e.g. glycerol or ethanol to chemically leavened dough as well as the challenge of monitoring gas release and bubble sizes in the oven should be addressed in future studies.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ifset.2016.03.006.

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3 Discussion

Saccharomyces cerevisiae serves as the primary leavening agent in the production of baked goods. Thereby the organism primarily serves as a tool to inflate the viscoelastic dough matrix, while the physiological state of the organism is mostly neglected. Yeast cells, however, dispose of technologically effective metabolites as a response to prevailing conditions during cell propagation, further processing and dough preparation. Not least due to the possibility to control yeast metabolites by the environmental conditions, yeast is a favorable model organism for numerous applications in medicine and bioengineering (Botstein *et al.* 1997). Nonetheless, high fluctuations can occur in the changes of type and quantity of metabolic products of *S. cerevisiae*, which can influence the dough structure beyond the intended inflation.

The present work provides a fundamental contribution to understand the destabilization of cereal based foams by the application of *S. cerevisiae*. The dough softening effect of yeast measured in a Z-kneading system was confirmed through oscillating in a rotational rheometer. The advantage of such a non-destructive fundamental rheological measurement in contrast to descriptive methods consists in the clarification of method- and process-independent material properties (Giesekus and Hibberd 1986). Since fundamental rheological parameters enable more insight into the microstructure and chemistry of the foam structure, the initial task of this study consisted in providing a fundamental rheological test method, which fits the needs of yeasted wheat dough. Based on existing oscillatory test methods, which are unsuccessfully applied for yeasted samples (Newberry *et al.* 2002, Salvador *et al.* 2006), the setting was adapted. The overall improvement consisted in a more gentle sample pre-treatment. Therefore, as a first step for improvement, the dough sample was fermented externally in a sample beaker at 30 °C and then transferred to the rheometer. Afterwards an equilibration time of ten minutes was performed to reduce the strain, which was induced by setting the gap. Moreover, using a crosshatched parallel plate/plate geometry prevented slipping over the dough samples as Newberry *et al.* described it in 2002 (Newberry *et al.* 2002). Due to the exclusion of method-dependent stress, the adjustments applied for current techniques enabled highly reproducible results to investigate the method-independent structural properties of yeasted wheat dough. The rheological data obtained in the following analysis confirm the weakening effect of varying yeast concentrations during a fermentation cycle of

135 min. The increasing fragility was noticeably reflected by a high susceptibility against pressure. The basis for subsequent investigations were the finding that immediately after kneading the loss factor tan δ increased asymptotically from 0.36 to 0.55 with linear rising yeast concentrations. The non-linear reduction of the elasticity indicates a yeast induced impairment of the structural integrity, which is limited by the dough system. The most likely candidates, which were analyzed in the course of this work are CO_2 and glutathione (GSH). While CO_2 causes a mechanical destabilization of the matrix and is limited by the availability of substrates (Dobraszczyk and Roberts 1994), GSH impairs the tertiary structure of the gluten network on the chemical level by disturbing the formation of native disulfide bonds (Gan *et al.* 1995). Finally, the interrelation between both metabolites was investigated (see Figure 6) to evaluate if a summation or an elimination of both mechanisms occurs during the breadmaking process.
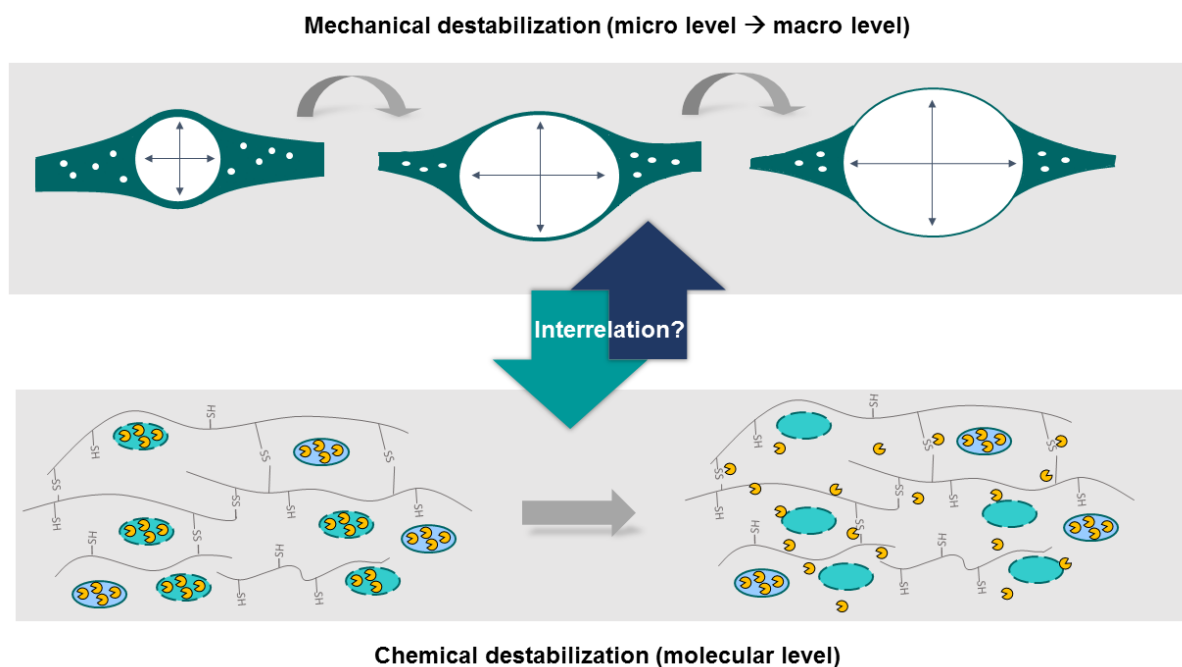

Figure 6: Assumed destabilizing interrelation between biaxially overstretched gluten strands by bubble growth (mechanical destabilization) and the depolymerization of the gluten network by yeast-introduced glutathione (chemical destabilization). Intact cells are shown as ellipsoid framed in blue, while lysed cells have a dotted line, and glutathione-molecules are represented as yellow-notched circles with ability to enter the dough medium to interact with disulfide bridges or to prevent the linkage of free thiol-groups.

By means of the Rheofermentometer, the impact of CO_2-formation kinetics and gas quantities on the structural integrity of the dough matrix was simulated via the application of varying numbers of colony forming units (cfu) of compressed (CY) and instant dry yeast (IDY). Due to the heat exposure of IDY it was assumed that both

trading forms show differences in type and quantity of their metabolic products and thus affect the dough structure to a different extent. The drawback of the Rheofermentometer, however, is the indirect measurement of the produced CO_2 volume as hydrostatic pressure on the one hand and missing information about CO_2-release kinetics on the other hand. Both were investigated by reprocessing the original Rheofermentometer data in chapter 2.3, to clarify the impact of gas release on the integrity of the matrix. By the addition of identical percentages of dry yeast, the number of cfu of CY and IDY was varied. Despite the fact that IDY has apparently ten-times less cfu than CY, the dough density after kneading was not lower than that for CY, and the density decreased with a higher slope. Moreover, despite less cfu in IDY, the v_{CO2} of 1630 ml/h was significantly higher than for CY with 1260 ml/h. These two factors suggest that IDY has a significantly higher vitality than fresh yeast samples with a considerably higher cell count. Since the dough medium was constant, differences in the type and quantity of metabolic products of both yeast forms have to be present.

Trehalose seems to be the most relevant reason for the activity increase of IDY, since the disaccharide acts as an energy reserve and is mainly accumulated during thermal and osmotic stress (Van Laere 1989, Hounsa *et al.* 1998). Thus, trehalose might shorten the adaption time of the cells to the prevailing conditions of the dough medium (Giannou and Tzia 2007). Glycogen provides as a further energy storage and is associated with high vitality of yeast populations. While it is generally accepted that trehalose protects the cells during stress, glycogen merely functions as a carbohydrate storage (Lewis *et al.* 1997, Singer and Lindquist 1998). Although glycogen is depleted by active yeast cells during storage it seems reasonable that the original glycogen content in dry yeast remains constant since the metabolism is inactivated by the reduced water content. Furthermore it seems plausible that zymase from lysed yeast cells catalyzes the fermentation of hexoses in the dough medium and thus simulates a higher yeast activity of IDY (Bajpai 2013).

In conjunction with the substrates provided by the dough medium, the total CO_2-volume of approximately 1500 ml per 315 g sample, however, was identical for both trading forms of *S. cerevisiae*. This indicates that rather the availability of phosphates, minerals, or amino acids limited the CO_2-production rather than enzymatically released hexoses. The time T_x, which is defined as time of porosity and describes the point of fermentation when gas escapes the dough matrix for the first time, differs significantly

for IDY and CY. Whereas for IDY the critical CO_2 content remained at an almost constant level between 538-564 ml per 315 g sample, the permeability of dough samples leavened with CY was reduced to a minimum of 372 ml per 315 g sample with rising gas formation rates. It seems that the application of IDY modifies the gluten network towards an improved extensibility. Since glutathione gets in the dough medium exclusively by lysed yeast cells and the number of cfu in IDY is ten times lower than that of CY, the reducing agent seems to be the most relevant candidate for the softening effect.
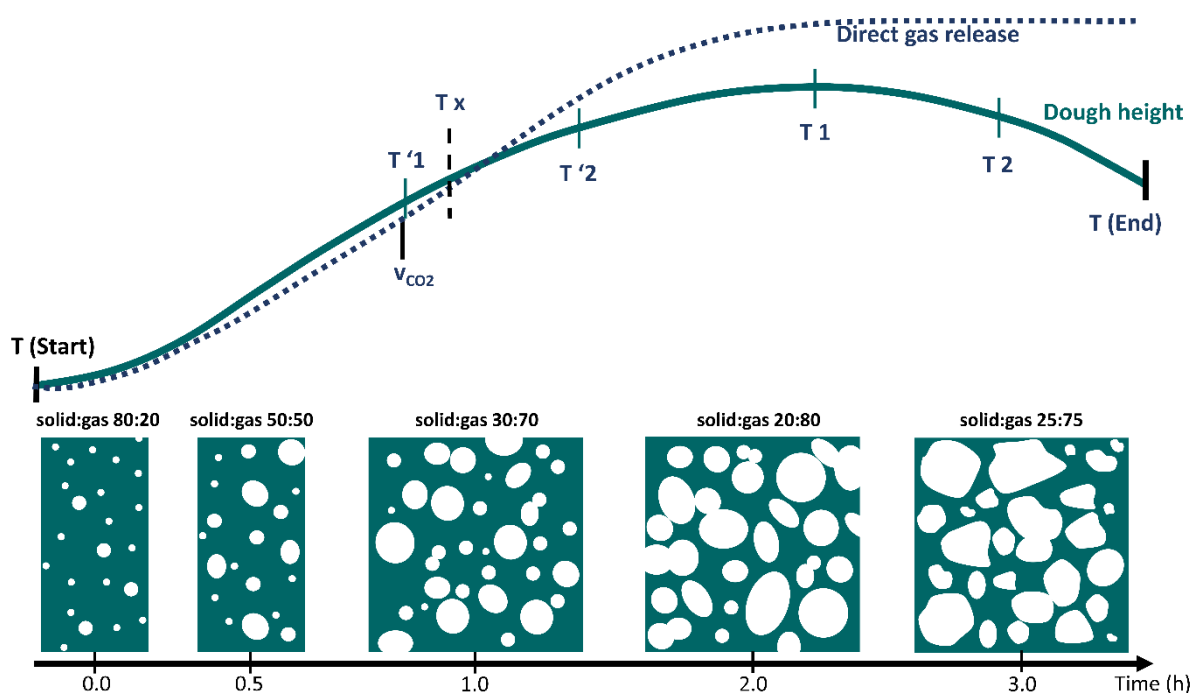

Figure 7: Gas-induced changes of the foam structure of yeast dough during a fermentation cycle of 3h at 30 °C at the example of 1% compressed yeast based on wheat flour in a Rheofermentometer. The size distribution of gas bubbles is represented at selected fermentation states and supplemented by the ratio of dough to gas. The dough–development curve (green line) and the direct gas release curve (blue dotted line) are represented with characteristic Rheofermentometer variables: T_1 Time to reach the maximum dough height, T'_1 Time to reach the maximum gas formation rate v_{CO2}, T'_2 Time to reach 90% of the maximum dough height, T_2 Time to reach 90% of the maximum dough height (after the maximum), T_x Time of porosity (gas starts to escape the dough matrix), v_{CO2} the maximum gas formation rate (slope of the tangent through the point of inflection).

Through the example of 1% CY (based on 100 g flour), Figure 7 summarizes the impact of the CO_2-formation kinetics and gas quantities on the structural integrity of the dough matrix during a 3h-fermentation cycle in the Rheofermentometer. Based on the bubble evolution monitored by Babin *et al.* (2006) via x-ray tomography, the schematic depiction of the cross-section through the dough matrix additionally describes the foam structure at different fermentation stages (Babin *et al.* 2006). It is obvious that the

development of the dough height is accompanied with changes of the gas cell structure and the changing ratio of dough to gas in favor of the fluid. After reaching the maximum dough height at T_1, the mechanical destabilization of the gluten network provokes the coalescence of gas bubbles. Consequently, the heterogeneity of the dough network increases and the CO_2 escapes by exceeding the gas retention capacity. Moreover, as presented by the blue dotted line, no further CO_2 is produced by the organism. This is mainly due to the limited substrate availability provided by the dough medium and the inhibition of the cell activity by an acid induced decrease of the dough pH below 5.5 (Graves *et al.* 2006).

Although, the gas retention capacity appeared to be improved by the quality and quantity of metabolic products of IDY, the final bread volume was considerably lower (3.3 ml/g) than with CY (3.9 ml/g). During thermal treatment, the evaporation of water and ethanol plus the bubble expansion caused additional mechanical stress for the partially GSH-ruptured gluten strands (Fan *et al.* 1999). To determine the hypothesis that predominantly dry yeast equivalent GSH quantities render the transformation process from dough to crumb, glutathione was measured in fresh and dried commercial yeasts and applied to non-leavened and chemically inflated dough.

The quantification of GSH was accomplished using a photometrical assay based on the Ellman's reagent in accordance with Anderson (1985) (Anderson 1985). The water-soluble GSH content of six compressed (CY) and 21 instant dry yeasts (IDY) was determined. Hence, without a forced disruption of the cell membrane, e.g. by a cell mill, the procedure ensures the quantification of the maximum GSH quantities, which passes from lysed cells into the liquid dough phase. The analyses demonstrated that the water-soluble content of GSH in CY ranges between 1.2 and 10.5 mg/g of yeast (db) and was, thus, significantly lower than the one of IDY (5.4-81.2 mg/g yeast (db)) which reached the dough medium. Moreover, a linear correlation (R^2 = 0.8483) between the amount of GSH and the proportion of dead cells was revealed. This confirms that rough processing conditions promote cell death (Sugiyama *et al.* 2000). Thus, drying can cause a remarkable GSH concentration of up to 8.4% (in terms of dry weight) in commercial products. This makes GSH appear an important component rather than a minor accompanying substance in *S. cerevisiae*. To prove that yeast equivalent glutathione quantities are sufficient to affect the integrity of the dough matrix subsequent structural analyses on non-leavened samples were conducted with 0.0,

7.3, 27.8, 58.6, and 76.2 mg GSH per 100 g flour. The reduction of dough development time and stability by 67% respectively 89% by the application of yeast equivalent GSH quantities were analyzed by descriptive rheological measurements in a torque-measuring Z-kneader. This is in accordance with Mauritzen and Stewart (1963) and Wieser (2007), who described that the sulfhydryl-disulfide interchange by GSH mainly occurs during the kneading process (Mauritzen and Stewart 1963, Wieser 2007). Furthermore, as represented by an asymptotic course, the destabilization cannot be enhanced with rising GSH quantities. This is in line with an asymptotic decrease of the complex shear modulus G* respectively an asymptotic increase of the loss factor tan δ gained with a rotational rheometer. As proven by Grosch & Wieser, the increase in the amount of glutathione has no further effect, since the number of cleavable disulfide bonds of glutenin chains in the gluten network is limited (Grosch and Wieser 1999). Hence, as proven by descriptive and fundamental rheological test methods, the hypothesized weakening effect of yeast equivalent GSH-quantities on non-leavened dough structures was confirmed. In this context, Figure 8 provides a graphical summary of the mode of action.
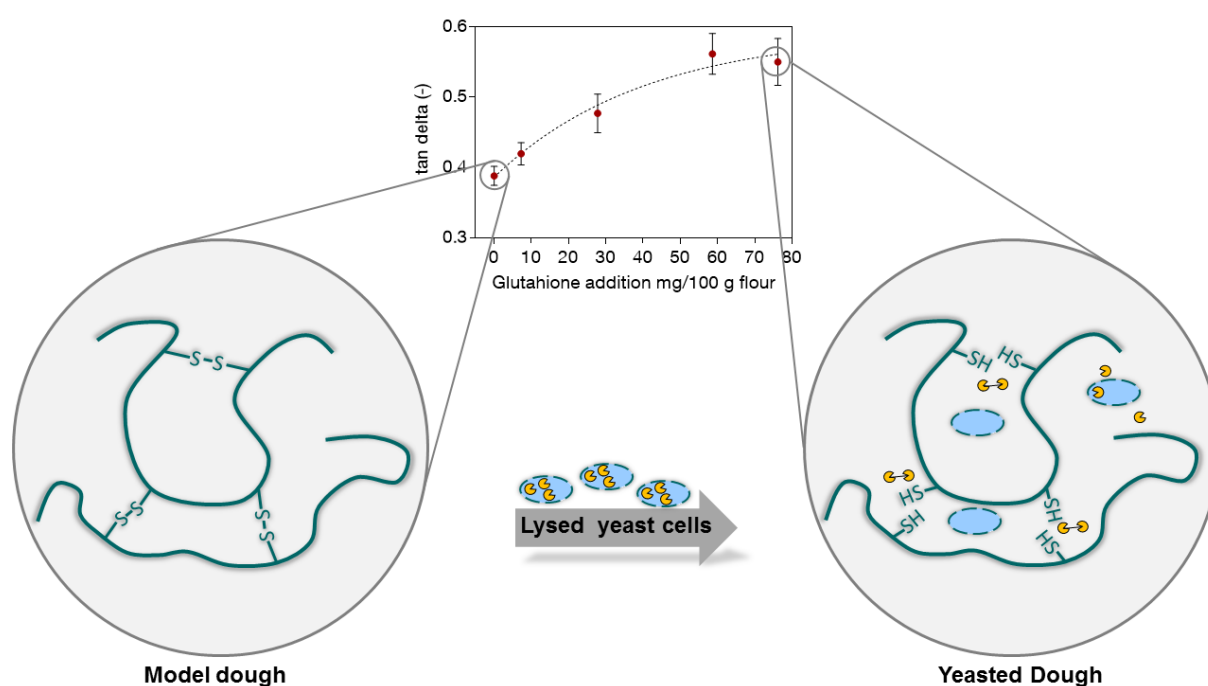
Figure 8: Impact of glutathione from yeast on the structural integrity of non-leavened wheat dough samples. Lysed cells have a dotted line; glutathione-molecules are represented as yellow-notched circles with ability to enter the dough medium to interact with disulfide bridges or to prevent the linkage of free thiol-groups.

However, it remains unclear if the disintegrative effect of GSH is valid in the presence of gas and to which extent breadmaking performance is impaired. By the application of GSH in combination with a chemical-leavening system based on sodium acid

pyrophosphate 10 (SAPP 10) and sodium bicarbonate ($NaHCO_3$) a weakening interrelation between both metabolites was examined independently from the yeast metabolism. The increase of the weakening coefficient proves that dry yeast equivalent GSH quantities reduced the gas retention capacity by up to 20.4%. However, the interrelation between GSH and the release characteristics of CO_2 on dough development and end product quality was not considered in the study presented in chapter 2.4. Therefore, within the scope of this work, further analyses aimed to clarify a dough weakening interrelation between GSH and CO_2.

Contrary to the before mentioned SAPP-based chemical-leavening system, CO_2 was generated by glucono-delta-lactone (GDL, $C_6H_{10}O_6$) and sodium bicarbonate ($NaHCO_3$). The release of CO_2 depends on the continuous cleavage of the gluconic acid ($C_5H_{11}O_5COOH$) from the lactone according to Equation 7:

$$C_6H_{10}O_6 + H_2O \rightarrow C_5H_{11}O_5COOH + NaHCO_3 \rightarrow C_5H_{11}O_5COONa + H_2O + CO_2 \qquad \textbf{Equation 7}$$
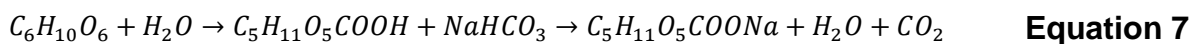
Due to the continuous acid release, GDL has similar leavening characteristics as yeast and is therefore more suitable for the breadmaking process than SAPP. Moreover, as valid for any chemical-leavening system, the stoichiometric reaction offers the advantage of a substrate-independent release of defined CO_2-quantities. The slight alkaline pH-shift due to undissipated reactants is the only drawback, which has to be considered in the context of interpreting structural data, since it strengthens the gluten structure (Jekle and Becker 2012).

Initially, the necessary concentration range of $NaHCO_3$ for imitating the CO_2 production of for *S. cerevisiae* was determined in a Rheofermentometer. As a result, the following $NaHCO_3$ concentrations of 0.45, 0.9, 1.35, 1.8, 2.25, 2.7, and 3.87 per 100 g flour were applied. Analogous to the trials with CY and IDY, the gas formation kinetics were determined by reprocessing the hydrostatic-pressure curves gained with the Rheofermentometer.

The selected concentration range of the chemical leavening system was suitable for simulating v_{CO2} (279.0 – 2283.2 ml/h) reached with compressed and instant dry yeast (300 – 1650 ml/h). Furthermore, the maximum formation rate of CO_2 was logically not affected by the presence of GSH, which implies that within typical dough process limits, the gas-forming reaction is independent from material properties of the matrix. Since

dough density after kneading was reduced with rising GSH concentrations, the gas development during kneading depended on the GSH-induced enhanced extensibility of the gluten network. Only the pH-induced strengthening of the gluten network by unreacted $NaHCO_3$ counteracts the improved inflation. The total gas volume in dough increased linearly with accelerated gas production. Thus, the ability of wheat dough to retain CO_2 exclusively depends on the velocity of CO_2-production and is not affected by GSH or by the material properties, respectively.

The effect of GSH on inflated dough matrices was analyzed independently from other yeast metabolites for the first time. Contrary to the results gained with descriptive and fundamental rheological methods, which revealed a significant dough softening, it was shown that the aeration of the dough matrix was overall improved by yeast equivalent GSH quantities. This is also reflected by the reduction of the time to reach v_{CO2}. Glutathione induces a reduction of the gluten network cohesion and improves the extensibility of the dough matrix. Moreover, the noticeable increase in dough stickiness, when protein-bound water is released, may support the solubility of CO_2.

Trials with chemical leavening systems therefore provide an opportunity to analyze the effect of individual or combined yeast metabolites. Besides, as represented by the reduction of the fermentation time by a factor of 12, the application of chemical-leavening agents offers the possibility for a significant reduction of the bread production period by 50%. Despite the possible alkaline or metallic aftertaste produced by $NaHCO_3$ as well as some acid carriers like sodium acid pyrophosphate (SAPP), chemically leavening provides the potential for the production of low-cost-high-quality baked goods to counteract rising global food shortages. This is congruent with the original reason Justus Liebig and Eben Norton Horsford developed baking powder in 1833. The overall objective was to avoid weight loss of up to 3% carbohydrates in the course of yeast fermentation to counteract bread shortage in Germany (Brose *et al.* 1996).

Up to here, the negatively assumed weakening interrelation between GSH and CO_2 could not be confirmed. The definitive statement, however, exclusively depends on the final loaf characteristics. Baking trials initially revealed that higher gas volume fractions in dough after resting also resulted in higher bread volume. Despite the positive effect of yeast equivalent GSH quantities for the aeration of wheat dough, the specific bread volume was significantly impaired. The following explanation provides a theoretical

approach for the possible mode of action, which has to be investigated in further studies. During the transition from dough to crumb, the structure stabilization is based on starch gelatinization by an irreversible loss of the native granule structure and gluten cross-linking at 50–70 °C (Babin *et al.* 2006). Especially the level of integration of the glutenin structure is affected dramatically by heat induced denaturation during baking. As postulated by Wieser (1998) the extractability in urea is reduced and most of the cysteine containing α/β- and γ-gliadins are covalently bound to glutenin polymers (Wieser 1998). Since GSH interfere with the formation of the gluten network, the heat-induced gluten cross-linking during baking becomes impaired. Accordingly, the rapid growth of bubbles during the first baking phase (oven rise) induced by thermal gas expansion and evaporation renders the bubble stabilizing 'strain hardening' of the gluten matrix (Dobraszczyk and Roberts 1994). Gas bubble fixation, therefore, occurs mainly by the increase in viscosity precipitated by starch gelatinization. The confirmation of the hypothesis can be generated easily via an inline imaging technique. As represented by Schirmer *et al.* (2014) the confocal laser scanning microscopy combined with a heating plate is suitable to illustrate microstructural changes during baking (Schirmer *et al.* 2014). Through the staining of the gluten network and the following detection of the expected reduced number of intersections between individual gluten strands the hypothesis can be confirmed.
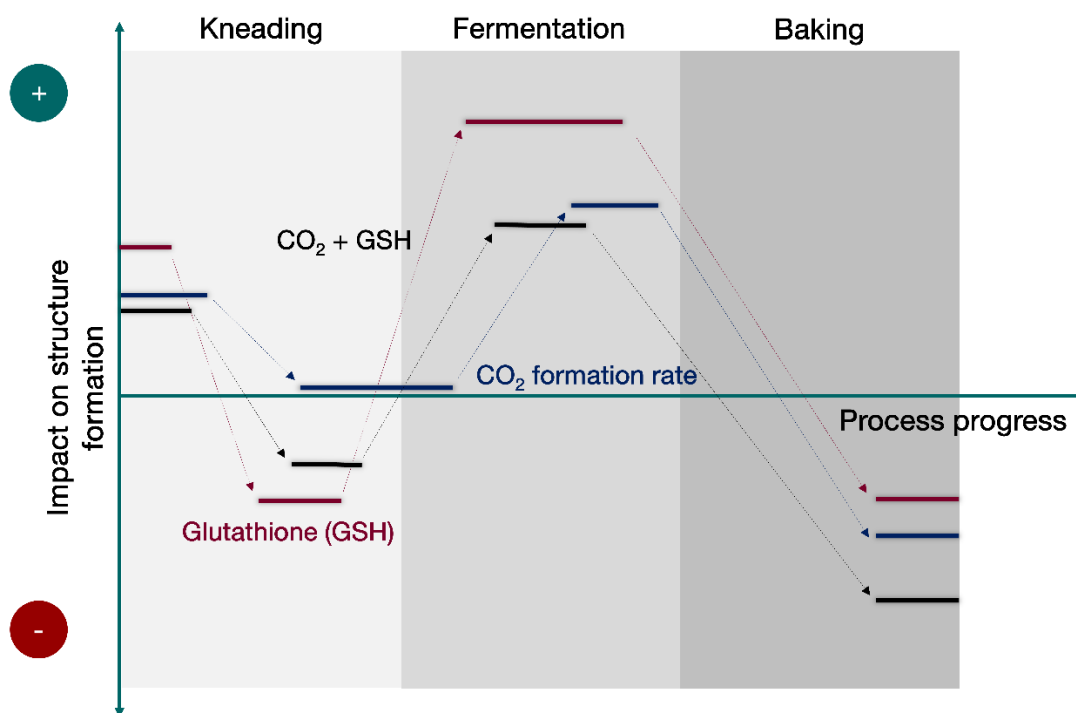

Figure 9: Impact of CO_2 formation rate (blue), glutathione (purple), and CO_2 formation rate + glutathione (black line) on the structural development during the main breadmaking process steps.

Figure 9 summarizes the individual and combined structural impact of GSH and CO_2 during the breadmaking process. While especially GSH from dry yeast impaired the processability during kneading, the gas-holding properties during fermentation were improved in the presence of the tripeptide. The effect of the CO_2 formation rate improved the fermentation process considerably, since high velocities enhanced the CO_2 volume, which was retained by the dough matrix. The hypothesis of a weakening interrelation between yeast-equivalent GSH quantities and CO_2 release kinetics became particularly evident during the baking phase, since the bread volume was reduced in the presence of GSH and high gas formation rates.

This study, moreover, demonstrates that especially the trading form of *S. cerevisiae* and the process conditions during yeast production define the GSH content in yeast. Controlling the process conditions could thus be an alternative to generate products with defined GSH contents to improve the overall baking performance. Besides, yeast can be applied as a hybrid-baking agent with clean label properties to control structuring processes of wheat dough and the resulting end products by providing specific compositions of metabolites. Overall, the successfully resolved weakening interrelation between CO_2 and GSH enables a mechanistic understanding of the technological activity of yeast, which goes far beyond the inflation of baked goods.

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5 Appendix

5.1 Reviewed Paper

1. <u>Verheyen, C.</u>, Albrecht, A., Becker, T., Jekle, M.: Destabilization of wheat dough: Interrelation between CO2 and glutathione, Innovative Food Science and Emerging Technologies 34 (2016), 320-325, doi: 10.1016/j.ifset.2016.03.006

2. <u>Verheyen, C.</u>, Albrecht, A., Elgeti, D., Jekle, M., Becker, T.: Impact of gas formation kinetics on dough development and bread quality, Food Research International 76 (2015), 860-866, doi:10.1016/j.foodres.2015.08.013

3. <u>Verheyen, C.</u>, Albrecht, A., Herrmann, J., Strobl, M., Jekle, M., Becker, T.: The contribution of glutathione to the destabilizing effect of yeast on wheat dough, Food Chemistry 173 (2015), 243-249, doi:10.1016/j.foodchem.2014.10.021

4. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Effects of S. cerevisiae on the structural kinetics of wheat dough, LWT - Food Science and Technology 58 (2014), 194-202, doi:10.1016/j.lwt.2014.02.050

5.2 Non-reviewed paper

1. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Baking performance of glutathione in yeasts, baking+biscuit 1 (2016), 54-57

2. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Backtechnologische Relevanz von Glutathion in Hefe. brot+backwaren 6 (2015), 52-55

3. <u>Verheyen, C.</u>, Peréz Alvarado, F., Sayed, A., Hussein, M.A., Jekle, M., Becker, T.: Study of the dough development by digital image viewing of the surface. Backtechnik Europe 1 (2014), 28-33

4. <u>Verheyen, C.</u>, Peréz Alvarado, F., Sayed, A., Hussein, M.A., Jekle, M., Becker, T.: Kameragestützte Untersuchung der Teigentwicklung. Backtechnik Europe 1 (2014), 30-35

5. <u>Verheyen, C</u>., Pèrez Alvarado, F., Sayed, A., Hussein, M.A., Jekle, M., Becker, T.: Untersuchung der Teigentwicklung mittels digitaler Oberflächenbetrachtung. Cereal Technology 1 (2014), 4-9

6. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Influences on dough development. baking+biscuit 3 (2012), 40-43. 147

5.3 Oral presentations

1. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Wieviel Gas verträgt der Teig? – Zusammenhang zwischen Teigeigenschaften und Gasbildungsrate, 5. WIG Frühjahrstagung, Freising, Germany, 2016-04-06

2. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Auswirkung differenter Gasbildungs-kinetiken auf die Materialeigenschaften von Stärke-Protein-Matrices, Jahrestreffen der ProcessNet-Fachgruppe Lebensmittelverfahrenstechnik, Erlangen, 2016-03-10

3. <u>Verheyen, C.</u>, Bernklau, I., Jekle, M., Becker, T.: Print it – Backen in der Zukunft, II. Bakers' Day 2016, Bremerhaven, 2016-03-03

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

5. <u>Jekle, M.</u>, Hackenberg, S., Verheyen, C., Elgeti, D., Tietze, S., Becker, T.: Hintergründe zur aktuellen Mehlanalytik, Getreidefachtagung, Freising, Germany, 2015-07-08.

6. <u>Verheyen, C.</u>, Bernklau, I., Jekle, M., Becker, T.: Brotbacken – Druckfrisch in die Zukunft, 9. Kolloquium der VDB Österreich, Schladming, Austria, 2015-06-15

7. <u>Jekle, M.</u>, Hackenberg, S., Verheyen, C., Elgeti, D., Tietze, S., Becker, T.: Vorschau zum Thema: Mehlqualität – sind unsere Analysen noch zeitgemäß?, VDB-Tagung, Freising, Germany, 2015-04-24

8. <u>Verheyen, C.</u>, A., Jekle, M., Becker, T.: Teigführung – Ein Prozessschritt zur Steuerung der Qualitätsmerkmale Struktur und Frischhaltung von Backwaren, 4. WIG Frühjahrstagung, Freising, Germany, 2015-04-22

9. <u>Verheyen, C.</u>, Bernklau, I., Jekle, M., Becker, T.: Gutes Brot noch besser machen: Getreideforschung 2050. Brotforum 2015, Weinheim,Germany, 2015-02-05

10. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Einfluss unterschiedlicher Teigführungsarten auf die Knusprigkeit von Kleingebäcken, 65. Tagung für Bäckereitechnologie, Detmold, Germany, 2014-11-12

11. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: The contribution of glutathione to the destabilizing effect of yeast on wheat dough, AACC International Annual Meeting 2014, Providence, USA, 2014-10-07

12. <u>Verheyen, C.</u>, Albrecht, A., Herrmann, J., Strobl, M., Jekle, M., Becker, T.: Einfluss hefetypischer Glutathionmengen auf die Weizenteigbildung, 3. WIG Frühjahrstagung, Freising, Germany, 2014-04-02

13. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Impact of phosphate raising agents on the structural kinetics of wheat dough, C&E Spring Meeting, Leuven, Belgium, 2013-05-29

14. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Einfluss mehleigener Stärke auf die Teigbildung und –reifung, Vereinigung der Backbranche, Frühjahrstagung, Freising, Germany, 2013-03-22

15. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Entwicklung eines chemisch gelockerten Weizenbrotes und rheologische Charakterisierung des Einflusses von Phosphattriebmitteln auf die Teigstruktur, 2. WIG Frühjahrstagung, Freising, Germany, 2013-03-20

16. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Reduzierende Stoffwechselprodukte von Hefen und deren Einfluss auf die Strukturkinetik von Weizenteigen während des Knetprozesses. Tagung für Getreidechemie, Detmold, Germany, 2012-06-21

17. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Einfluss variierender Energieeinträge auf die Strukturbildung von Weizenteigen. 1. Frühjahrstagung des WIG, Freising, Germany, 2012-03-28

18. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Einfluss verschiedener Prozess- und Produktparameter auf die Teigentwicklung und die Qualität von Weizenbroten, Ausschuss für Bäckereitechnologie, Minden, Germany, 2012-03-22

19. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Methodik zur Erfassung rheologischer Kenndaten hefegelockerter Weizenteige während der Gare. Tagung für Getreidechemie, Detmold, Germany, 2011-06-28

20. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Characterization of the rheological development of yeasted dough during fermentation. 10th European Young Cereal Scientists and Technologists Workshop, Helsinki, Finland, 2011-05-25

5.4 Poster presentations

1. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Characterization of Yeasted Wheat Dough during Fermentation, 1. Frühjahrstagung des WIG, Freising, Germany, 2012-03-28

2. <u>Verheyen, C.</u>, Jekle, M., Becker, T.: Methodenabhängiger Dichteverlauf von Weizenteigen für verschiedene Trockenhefemengen der Species *S. cerevisiae*. 18. Internationale Tagung IGV, Nuthetal, 2011-05-09

5.5 Curriculum Vitae

Christoph Verheyen
M.Sc.
*30.10.1984

Work Experience

Since 01/2011	PhD Student, Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, Freising, Germany

Education

09/2008 - 11/2010	M.Sc., Master of Science in Food Technology and Biotechnology, Technische Universität München, Freising, Germany
09/2005 - 07/2008	B.Sc. Bachelor of Science, Ostwestfalen Lippe University of Applied Sciences, Lemgo, Germany
08/1995 - 06/2004	Abitur, Freiherr vom Stein Gymnasium, Kleve, Germany