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Fundamental studies on sodium reduction in processing cereal-based foods

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Nur Begeisterung hilft über die Klippen hinweg, die alle Weisheit der Erde nicht zu umschiffen vermag.

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Cᴏɴᴛᴇɴᴛs

SUMMARY

The political pressure to reduce NaCl in cereal-based food products is not only evident and increasing, but appears to be well-founded. Dietary sodium reduction is an important factor in the prevention of hypertension and related cardiovascular diseases. Most of all, cereal products are particularly affected by regulations, since ca. 25% of the required sodium intake per day and person is taken up by cereal products. Therefore, cereal products are regarded as the sodium main supplier in the human diet.

However, the traditional use of sodium chloride (NaCl) fulfils various important rheological, technological, sensory and preservatory properties in the manufacturing of baked goods. Thus, reducing NaCl in cereal-based products will have consequences for food quality that must be taken into account. These consequences for cereal-based foods are analyzed stepwise and in detail in this work to establish the necessary background knowledge to reduce sodium without changing product quality.

The rheological properties of wheat dough for yeast-leavened products were tested at different levels of NaCl addition ranging from 40 to 0 g NaCl kg^{-1} wheat flour. Highly significant (P $\leq$ 0.001) differences due to NaCl reduction were determined by the stickiness test and the examination of the creep test using the Burger model in particular. Rheological changes measured in the creep test seem to depend on protein charge shielding due to NaCl interaction, which alters gluten network formation. A decrease in cross-linked gluten molecules with less NaCl was confirmed by confocal laser-scanning microscopy (CLSM).

Complementary to rheological behavior, proofing ability and end product quality of these wheat dough (40 to 0 g NaCl kg^{-1}), crumb firmness and rate of staling decreased with decreasing NaCl levels. An increase in bread volume was observed based on the increased yeast leavening ability resulting from decreasing NaCl. Significant (P $\leq$ 0.05) linear relationships (r $\geq$ 0.829) between Rheofermentometer results, bread volume, and crumb firmness were found, suggesting a predictability of bread quality by measurement of gas release. Higher retrogradation (measured by differential scanning calorimetry) was observed with low NaCl levels for the first time. The retrogradation effect is based on the theory that

NaCl probably leads to Na^+ inclusion in starch molecules during storage and reduces retrogradation.

The extent to which this theory of NaCl induced retrogradation is relevant was analyzed using a model starch-water system as a function of various sodium replacers. Starch-water suspensions with various chloride salts (LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, and NH4Cl) were heated in a DSC until starch gelatinization, stored from 0 up to 504 h at 8 °C, and reheated again. The Avrami equation was used to evaluate the rate of starch re-crystallization, and indicated that the starch re-crystallization rate (k) is significantly ($p < 0.01$) reduced with the addition of a cation. Furthermore, bivalent cations such as Ca^{2+}, Mg^{2+} decreased the starch re-crystallization rate (k) more than univalent cations (Li^+, NH_4^+, Na^+, and K^+). This result may be based on two theories: (1) the above stated theory about starch as an ion exchanger and (2) the theory that cations with higher charge densities show greater hydration, and, therefore, lower a_w-values, than cations with lower charge densities. The results illustrate important and novel results for predicting starch quality change when using sodium replacements.

This work displays, for the first time, a comprehensive scientific study of the effect of NaCl on wheat dough and bread by means of a number of rheological and technological measurements, over a wide range of NaCl levels, to obtain an improved understanding of the effect of NaCl on wheat dough and yeast-leavened products. Furthermore, the results achieved by this study provide a valuable basis for designing functionally effective sodium replacers. Additionally, the current work provides also novel results in the field of starch quality changes due to the use of sodium replacers.

ZUSAMMENFASSUNG

Der politische Druck zugunsten einer Natriumreduktion in Lebensmitteln steigt seit Jahren unverkennbar an. Die Reduktion der durch Lebensmittel aufgenommenen Natriummenge wird als wichtiger Faktor zur Prävention von Bluthochdruck und damit verbundenen Herz-Kreislauferkrankungen genannt. Insbesondere getreidebasierte Lebensmittel stehen bei der geforderten Natriumreduktion im Vordergrund, da diese bis ca. 25% der täglichen Natriumaufnahme ausmachen können. Eine Natriumreduktion erweist sich jedoch als komplex, da Natriumchlorid (NaCl) essentielle rheologische, technologische, sensorische wie auch konservierende Effekte in Backwaren erfüllt. Es zeichnen sich somit nicht zu vernachlässigende Auswirkungen einer Natriumreduktion auf die Backwarenqualität ab. Diese Auswirkungen werden in der nachfolgenden Arbeit schrittweise und detailliert aufgezeigt, um die notwendigen Hintergründe zu erschließen, Natrium ohne Qualitätseinbußen zu reduzieren.

Die rheologischen Eigenschaften von Weizenteigen wurden bei verschiedenen NaCl Konzentrationen (40-0 g NaCl kg^{-1} Weizenmehl) ermittelt. Hochsignifikante Unterschiede durch eine Natriumreduktion konnten insbesondere durch Kriechkurven und deren Auswertung ermittelt werden. Rheologische Veränderungen beruhen wahrscheinlich auf einer verminderten Ladungsabschirmung der Glutenmoleküle durch NaCl und somit auf einer geringeren Proteinquervernetzung, welche in einem Konfokalen Laser Scanning Mikroskop (CLSM) nachgewiesen werden konnte.

Ergänzend zu den rheologischen Messungen wurde das Gärverhalten der Weizenteige sowie die Backwarenqualität mit 40-0 g NaCl kg^{-1} Mehl experimentell untersucht. Eine verminderte NaCl Zugabe führt zu sinkender Krumenfestigkeit, langsamerem Altbackenwerden sowie eine Erhöhung des Gebäckvolumens, welche auf einer Zunahme der Hefegäraktivität zurückzuführen ist. Die Annahme, dass die Gebäckqualität durch Messung der Gasfreisetzungskinetik während der Gare vorhergesagt werden kann, wurde durch lineare sowie signifikante Zusammenhänge (r ≥ 0.829) zwischen der Gasfreisetzung und der Gebäckqualität bestätigt.

Ferner konnten neue Erkenntnisse über die Stärkeretrogradation (gemessen mittels Differential Scanning Kalorimetrie, DSC) erzielt werden. Es zeigt sich erstmals, dass eine Reduktion von NaCl eine erhöhte Rekristallisation hervorruft. Eine Erklärung hierfür könnte die Theorie sein, dass Stärke als schwacher Ionenaustauscher fungiert, und daher Na^+ Ionen in die Stärke einlagert werden, welche die Retrogradation stören. Um zu überprüfen, inwiefern die Theorie für die Retrogradationsveränderungen durch NaCl und Natriumsubstitute möglich ist, wurden Stärke-Wasser Systeme mit verschieden Natriumersatzstoffen ($LiCl$, $NaCl$, KCl, $MgCl_2$, $CaCl_2$ und NH_4Cl) versehen, in der DSC erhitzt, gelagert und die Verkleisterungs- und Retrogradationseigenschaften ermittelt. Durch die Verwendung der Phasenumwandlungstheorie nach Avrami konnte gezeigt werden, dass die Rekristallisationsrate (k) durch die Zugabe von Kationen signifikant erniedrigt wird (unabhängig welche Kationen eingesetzt werden). Weiterhin zeigten zweiwertige Kationen wie Ca^{2+}, Mg^{2+} eine stärkere Reduktion der Rekristallisationsrate (k) im Vergleich zu einwertigen Kationen (Li^+, NH_4^+, Na^+, und K^+). Dieses Ergebnis kann auf folgenden zwei Theorien basieren: (1) auf der zuvor genannten Annahme, dass Stärke als Ionenaustauscher fungiert und größere Ionen die Retrogradation stärker beinträchtigen als kleinere, sowie (2) auf der Hypothese, dass kleinere Ionen aufgrund höherer Ladungsdichte eine stärkere Hydratation aufweisen. Diese Ergebnisse zeigen wichtige und vor allem neuartige Erkenntnisse zur Vorhersage der Stärkeveränderung bei Nutzung von Natriumersatzstoffen.

Zusammenfassend stellt diese Arbeit erstmals eine umfassende, wissenschaftlich-fundierte Studie dar, welche die Effekte von NaCl auf Weizenteigen und –backwaren durch eine Vielzahl an rheologischen und technologischen Messungen und einer Bandbreite an NaCl-Konzentrationen und Natriumsubstituenten analysiert. Hierdurch wurde ein tiefes Verständnis über die detaillierten Vorgänge von NaCl in Weizenteigen und –backwaren erworben, welche bei der Entwicklung geeigneter Natriumersatzstoffe für Hefe-gelockerte Lebensmittel essentielle Hilfestellung leistet. Weiterhin zeigt diese Arbeit neuartige Ergebnisse im Bereich der Stärkeveränderungen durch verschiedene Natriumsubstitute auf.

1.   INTRODUCTION

1.1 Sodium chloride in baked goods

The usage of sodium chloride (NaCl) in food has come under critical discussion. It is supposed that an increased dietary NaCl intake is a potential but not the primary reason for hypertension and related diseases. In spite of this, cardiovascular diseases caused by high blood pressure (stroke, cardiac infarction and cardiac insufficiency) are regarded worldwide as the most common causes of death [1]. Furthermore, the daily dietary intake of NaCl in Western countries is excessive, and a gradual reduction is highly desirable [2].

To maintain physiological processes, an adult requires an average daily intake of approximately 1.5 g NaCl per day [3]. However, in Western Europe men consume approximately 9-14 g per day and women 7-10 g [4]; and thus, the WHO recommend an average daily consumption of 5 g of NaCl [5]. As such, health claim regulations have been implemented in Europe to define the relationship between substances (such as sodium) and the risk of a disease or health-related condition [6]. NaCl is a disqualifying ingredient in the health claims regulation [7, 8], meaning food labeling regarding nutritional value is not permitted for foods that contains excessive sodium content.

Up to around one-third of the required sodium intake per person per day comes from starch-based products such as bread [9, 10]. Therefore, starch-based products are regarded as the main sodium suppliers of the human diet. A capable and cost-effective solution for decreasing death from coronary heart disease and stroke induced by hypertension is to reduce the quantity of NaCl in food, and starch-based products such as baked goods in particular.

However, the traditional use of NaCl in food provides essential preserving, rheological, technological, as well as sensory effects [11]. The sensory effects are not only founded on the salty taste elicited by the sodium ion [9], moreover NaCl contributes to general taste enhancement. That is, the addition of NaCl increases sweetness and hides metallic, bitter or other negative flavors [12]. Salt perception also depends on the viscosity and texture of the

surrounding matrix [13]. The salty taste can only be perceived if the salt crystals are in a solved, unbound form and reach the salt receptors quickly during consumption. NaCl also has a considerable effect on yeast-leavening ability as well as the rheological behavior of wheat dough [11]. Rheological changes in wheat dough directly influence the quality of baked goods. Thus the reduction of NaCl in bakers' recipes will influence the global manufacturing process of baked goods.

Rising food awareness, the ambition of the legislator to influence the consumption habits of the population positively and the fact that the daily dietary sodium intake is mainly caused by starch-based products, occasioned the food industry to react. From this finding, it can be deduced that the political pressure to reduce sodium in food, or rather in baked goods, is elevated. A broad, step by step knowledge about the rheological and technological effects of reducing sodium chloride in baked goods is therefore of great interest to all manufacturers of starch-based products. Thus, the following chapters depict possibilities and previous research useful for analyzing this rheological and technological effect while reducing the levels of NaCl and the usage of sodium replacers.

1.2 Wheat flour ingredients and their impact on dough and end-product quality

To analyze the rheological and technological effect while reducing the levels of NaCl or while usage of sodium replacers it is important to have a large fundamental knowledge about wheat flour ingredients and the processing of starch-based foods. Concerning the inherent parts of wheat grain ingredients the starch fraction dominates (67-70 g 100 g^{-1} grain), followed by proteins dominates (12-14 g 100 g^{-1} grain), total dietary fibers (10-13 g 100 g^{-1} grain), lipids (3 g 100 g^{-1} grain), and mineral substances (2 g 100 g^{-1} grain) [14, 15]. The characterization of each fraction will be discussed in following chapters.

The process chain of the production of baked goods can be split into three main process steps as well as the subsequent storage. Whereat, every process step features different influence on the end-product quality and is substantially influenced by different ingredients. The first two process steps (Figure 1) feature typical effects on the end-product by influencing pore distribution in the end-product, and with this the crumb structure, due

to the formation of the protein network during the mixing process. In the subsequent proofing phase, the protein network substantially influences the volume of the end-product due to its gas retention property. During the thermal influence of the baking process, the starch attains an important factor concerning the composition containing effect on the end-product, while the protein loses its impact for the end-product structure. By denaturing the proteins, water is released and this water is mainly used by the starch for gelatinization [16]. During gelatinization, starch granules multiple up to 3x of its original size. During the cooling process and the subsequent storage of the end-product, a re-crystallization process of the starch appears which substantially influences the quality of the end-product. Because both flour fractions (protein and starch) influence the end-product structure, the effects of modifying these two ingredients on the end-product are thoroughly characterized in the following.

Mixing Process
- Formation of a **protein** network which defines the dough structure as well as the dough rheology.

Proofing
- The **protein** network influences the gas holding properties and characterizes thus the volume of the end-product.

Thermal Treatment
- Protein denatures and releases water, which is taken by the starch for gelatinization. Thus, **starch** contributes to the end-product quality.

Storage
- **Starch** retrogradation during storage correlates with quality reduction in the end-product.

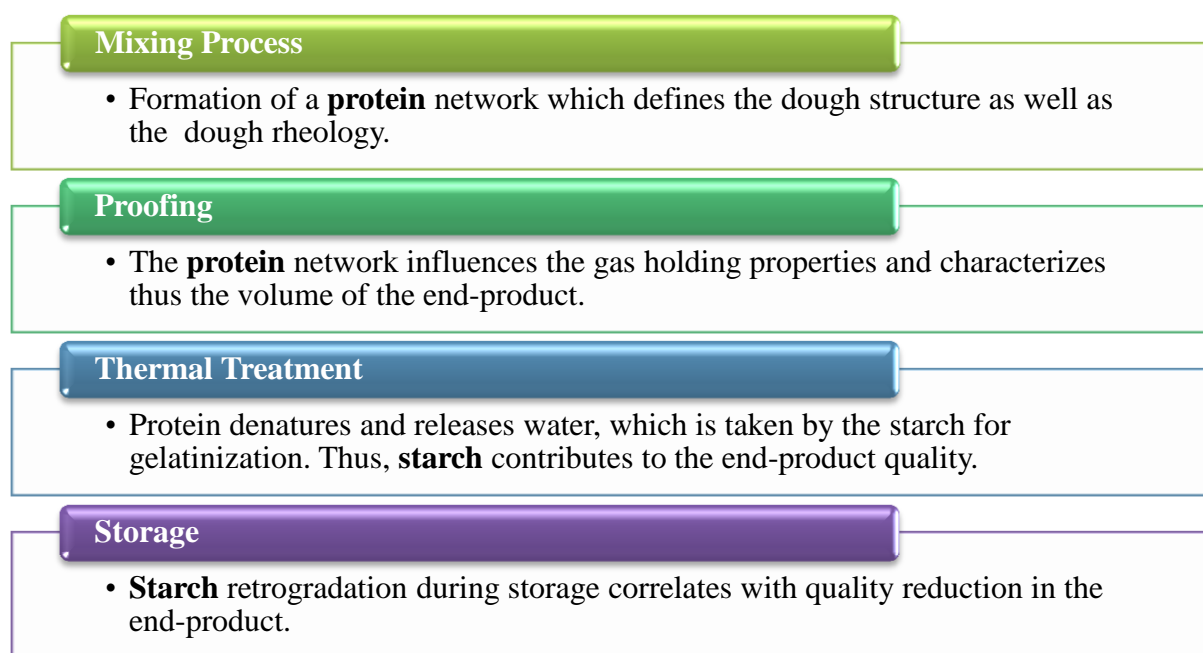**Figure 1: Schematically classification of the essential grain ingredients of wheat and their structural effects in the end-product.**

1.2.1 Protein characterization

The structural properties of thermally treated flour-water systems, such as bakery products, are, in addition to the starch fraction, decisively based on the protein matrix. Wheat proteins play an essential role in achieving porous, voluminous, starch-based

products [17-19]. On this occasion the continuous visco-elastic protein network (gluten) provides an essential function for achieving high quality end-products [14]. Gluten is formed by mechanical energy input, during the mixing process, acting on glutenin, gliadin, and lipids. In combination with this protein composition, the gas retention ability of baked goods is primarily based on the yeast metabolic fermentation product (CO_2) of wheat dough. This leads to a porous, fluffy and elastic crumb with high end-product volume [20]. A gluten-starch matrix prevents the coalescence of gas bubbles in the flour-water system during the proofing phase [21]. If this distinctive gluten network is absent during dough manufacture, due to poor protein quality, quantity or imperfections, the gas retention ability often decreases during proofing and baking. As a result, baked goods are formed with low volume and low crumb porosity which results in high crumb firmness [22].

In wheat dough, the typical protein network structure is formed by the interlinking of the glutenin subunits to highly molecular aggregates and by the interaction of the polymeric glutenins with monomeric gliadins via application of energy input during mixing. Thus, wheat proteins are defines the visco-elastic properties of the wheat dough. The visco-elasticity is based on the elastic qualities of fibrillar glutenins [23] which form a polymer network system with the gliadins, and which are available as monomers and are responsible for the viscous functions of the dough [14, 23-25]. To be able to characterize the quality differences of wheat proteins, these are split up in four groups by means of Osborne fractionation: albumins (soluble in water), globulin (soluble in salt solution), gliadin (soluble in 70% ethanol), and glutelin (partially soluble in acid or base). Gliadin (prolamin) and glutelin (glutenin) constitutes approximate 80% of the total quantity of wheat proteins in the flour [26, 27].

1.2.2 Starch characterization

Starch is the main component of flour and therefore the most-represented carbohydrate in dough. Further, it is given in the form of starch granules, whose size and composition influence dough manufacturing and end-product quality. Starch consists of approximately 17-28% linear (amylose) and approximately 83-72% branched (amylopectin) α-glucanes whose brick is α-D-glucose [24]. Nevertheless, selected starch can contain up to 100% amylopectin (waxy starch) or up to 70% amylose (amylo-starch). Amylose with 500-600 monosaccharide units illustrates a different degree of polymerization and a smaller

molecule in comparison to amylopectin (degree of polymerization of 300.000-3.000.000). The linear chains of amylose and amylopectin are constructed by α-(1,4) linked D-glucose units. Additionally, amylopectin features α-(1.6) branching points and hence builds up larger associations. At the molecular level it is supposed that amorphous as well as semi-crystalline areas alternate outwardly from the inside of the starch granules. Thus, typically concentric layers are visually detectable. Amorphous areas of the starch granule which are arranged less densely also consist, in particular by amylose [28]. The semi-crystalline layers consist of thicker parallel amylopectin chains and less thick amylopectin chains with embedded amylose helices [29].

Concerning the size distribution of the starch granules, two types are characteristic of wheat, since wheat starch is non-uniform in size: (1) small starch granules with a diameter of 2-10 µm (B-type) and (2) large starch granules with a diameter up to 63 µm (A-type) [30]. Furthermore, larger starch granules exhibit a lower gelatinization temperature, lower water binding properties, and lower resistance towards enzymatic degradation than smaller granules [31, 32]. The starch granules' size distribution and enzymatic degradation are reflected in the sometimes difficult baking behavior of e. g. rye products. Further, a significant effect of granule size distribution on dough rheology as well as on pasting properties has been shown [33-38]. Other cereals, such as corn, consist of homogenously distributed starch granules (A-type starch) with similar amylose content to wheat starch (25%) [15].

The principal meaning of the starch during thermal treatment is based on the transformation of a starch-water matrix called gelatinization. Through water addition, starch hydrates (swells) and absorbs in cold conditions up to 50% of its dry mass in water and the starch granules diameter increases by about 30-40% [39]. As a result of the heating process, specifically the subsequent gelatinization, the structure of starch changes irreversibly from a crystalline to an amorphous state. At the beginning of the gelatinization process, water diffuses in the non-crystalline starch groups and both the amylopectin double helices and the crystalline areas dissolve [40]. Following this, the starch granules feature a heavily swollen and deformed structure as well as partial dissolution, and amylose from the starch granules is extracted (amylose-leaching). Through the rising temperature in the starch suspension, viscosity is greatly increased and a starch paste is

formed. Upon cooling and during the storage, the gelatinized starch molecules reshuffle themselves in a complicated process and reform as indissoluble, regular, and partially crystalline structures (retrogradation) [41, 42]. Retrogradation contains an entirely reversible re-crystallization of amylopectin and a partially irreversible re-crystallization of amylose [43]. In principle, this re-crystallization process is based on the formation of hydrogen bonds between the hydroxyl groups of adjoined starch molecules [44]. Additionally, the original water retention of starch gels is reduced. Thus, water is resigned from the starch gel (syneresis) and colloidal systems are torn into two phases: fluid and inherent parts [44]. These processes cause a solidification of starch gels. Concerning retrogradation, a distinction is made between rapid amylose retrogradation, within a few hours, and amylopectin retrogradation, which occurs over several days or weeks [45].

Various publications describe a relationship between starch retrogradation and bread staling [46-49]. Retrogradation is a complex phenomenon that has yet to be fully understood as many factors influence starch retrogradation. The most important influences on retrogradation are the starch source [23], the amylose or amylopectin amount [50, 51], the molecular size [51], the temperature and the water amount [52], pH [53], and various additions, such as salts [27, 24]. However, retrogradation kinetics of starch with various salts are not analyzed in the past, and are therefore interesting for future works.

1.3 Methods for analysing changes in flour-water systems

Various additives (e.g. NaCl or other chloride salts) have an effect on the rheological structure of dough and on the quantitatively measurable end-product quantities. Thus, in the following chapter methods for analyzing changes in flour-water systems are presented.

Therefore, this chapter is subdivided into rheological and microscopic (CSLM) analyses of the dough structures, the technological measurements which characterize end-product quality both after baking and during storage, and thermal analyses for analyzing the gelatinization and re-crystallization (retrogradation) processes of starch.

1.3.1 Rheological analysis

Rheology is the science of the deformation and flow of matter. Commonly (in bakery and milling industries) dough rheological properties are empirically analyzed using z-blade mixers (e.g. Farinograph) or load-extension tests (e.g. Extensograph). Scientifically fundamental rheological measurement using a Rheometer improves insight into dough properties such as viscosity and elasticity. Regarding the effect of NaCl on wheat dough, some work was done with empirical rheological analysis. However less work were published with fundamental rheological analysis. Therefore, a key aspect of this work was the comparison of fundamental rheological methods with empirical rheological methods in wheat dough with different NaCl amounts.

Rheological constitutive equations use stress and deformation in respect to each other to define the material qualities. The common equation of a flow curve is given as

$$\sigma\,(\dot{\gamma}) = \mu\,(\dot{\gamma})\,\dot{\gamma}, \tag{1}$$

where σ is the shear stress, γ is the shear rate and μ is the constant of proportionality for a Newtonian fluid and is called viscosity [54]. If μ is constant, the equation (1) follows the Newtonian equation (illustrated by a damper), which is used to characterize the viscous flow of liquids, whereby, the shear stress is directly proportional to the shear rate as

$$\sigma = G\,\dot{\gamma}. \tag{2}$$

Hooke´s law, illustrated with a spring, is used to describe elastic solid states. A sample obeys Hooke´s law when force is applied to a solid sample and the resulting stress versus strain forms a straight line through the origin [54]:

$$\sigma = G\,\gamma, \tag{3}$$

where G is the shear modulus and γ is the shear strain. However, this law is restricted to small deformations since such materials do not flow and are linearly elastic.

The rheological qualities of flour-water systems, like dough, are defined as visco-elastic material, since dough presents behavior between Newtonian fluids and Hookean solids. The nature of such models is that they are time dependent due to its visco-elasticity, and this is in contrast to both Newtonian and Hookean behaviors. Common measurements to

describe time dependent visco-elastic behavior are dynamic oscillation measurements (dynamic rheometry) as well as creep-recovery measurements. Thus, visco-elastic systems illustrate viscous parts (dampers) in combination with elastic parts (springs) and the analysis of such complex systems is therefore a challenge.

To study the visco-elastic properties of bread dough following two methods are commonly used: the dynamic oscillatory tests and the creep recovery test. The oscillation test and the creep recovery test complement each other as the oscillation test provides the short term response of dough, while the creep time test provides the long term response [55]. Thus, in addition to the creep-recovery tests performed in this work, frequency measurements were taken to examine the visco-elastic behavior in wheat dough with different amounts of NaCl.

Dynamic rheometry

The viscous and elastic fractions of a material may be analyzed in oscillation experiments [56-58]. The sample is clamped between two plates (Figure 2) and a sinusoidal deformation (γ) with an angular frequency (ω) is typically applied to the sample by the movement of the upper plate. The strain in the sample between the plates is supposed to be a function of time and is defined as

$$\gamma = f(\gamma_0, \omega, t) = \gamma_0 \sin(\omega t), \qquad\qquad (4)$$

where γ_0 is the amplitude of the strain and ω is the frequency. An elastic material transfers the stress in direct proportion to the shear strain (γ) on the force sensor, thus the phase angle (δ) is zero between exciter signal and respondence signal.
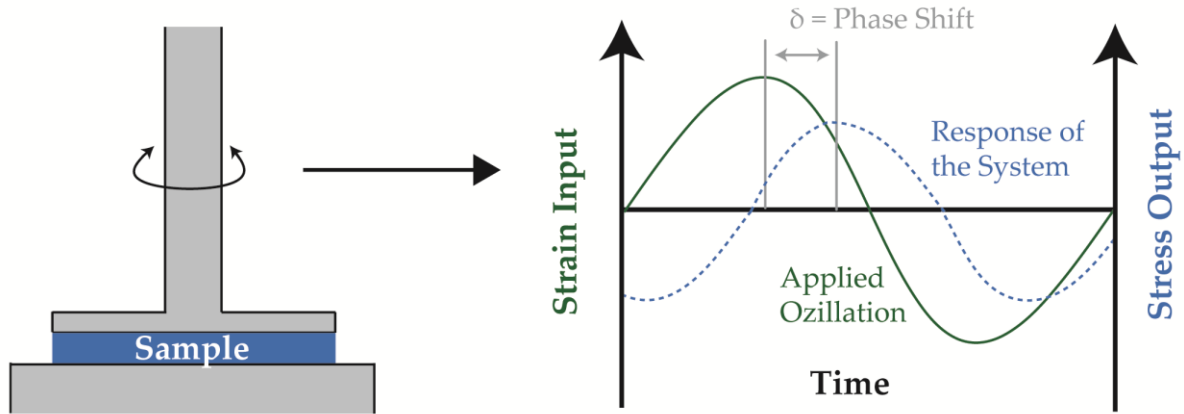
Figure 2: On the left: sample clamped between two plates in a Rheometer. On the right: amplitude alteration and phase displacement with the sinusoid deformation of a visco-elastic sample in the oscillation experiment.

Visco-elastic properties, by which dough systems are distinguished, show a phase angle (δ) of $0<\delta<90°$ and viscous materials show a response of $\delta = 90°$. With small strain amplitude, the following shear stress is produced by the strain input as

$$\sigma = \sigma_0 \sin (\omega t + \delta), \tag{5}$$

where σ_0 is the shear stress applied to the sample and δ is its phase angle. By trigonometric transformation of equation (5) the material function can be identified as

$$\sigma = \sigma_0 \sin \omega t \cdot \cos \delta + \sigma_0 \cos \omega t \cdot \sin \delta. \tag{6}$$

In an experiment following material functions are measured:

$$G' = \frac{\sigma_0}{\gamma_0} \cos \delta \tag{7}$$

$$G'' = \frac{\sigma_0}{\gamma_0} \sin \delta, \tag{8}$$

where G' is called the shear storage modulus and accounts for elasticity, G'' is titled the shear loss modulus and defines the viscosity. The quotient from G' and G'' is called loss factor (tan δ) is used to define the visco-elastic behavior of a sample. The loss factor shows an index for the elasticity of the test. The smaller the loss factor, the more elastic the substance is (equation (8)) [57]:

$$tan\ \delta = \frac{G''}{G'}.$$ (9)

The quotient from the shear stress amplitude (σ) and the deformation amplitude (γ) is defined as the absolute value of the shear complex modulus ($|G^*|$) (equation (9)). The greater the shear complex modulus, the more stiffly (firmly) the material behaves. Furthermore, it was observed that in polymer systems tan δ has high values for dilutes such as dough (>0.3), medium values (0.2-0.3) for amorphous polymers, and low values (~0,01) for glassy crystalline polymers and gels [54].

$$|G^*| = \frac{\sigma_0}{\gamma_0} = \sqrt{(G')^2 + (G'')^2}.$$ (10)

Two dynamic rheology measurements are common employed for practical use: variation of amplitude (amplitude sweep) and variation of frequency (frequency sweep). To guarantee that the material is tested within the linear visco-elastic range (LVE), this range is usually first determined using an amplitude sweep test before the frequency sweep is performed. The measuring principle of an amplitude sweep is based on the fact that the oscillation frequency is held steady while the amplitude is varied. The LVE is the range where the material will not be destroyed and were G' and G'' will be parallel and constant as a function of the amplitude. In the LVE the deformation and the load of the material is low so that the changes to the substance are reversible [57]. The maximum applicable limit value of the amplitude (γ_0) is determined before using a frequency sweep and should not be exceeded in the following experiments. The most common, following oscillatory measurement test is the frequency test as it allows for the study of a sample's viscous and elastic behavior through varying the frequencies that provide a constant strain or stress [54].

Creep-recovery measurements

If a constant stress ($\sigma_{constant}$) is applied to an ideal elastic material (with the absence of viscous qualities) the material will return to its original form. However, an ideal viscous material shows the constant flow by which a linear response to the stress is determined. This answer is based on the inability of the material to recover from the applied tension. Visco-elastic systems (as dough) show a non-linear response to the applied stress in that they have the ability to restore some structures using stored energy. Hence, the recreational

function shows a partial restoration of the original form. Idealized creep and recovery curves are shown in Figure 3.
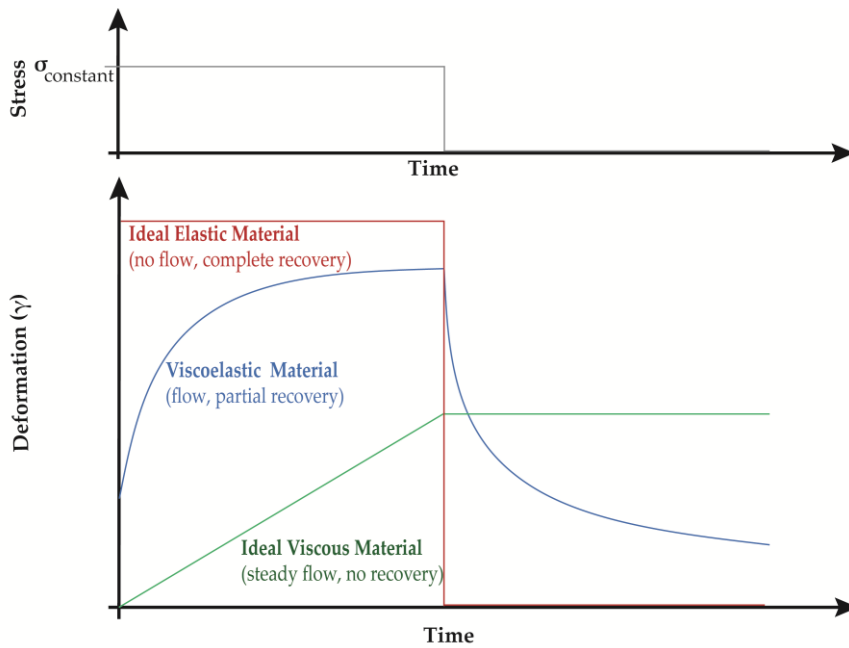
Figure 3: Schematic illustration of the various systems of a creep recovery test [9].

Such experiments with applying constant deformation or stress on the material are called creep experiments. Here, a steady stress is applied to the material, whereby the temporal changes of the resultant strain with which the material reacts to the deformation is measured (creep phase). A sample is measured in the LVE if the compliance curves are independent of σ. Creep data can be shown as a creeping compliance function (10) [54]:

$$J(t) = \frac{\gamma(t)}{\sigma_{constant}}.$$
(11)

Mechanical models

The overall shear stress (σ) caused by the deformation is the sum of the single shear stress described by the sum of the not time dependent Newtonian model (equation (2), viscous behavior) and the not time dependent Hooke's model (equation (3), elastic behavior) as

$$\sigma = f(\gamma, \dot{\gamma}) = G\gamma + \mu\dot{\gamma}.$$
(12)

In creep measurements, in which the material is allowed to flow after a constant shear stress (σ) is applied, the change of the strain over time is zero ($d\sigma/dt = 0$). The solution of

the equation (12) (by the derivation and solution of the differential equation) shows with the initial strain is zero ($ɣ = 0$ with $t = 0$):

$$ɣ = f(t) = \frac{\sigma_0}{G} \left(1 - exp\left(\frac{-t}{\lambda_{ret}}\right)\right), \tag{13}$$

where λ_{ret} is the retardation time ($\lambda_{ret} = \mu/G$) and defines the time required to reach a defined value of 63.2% (1-1/e) of the final value. For Hooke's solids, the retardation time is zero because the maximum deformation is reached immediately upon applying the tension. Whereas the time to reach the maximum strain of the visco-elastic material is delayed (retarded).

The Burger Model was created to model visco-elastic properties which are time dependent. Nevertheless, the Burger model, which is composed of the in series connected Kelvin (represented by a purely viscous damper and purely elastic spring connected in parallel) and Maxwell Models (represented by a purely viscous damper and a purely elastic spring connected in series), may be used to define elastic delays in biological systems (Figure 4) [54].

Maxwell   Kelvin   Burger
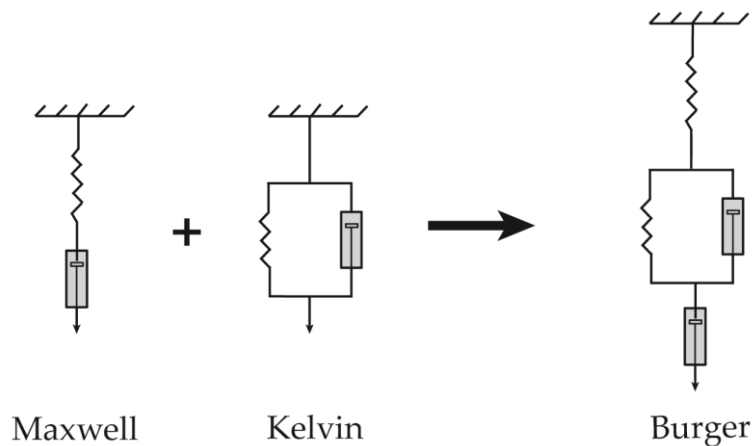
Figure 4: The sum of Maxwell model (represented by a purely viscous damper and a purely elastic spring connected in series) and Kelvin model (represented by a purely viscous damper and purely elastic spring connected in parallel) leads to the burger model (represented by a Maxwell and Kelvin model connected in series).

The data which follow this mechanical analogue of Burger, demonstrates an initial elastic behavior on account of its free spring, a retarded elastic behavior which correspond to the

parallel spring-damper model and a Newtonian flow behavior during long time periods (long time viscous flow) which is referred to the free damper (Figure 4) as

$$\gamma = f(t) = \frac{\sigma_0}{G_0} + \frac{\sigma_0}{G_1} \left(\sqrt{1 - exp\left(\frac{-t}{\lambda_{ret}}\right)} + \frac{\sigma_0\, t}{\mu_0} \right). \tag{15}$$

The Burger Model can be defined as creep-compliance; it is divided by a constant stress and under the consideration of the retardation time as

$$\frac{\gamma}{\sigma_0} = f(t) = \frac{1}{G_0} + \frac{1}{G_1} \left(1 - exp\left(\frac{-t}{\lambda_{ret}}\right) + \frac{t}{\mu_0} \right). \tag{16}$$

The results can be also expressed as a function of creep compliance as

$$J = f(t) = J_0 + J_1 \left(1 - exp\left(\frac{-t}{\lambda_{ret}}\right) + \frac{t}{\mu_0} \right). \tag{17}$$

whereby, J_0 is initial compliance which correspond with Hookean spring, J_1 is the retardation compliance which correspond with the Kelvin model, λ_{ret} defines the retardation time of the Kelvin component and μ_0 the Newtonian viscosity of the free damper (Figure 5) [54].

Figure 5: Schematic illustration of a typical creep curve and the parameters of the Burger Model (equation (17)) [54]. J_0 is initial compliance, J_1 is the retardation compliance, λ_{ret} defines the retardation time and μ_0 the Newtonian viscosity.

1.3.2 Microstructure – Confocal Laser Scanning Microscopy (CLSM)
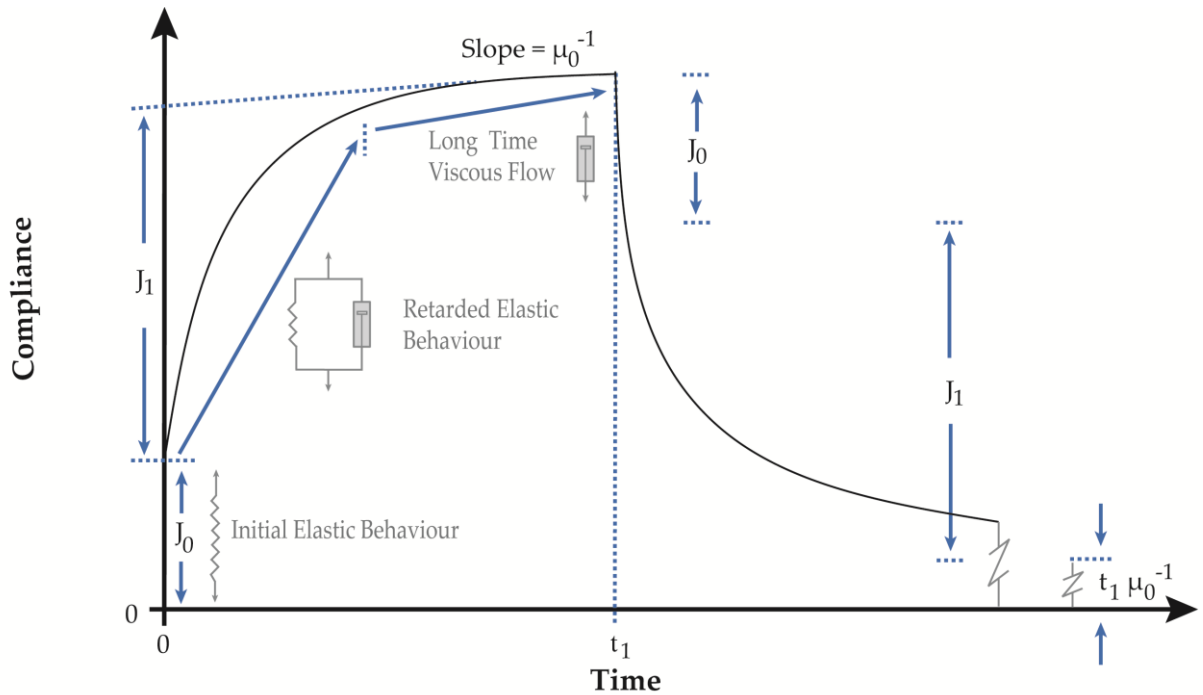
The quality of a yeast-leavened product in the dough state can be also analyzed by its microstructure. It has already been illustrated, that the formation and alteration of protein networks by different ingredients such as salts or water [59] can be characterized by the microstructure of dough [60-63]. These papers also discussed a link between the microstructure, or rather the molecular properties, and the apparent macroscopic properties of the end-product. Thus, characterizing dough microstructure by microscopy helps to quantify and predict end-product quality.

In order to illustrate the microstructure, various microscopy formats can be used: transmitted light microscopy, electron microscopy, atomic force microscopy or confocal laser scanning microscopy (CLSM). CLSM is a further development of light microscopy [64, 65], and features several advantages compared to other microscopy species. Thus, CLSM serves as a modern instrument for analyzing biological structural systems [66]. In contrast to standard light-microscopy it is possible, through the confocal construction and with the usage of selective fluorescence dyes, to achieve a noninvasive, highly sensitive representation of the inside of the sample. Furthermore, the use of CLSM allows for simultaneous yet separate analyses of various phases (protein, starch, fat, or pentosan) through different laser excitations and different detectors.

The confocal microscope is based, in this case, on the principle that the picture is brought into focus by a light source aimed at a specifically defined depth inside the sample [67]. The movable, reflecting laser system scans the object line by line, point for point. This principle is in contrast to the classic light microscope which illuminates the whole sample. The fluorescent light emitted is then absorbed by a detector and shown as a digital image. Nevertheless, to receive accurate fluorescence the sample must be flooded with dye that binds specifically to the structure being examined (protein, starch, fat, or pentosan). This dye is then stimulated by the laser using characteristic wavelengths to elicit reflective light at the defined wavelength (Figure 6) [64, 68].
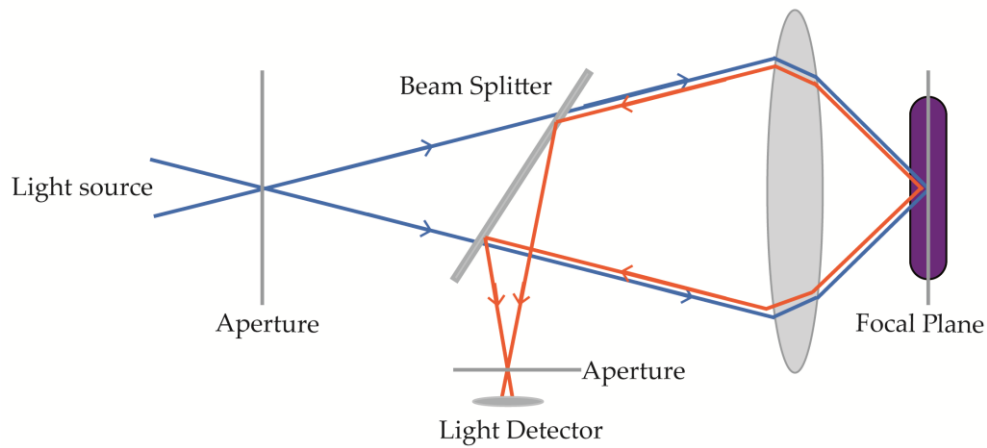
Figure 6: In the CLSM a parallel laser beam (blue) moves over the sample. The fluorescence light emitted by the sample passes through a dichroic mirror and is focused onto the pinhole (red). The information collected is strengthened in the photomultiplier and a digital picture is provided.

The sample beyond the focus of the defined objective is not shown in the CLSM image [69, 70]. The use of a pinhole along the light path allows the diameter of this pinhole to be adjusted and thereby determines the intensity of the light that reaches the detector; in turn, this enables the specification of the level brought into focus, limiting the sample to a specified depth. If several pictures are joined along the z-axis an optical serial section picture arises which illustrates the spatial structures of the sample, i.e. Figure 7 [66, 68, 71].
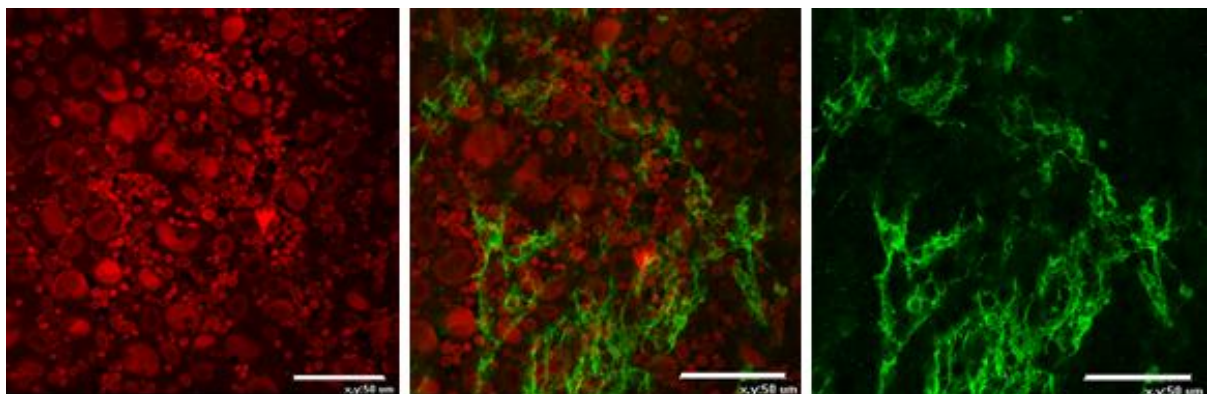
Figure 7: Two-dimensional images of wheat dough without NaCl. The red phase reflects the starch granules distribution (on the left). The green phase shows the protein structure (on the right). In the middle picture both phases of the same sample are visualized combined. Two different dyes are used: Rhodamine B for proteins (green) and Nile blue for starch (red).

1.3.3 Macrostructure – Baking

To achieve optimum end-product quality, it is essential to consider the baked goods' macrostructure. During the energy input in the mixing process, the raw materials (flour, water, and yeast as well as other ingredients) form a multiphase system. In the mixing process two important steps occur: a) the homogenous dispersion and hydration of the compounds and b) the elemental kneading process that forms the visco-elastic wheat dough structure. Afterwards, the dough system is primarily composed of a protein network in which starch and gas micelles are embedded [14, 72]. The unique visco-elastic properties of the protein network enable gas retention. The theory of gas cell growth is as follows: Gas nuclei are first drawn into the dough in the aeration process that results from intensive kneading [73]. During proofing, the volume of the dough increases due to the yeast's leavening ability and gas cell growth. The extent of gas retention in wheat dough is closely related to the development of the gluten matrix. Well-developed protein usually retains more gas than less developed dough. The gas retention effect, or rather foam stability, could be explained by the Gibbs-Marangoni effect, which defines mass transfer between two fluids along an interface as being a result of surface tension gradient. Gas cell stability depends on the competition between the proteins, lipids, and other surface active components in the dough since they control cell lamellae stabilization. It has been noted that the gluten matrix in the dough, in particular, stabilizes the gas cell membrane [74]. Gas retention capacity in wheat dough can be measured by e.g. a Rheofermentometer which analyses both dough development and gas release. In the case of temperature dependence, the Gibbs-Marangoni (Mg) effect can be titled thermo-capillary convection as

$$Mg = -\frac{d\gamma_S}{dT} \frac{1}{\mu\alpha} \ L \ \Delta T, \tag{18}$$

were γ_S is the surface tension, α is the thermal diffusivity, μ is the dynamic viscosity, L the characteristic length, and ΔT is the temperature difference. Since NaCl may change the surface tension as well as the viscosity of the system, the thermo-capillary convection may change, too. According to the foam structure a specific gas pressure occurs. This pressure is defined by the Young-Laplace equation [74]:

$$\Delta p = \frac{4\gamma_S}{r}, \tag{19}$$

where γ_S is the surface tension, p is the pressure, r is the surface flection. The increment in temperature during baking leads the gas volume to expand and water solubility to decrease, which follows the following equation for ideal gases [69]:

$$P\,V = n\,R\,T, \tag{20}$$

where P is the absolute pressure of the gas, V is the volume, n is the amount of substances in the gas, R is the ideal or universal gas constant, and T is the temperature.

The transformation from dough to stable crumb is achieved by thermal treatment. The transition of dough to the end-product is characterized by volume increase, water loss and rheological changes. Above approximately 50 °C, the dough viscosity decreases as a result of starch swelling and amylose leaching [75]. During the baking process, yeast produces carbon dioxide and ethanol which expand and contribute, along with the evaporating water or steam, to volume increase [14]. Gas cells expand during baking because of thermal expansion [76] and the walls of the gas cells become thinner. In parallel, the proteins denaturize and the nascent water may be absorbed by the starch for swelling and gelatinization. Thus, dough transitions from foam structure into a spongy structure that is a porous system of fine gas cells divided by thin walls. The amount, size, shape, and orientation of gas cells, as well as the gas cell wall thickness, all affect the end-product's properties [77].

High baking temperatures (ca. 70-80 °C) cause the dough-gas matrix to rupture: the open cell construction changes from a compact cell foam to an open cell sponge and gas release increases [78]. Thus, the specific volume of the product increases. At approximately 80-90 °C, a stable crumb network is formed (by partial starch gelatinization and protein denaturation). In addition to the structural changes that result from heating, bread baking may be explained from the perspective of the transport phenomena as a simultaneous heat and mass transfer in the porous matrix dough [79]. Thus, the literature defines a wheat bread crumb as a biochemical, inactive colloid system which consists of denatured protein and partially gelatinized as well as partially coherent starch granules [80]. However, in other flour systems such as rye, the structure providing protein network is absent and the crumb structure is based on pentosan and starch characteristic [81]. In summary, loaf

volume depends on the combination of dough enlargement through gas expansion and the gluten-starch matrix before it bursts [76].

Therefore, from a technological and sensory point of view, a well-developed, even and not to compact pore structure seems to be one of the key factors for creating an attractive product. The pore structure of the crumbs, the end-product volume, and taste perception are influenced by the pore structure of the initial dough and is, thus, crucial to consumers' purchasing decisions.

Hence, in addition to rheological dough analysis or microscopic characterization, differences in the protein network that result from modification by NaCl can be determined through the consideration of the macrostructure (end-product volume and crumb texture). As such, a laser volumeter can be used to determine the end-product volume. Texture Profile Analysis (TPA) is used to study crumb texture. This uniaxial compression method is based on the principle of a cylindrical plunger which moves at a defined speed to a specific depth in the sample. The measurement of the strength across two-plunge compression process enables about the definition of crumb characteristics like firmness and springiness.

1.3.4 Thermally determinable structural properties – Differential Scanning Calorimetry (DSC)

The methods of thermal analysis include physical parameters which deliver statements about chemical processes as a function of the temperature and/or the time; whereby, the sample is subjected to a controlled temperature program. Importance tests with thermal analysis methods are differential thermal analysis (DTA) and dynamic differential scanning calorimetry (DSC). In particular, DSC has been developed as an efficient method for establishing the thermo-dynamic parameter. The application range of DSC varies from material characterization to investigate the long term stability of active pharmaceutical substances.

Power compensated differential scanning calorimetry (DSC) determines the difference between temperatures in reference and sample areas in two separate ovens. The reference substances are materials or air which do not change in temperature in the area considered. Both test and reference area are heated with the same program and the difference required to maintain in both ovens at the same temperature is measured. With steady heat addition

a transformation process occurs which is both typical for the sample and which features a disturbance of the stationary state. The electric power required for this process of transformation is logged as a function of the temperature. The area of application for typical DSC equipment is at temperatures between -180-800 °C and rates of heating of approximate 5-20 °C min^{-1}.

The total amount of the endothermic or exothermic heat measured in a material during a defined time (t) or temperature (T) range is called heat quantity (ΔQ) [82]. The heat quantity accompanies chemical or physical transformation and/or changes in temperature and is defined as

$$\Delta Q = \int_{t_1}^{t_2} \frac{dQ}{dt}\, dt, \tag{21}$$

where dQ/dt defines the heat flow. For typical starch-water DSC experiments, approximately 20 mg of sample (e. g. starch-water suspension) as well as a reference are given in small aluminum pan and sealed. Afterwards, the sample is heated at a steady rate in excess of the melting or gelatinization point. Quick cooling after this first heating converts the sample into a re-crystallized state (here: retrograded starch). The second rate of heating allows for the quantitative capture of retrograded structures which are illustrated as endothermic melting signals.

In typical DSC measurements the onset temperature, peak temperature, end temperature, and gelatinization enthalpy (ΔH) characterize the sample. Regarding starch retrogradation, gelatinization enthalpy (ΔH) measured after storage of gelatinized starch features the amount of re-crystallized starch. The analysis of starch retrogradation seems to be of great importance for characterizing starch based foods since a strong relationship between starch re-crystallisation and staling or bread firming has been discussed in several publications [46, 47, 83, 84].

In previous studies, it has been reported that the Avrami equation is regarded as a useful tool for studying the kinetics of starch retrogradation during staling. Armero and Collar [85] further discussed that if the Avrami parameters are adequately used the equation will provide a good basis for comparing other starch staling processes. The Avrami equation enables the re-crystallization of starch gels, starch retrogradation, and

bread staling to be modelled. In some studies the crystallization rate constant (k) has been determined as when the modus of nucleation and growth are assumed. Other authors use Avrami plots to analyse the Avrami index (n) and gain insight into the mechanism of crystallization [86].

The crystallization process consists of nucleation and crystal growth. In detail, nucleation is the stage where the solute molecules start to assemble into very small agglomerates [87]. These clusters stabilize at defined operating conditions (such as temperature or supersaturation) and generate the nuclei [88]. However, if the clusters do not become stable, they re-dissolve. To avoid dissolution, the clusters need to reach a critical size in order to become stable nuclei. During nucleation the crystal structure that refers to the relative arrangement of the atoms, but not the macroscopic characteristics of the crystal (size and shape), will be defined even if the results show an internal crystal structure. Crystal growth is the subsequent growth of the nuclei that occurs after critical cluster size is reached, and depends on the supersaturation. Depending on the process, either nucleation or growth may prevail. As a result, crystals with different sizes and shapes are achieved [88].

The received gelatinization enthalpy (ΔH) will be fitted to the Avrami equation [89, 90] by a non-linear procedure equation for analyzing the form and gradient of crystallization. This equation was derived from the assumption that starch retrogradation is a measure of the increasing degree of starch re-crystallization, and that the rate of crystallization is described by the Avrami equation. Re-crystallized starch (expressed as gelatinization enthalpy after storage of the sample), subjected to storage time, and was fitted to the Avrami equation:

$$\Theta = \frac{(A_\infty - A(t))}{A_\infty - A_0} = 1 - e^{(-kt^n)}, \tag{22}$$

where Θ is the fraction of the total change in gelatinization enthalpy of retrogradation still to that occur (or the fraction of not re-crystallized starch remaining after time as illustrated in Figure 8). A_0, A_t and A_∞ are experimental values of the property at time zero (A_0), at time t (A_t), and infinity (or limiting value after 504 h, A_∞), k is a constant rate (in this case equally with the re-crystallization rate of starch), and n is the Avrami exponent which is characteristically related to the way the nucleation of crystallites, and their subsequent growth, occurs.
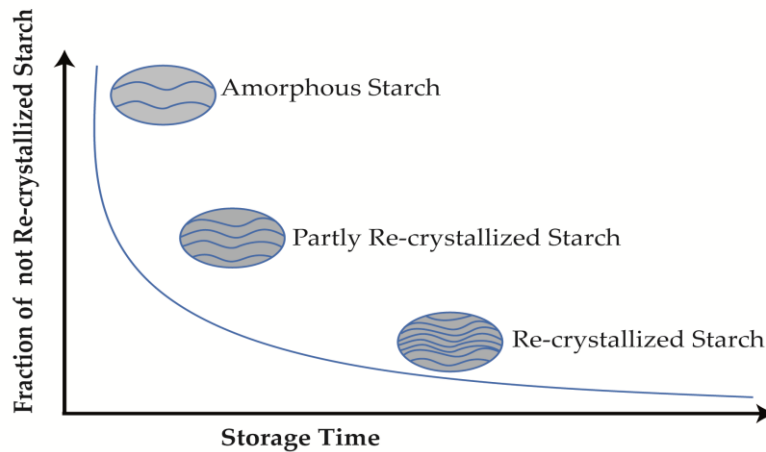
Figure 8: Schematic illustration of the gradient of starch retrogradation for analysis by Avrami-equation.

1.4 Sodium chloride - Backgrounds

1.4.1 Sodium chloride and its relationship with hypertension

An excessive NaCl intake has been presented as a cause for hypertension in various ways and from various perspectives such as epidemiological, intervention, and numerous experimental animal studies. As a whole, these studies propose that the sodium ion has negative effects on the human body. Sodium is one of the major cations in human extracellular fluids and is an important substance in every fluid compartment of the human body, just like animals. Sodium ions feature influence on osmotic pressure, membrane potentials, enzyme activities, transport processes, and buffering [9]. In healthy organisms the sodium ion concentration is held very steady by complex r processes.

Contemporary, huge population studies have contributed to the 100-year conflict: the possible effect of sodium on blood pressure. Some studies indicate a moderate, but significant correlation between sodium intake and blood pressure, whereas others found no correlation. In the following, the main studies regarding this discussion are summarized. Luis Dahl et al. [91, 92] illustrate, in an epidemiological study, a positive linear relationship between the prevalence of hypertension and the mean sodium intake within five populations in 1960 [91]. Thus, he suggests that high blood pressure is common among groups who regularly eat salt in amounts well in excess of the daily requirement. Parfrey et al. [93] indicate that the blood pressure of people with mild essential hypertension (average

diastolic blood pressure 90-110 mm Hg) was lowered by moderately reducing dietary sodium and increasing potassium. However, this effect was not observed for normotensive patients (diastolic pressure less than 85 mm Hg). The Intersalt study is considered to be the most comprehensive study concerning the correlation of various factors and hypertension as it reports on 52 study centers with 200 participants each [4]. This study indicates that obesity and alcohol consumption have a more significant impact on blood pressure than an excessive NaCl intake. The relationship between high blood pressure and NaCl intake could only be confirmed by including population groups with an unusual life style (too much alcohol, nicotine and less movement) [94]. However, a possible connection between NaCl consumption and raised blood pressure was discovered in older people. In the DASH-study [95], similar results were observed where healthy nutrition, with a lot of fruit and vegetables (however, with an unchanged NaCl intake), led to a lowering the blood pressure [96, 97]. Additionally, on the DASH diet, blood pressure could be lowered even further with a diminished NaCl intake [97]. Graudal et al. [98] illustrate, that diminished NaCl intake can lower high blood pressure, however, is not essential in healthy people. Following this, He & MacGregor [99] show a significant influence on the reduction of blood pressure in hypertensive (critical raised blood pressure values) and normotensive (normal blood pressure) people by reducing sodium intake. Therefore, the number of cardiovascular deaths could be lowered by about 4-14% annually [99]. Furthermore, a reduction of NaCl consumption has been linked to lowering the risk of stomach cancer, improved bone mineralization, and the prevention of kidney stones [9]. A detailed collocation of other studies as well as a critical exposure of their errors in realization and interpretation can be found in the work of Taubes [100]. In summary, Taubes indicates that the decrement of dietary sodium intake reduces high blood pressure in 'salt-sensitive' people. This in turn positively affects the appearance of cardiovascular complications [7]. However, other measures such as weight reduction, reduction of alcohol, increased exercise, stress reduction, and change in diet contribute to a reduction in hypertension [7].

Contrary to these studies, other studies present the opposite. Several countries have a high sodium intake with no evidence to suggest increased blood pressure as in Asia [101]. Asian adult populations show a mean intake of >200 mmol per day (≈11.78 g NaCl per day) [102] based on a high intake of soy sauce, miso soup, and pickles [102-103]. Cameroon, Ghana,

Samoa, Taiwan, Tanzania, Uganda and Venezuela present a sub-optimal sodium intake [102]. Thus, sodium intake differs markedly around the world. In parallel, negative physiological effects might also occur as a result of NaCl reduction, such as decreased vascular elasticity, increased cholesterol, chronic exhaustion, and raised insulin resistance [104, 105]. Gomi et al. [106] reported that stringent dietary sodium reduction degrades insulin sensitivity by increasing sympathetic nervous activity in patients with primary hypertension. One of the most recent studies concludes that their study does not support the general recommendation to reduce sodium intake [107]. They accompanied 3.700 adults for seven years and find the highest amount of mortal heart attack and strokes among the group with the lowest urinary sodium excretion per day. Therefore, sodium reduction may not prevent cardio vascular disease.

In summary, sodium reduction is controversial, and less conclusive evidence exists to support the idea that sodium reduction improves human health. However, due to the regulations and because some studies proposed that the sodium ion has negative effects on the human body in western countries, the food industry is forced to reduce NaCl levels in food products.

1.4.2 Levels of NaCl in baked products and targets for reduction

In western countries, about 75% of the daily sodium intake is consumed from manufactured foods [102], while the sodium intake from salt added by the consumer during or after cooking is only a small part of the total NaCl intake [9]. Further, 25-35% of the daily sodium intake from these processed goods comes from cereals and cereal products and are regarded as the main supplier of sodium in the daily dietary [9, 10]. Meat and meat products and dairy products deliver 26% and 8% of the daily sodium intake respectively [9]. Therefore, primarily in Western Europe and the USA, baked goods are being considered critically according to planned and actual food regulations (e. g. health claims [108]).

For the maintenance of the physiological processes, an average everyday NaCl intake of an adult of approximate 1.4-4.0 g NaCl per day [3, 9, 109, 110] is considered normal. However, the Intersalt study [111] estimated the NaCl intake per person per day to be 7.9-10.5 g NaCl in Western Europe. Other studies show daily sodium chloride intakes of 10-12 g in

Europe [9, 10, 110]. Thus, sodium intake is in excess of physiological needs. Consequently, the world health organization recommends a sodium intake of <85 mmol per day (< 5 g NaCl per day) for adults [112]. Other institutions advocate 6 g NaCl per day [110, 113]. However, the long-range aim is to reduce the daily sodium chloride intake to 3 g per day [9].

Consequently, the governments of several European countries have developed proposals for the target levels of NaCl in many foods [108, 114]. Finland has developed a comprehensive public health campaign in collaboration with the food industry and received marked reductions in sodium intake. As such, a significant (p<0.001) annual decrease in the sodium intake of men and women was observed which coincided with a decrease of ~60% in coronary heart disease and stroke mortality between 1979 to 2002 [115, 116]; recent studies on sodium intake have confirmed this [117]. Other regulations have been put in place in UK, where major retailers, manufacturers, caterers, and trade associations have decreased the NaCl content of various processed foods [102]. Further, a regulation on nutrition and health claims made on foods was adopted in Europe in 2005. Health claims on food labels are claims by manufacturers of food products that their food will decrease the risk of developing disease [6]. Therefore, a claim that a food is healthy because it is low in sodium/salt may only be made when the product contains less than the defined sodium amount: 3 g NaCl per kg flour. However, bread normally contains around 15-25 g NaCl per kg flour. Further, a claim stating that the content of sodium has been reduced may only be made when the reduction in content is at least 25% compared to a similar product [6]. Therefore it's a challenge to reduce the sodium amount in baked goods

Reducing the overconsumption of sodium chloride leads to food products that taste more unpleasant and offer problems for the food industry through changes in manufacturing and end-product quality. Therefore, the baking industry requires detailed, basic knowledge about how product quality changes with reduced NaCl.

1.5 Sodium chloride - purpose of this study

The political pressure for the reduction of sodium chloride in food products, and baked goods in particular, is increasing. Therefore, the establishment of a permissible reduction in the level of NaCl that still allows dough to develop adequate rheological properties and yield high quality bread remains a formidable challenge and requires critical analysis.

However, only poorly detailed scientific knowledge and explanations regarding the impact of various levels of sodium on the dough behavior, or rather its fractions (proteins and starch) and thermal treated end-product, exist. To date, scientific studies only compare selective levels of NaCl in dough with mainly empiric rheology. Fundamental rheology (dynamic rheometry) as well as the measurement of stickiness has not been sufficiently analyzed. Examinations of baked goods have been severely restricted regarding loaf volume through the use of just 3-4 levels of NaCl. Consequently, knowledge about NaCl in dough systems, while substantial, is both incomplete and contradictory. Likewise, it is not entirely clear which rheological methods is best suited to analyze starch-based dough and sensitive enough to distinguish subtle differences in dough properties when incremental changes in the level of NaCl are made.

Furthermore, there is no scientific knowledge about the impact of sodium chloride on the staling rate of wheat bread crumbs. Only with a fundamental knowledge of the impact of sodium in dough and yeast-leavened products will it be possible to reduce and replace sodium with suitable substances.

Additionally, the effect of NaCl and sodium replacers (as chloride salts) on starch recrystallization (retrogradation) may be important in predicting, or rather explaining, alterations in the crumb, or rather the starch particles, during the storage of starch-based foods. As a conclusion, only through a detailed understanding of the interaction mechanisms of sodium in starch-based food systems will it be possible to achieve high quality end-products with good manufacturing properties and less crumb staling.

Therefore, the aim of this work is to systematically analyze the effects of NaCl on both protein and starch in flour-water systems, otherwise termed dough, and their thermally

treated end-product. To achieve this comprehensive knowledge, this work is structured in three main parts:

1.   Global review of NaCl and its sensory, preserving, and technological impact on yeast-leavened products

2.   A detailed analysis of the effects of a wide range of NaCl levels (0-40 g NaCl kg^{-1} wheat flour) on a variety of rheological and technological properties of wheat dough systems, as well as the microscopic structure of proteins.

3.   Starch re-crystallization kinetics as function of various chloride salts which may function as sodium replacer.

A firm understanding of these interaction mechanisms will enable a reduction in the sodium content of baked goods through the discovery of a suitable salt substitution without decreasing consumer product acceptance.

2.   RESULTS

2.1 Paper Summary

CHAPTER A   REVIEW: SODIUM CHLORIDE – SENSORY, PRESERVING AND   **42**
TECHNOLOGICAL IMPACT ON YEAST-LEAVENED PRODUCTS

Salted, yeast-leavened products are a major source of dietary sodium, thus the baking industry needs to explore ways to reduce sodium in its products. Therefore, the impact of NaCl on shelf-life, rheology, and technological attributes as well as the sensory quality of yeast-leavened products needs to be understood. This review suggests paths of future research, including the overall impact of various NaCl levels on dough rheology and end-product quality (yeast leavening rate, crumb texture, staling rate and retrogradation) and a global, detailed consideration of the effect of a comprehensive range of NaCl on the main flour components (starch and protein).

CHAPTER B   IMPACT OF SODIUM CHLORIDE ON WHEAT FLOUR DOUGH
AND YEAST-LEAVENED PRODUCTS.

SECTION 1   I. Rheological attribute   **60**

This study compares fundamental dough properties with empirical rheological measurements to gain an improved understanding of dough rheology resulting from reduced NaCl. Flour-waters systems were mixed with varying levels of NaCl and the rheological, microscopic (CLSM) parameters were analyzed. Rheological changes were reflected in particular by a decrease in dough firmness and elasticity with NaCl reduction. Protein modification was distinct both under the CLSM and as manifested in rheological changes. A new finding of the current paper is that decreasing NaCl decreases analytical dough stickiness. This result was measured for the first time, and was unexpected as it conflicts with the haptic impression. Subsequently, a theory for this result is presented.

Section 2   II. Baking quality parameters and their relationships   **68**

NaCl levels were reduced and the technological parameters analyzed in thermally treated wheat flour-waters systems. A volume decrease with rising NaCl reduction and a corresponding increase of crumb firmness and rate of staling were determined. These results are most likely based, on the one hand, on changes in the protein structure, and on the other hand, on the inhibition of yeast activity by salt ions. Furthermore, exciting results were achieved as NaCl reduction increased crumb retrogradation. To explain this finding, starch is considered as a weak ion exchanger, by which larger sodium ions replace hydrogen ions, thereby reducing retrogradation.

Chapter C   **Starch re-crystallization kinetics as a function   76
of various cations**

Starch-waters systems were produced with different chloride salts (Ca^{2+}, Mg^{2+}, Li^+, NH_4^+, N^+, K^+), the thermally determinable gelatinization and re-crystallization properties were analyzed, and retrogradation kinetics were examined by Avrami equation. With the addition of NaCl, gelatinization enthalpy increased in comparison to the reference and the gelatinization temperatures decreased. This is based in particular on ions' reduction of the a_w-value. Furthermore, for the first time it was shown that all cations, bivalent cations in particular, demonstrate a decrease in starch re-crystallization. Consequently, some theories about this phenomenon are presented.

2 .2 Review: Sodium chloride – sensory, preserving and technological impact on yeast-leavened products

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ABSTRACT

Dietary sodium reduction is an important risk factor in the development of hypertension. Since salted, yeast-leavened products are a major source of dietary sodium, the baking industry needs to explore ways to reduce sodium in its products. Therefore, the impact of NaCl on shelf-life, rheology and technological attributes as well as on the sensory quality of yeast-leavened products needs to be understood. This review offers essential background knowledge about the effects of NaCl reduction on dough and yeast-leavened products as well as techniques for reduction. Further, this review suggests paths of future research, including the overall impact of various NaCl levels on dough rheology and end-product quality (yeast leavening rate, crumb texture, staling rate and retrogradation) and a global, detailed consideration of the effect of a comprehensive range of NaCl on the main flour components (starch and protein).

Keywords: sodium reduction, sodium replacers, bread, dough

INTRODUCTION AND PURPOSE OF THE REVIEW

Excessive sodium intake is in different studies shown to be a significant risk in human health since hypertension can be related to high sodium consumption (Graudal et al., 1998, Sacks et al., 2001,

Cutler et al., 1997, Elliott et al., 2007). In the literature, several effects of sodium on the humans have been described. Sodium intake has a high effect on coronary heart disease (CHD) (He et al., 2000, Cook et al., 2007), stroke (Xie et al., 1992) and non-cardiovascular diseases (Tarek and Graham, 1996), and around 12.8% death of the global total around the world were traced back to a non-optimal blood pressure (high blood pressure) in 2000 (Lawes et al., 2006a, Lawes et al., 2006b). He and MacGregor (2003) estimated that if global sodium chloride intake will be reduced from approximately 10 g d^{-1} to 3 g d^{-1}, around one quarter deaths from coronary heart disease and one third deaths from stroke in the United Kingdom could be prevented. Furthermore the existing suggestion of reducing sodium chloride intake from approximately 10 g d^{-1} to 5 g d^{-1} have a meaningful effect on blood pressure and resulting disease, however, a reduction to 3 g d^{-1} will have additional great effects (He and MacGregor, 2003). Therefore the long target for worldwide sodium chloride intake should be 3 g d^{-1} instead 5 or 6 g d^{-1} (He and MacGregor, 2003).

Sodium is contained in several ingredients (such as water) and medicine. However, the greatest contributor in processed foods to sodium intake is the salt sodium chloride (NaCl) (Kilcast and Angus, 2007, He and MacGregor, 2003). Thus, an efficient and cost-effective way to decrease heart disease and stroke is to reduce the amount of NaCl in processed food. Consequently, the food industry is trying to reduce NaCl in foods by replacing sodium with other substances. However, the replacement of NaCl in food is complex, in particular in yeast-leavened products. Thus, it is well known that the addition of NaCl to yeast-leavened products as wheat dough modifies wheat dough structure as well as yeast´s ability during proofing (Lynch et al., 2009, Kilcast and Angus, 2007). To reduce NaCl it is therefore important to understand the comprehensive mechanism of NaCl in foods. With fundamental knowledge about alteration in food fractions and in particular in yeast-leavened products, the reduction and replacement of NaCl could be made possible. Thus, the following chapter discusses the effect of NaCl in food and in particular in yeast-leavened products.

EFFECTS OF NaCl IN FOODS

The traditional use of NaCl in food combines essential preserving, technological, as well as sensory effects (Brady, 2002). However, the effect of salt reduction will vary from food to food such that each food will have to be considered individually. The main effects will be discussed in the following reviews.

Sensory perception of NaCl

NaCl provides the typical salty taste in food and contributes to the overall taste improvement of food. In detail, the sodium ion is thought to exhibit the salty taste. However, sensory perception is not based on the salty taste alone. The addition of NaCl also increases the ability to taste sweetness and hides metallic, bitter or other negative flavours (Miller and Hoseney, 2008). Omission or reduction of NaCl results in foods that are less flavourful, or rather tasteless.

Typical salt perception in the oral space is based on an ion canal called 'ENaC' (epithelial sodium canal), which is permeable for sodium ions (Figure 1). Due to a concentration slope on the tongue, the sodium ions flow through the ENaC and reach a depolarisation of the membrane.

Through this, Na^+ / K^+ canals are opened and an action potential is caused. The action potential leads to an emission of Ca^{2+} ions which releases neurotransmitters and activates primary sensory neurons which pass the signals on to the brain.

They are processed in the gustatory cortex, are compared to other impulses, and then the taste impression is interpreted as 'salty'. Furthermore, the anions of the sodium salts can cause other additional gustatory nuances and thus sodium salts are potent taste stimuli. The highest discernible Na^+ salt is NaCl because only Cl^- is small enough to diffuse through the tight junctions to the taste buds and thereby create this depolarising effect (anion paradox) (Greger and Windhorst, 1996, Silbernagl and Despopoulos, 2003). However, other chemical molecules, such as the halogenide anions (Br^-, I^-) or metal cations (Li^+, K^+, Mg^{2+}) cause a similar salty taste. Whereby, small ions like Li^+ and Na^+ can stimulate two various receptors (Ossebaard and Smith, 1995), bigger ions such as K^+ only stimulate one of the receptors (Voilley and Etiévant, 2006). Furthermore, the disposal of aromatic components and the repulsion of volatile aromatic molecules are promoted by the

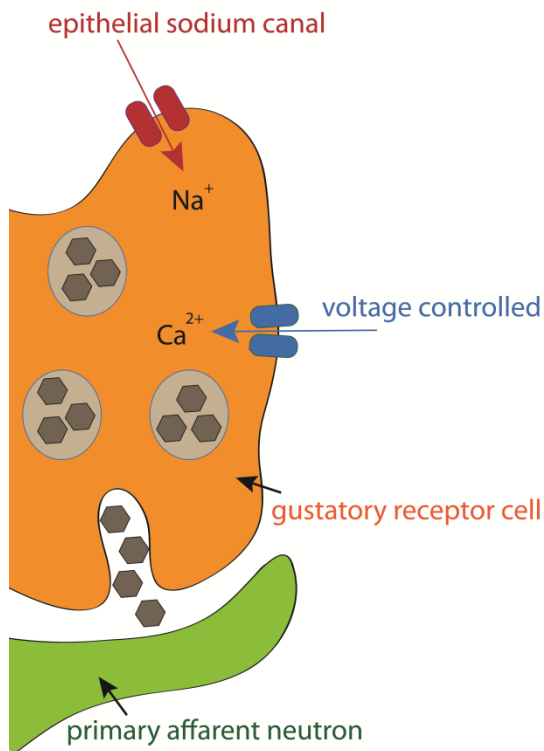use of NaCl, by which the perception of other taste character is intensified (Voilley and Etiévant, 2006).

Figure 1: Schematically illustration of the salt perception in the oral space. Due to a concentration slope on the tongue, the sodium ions flow through the epithelial sodium canal (red) and reach a depolarisation of the membrane (yellow). Through this, Na+/ K+ canals are opened and an action potential is caused. The action potential leads to an emission of Ca2+ ions (blue) which releases neurotransmitters (brown hexagon) and activates primary sensory neurons (green) which pass the signals on to the brain. They are processed in the gustatory cortex, are compared to other impulses, and then the taste impression is interpreted as 'salty'

Salt perception is not only determined by the sodium levels in food matrices. It has been discussed, that sodium interacts with other food components and thus the perception of sodium differs from food to

food (Walsh, 2007). Taste perception difference may come from the complexity of the media in which sodium exist. It has been reported that a one-third reduction in the sodium content of beef stew was not significantly recognised by the sensory panel; however, a one-third reduction in sodium content in simple dishes like mashed potatoes does significantly decrease the saltiness (Malherbe et al., 2003). Further salt perception depends on the texture of the food matrices; however, texture plays a less important role than the complexity of foods (Kroeze, 1990). The perception of salt depends, in this instance, on the viscosity and the texture of the surrounding matrix (Pangborn and Szczesniak, 1974). Since the taste of salt can only be perceived if the salt crystals are in a dissolved unbound form and reaches the salt receptors in the oral space during consumption quickly. The intensity of the sensory salt perception are in direct connection with the solubility rate of the salt in the food medium. A fast solubility can strengthen the salty taste, so that lower NaCl concentrations can be added to food with the retention of a consistently salty taste.

The interaction of salt molecules with the components of the dough system and the

manner of the distribution of the salts in the dough matrix therefore influence the sensation (COST Action 921, 2006, Drewnowski, 1994). Thus, changed structural conditions in the matrix of baked good change the mass transfer and thus the disposal of the ions responsible for salty taste (Voilley and Etiévant, 2006, Pangborn and Szczesniak, 1974, Pozo-Bayón, 2006).

Preserving effects

Sodium chloride has historically been used to preserve food. However, with the introduction of modern refrigerators in the 20th century the need for preservation techniques has been changed. The antimicrobial activity of salt is mainly based on its effects on water activity (a_W). The water activity of a system (as food) is defined as the ratio of the partial pressure of water in the atmosphere in equilibrium with the system (p) compared with the partial pressure of the atmosphere in equilibrium with pure water at the same temperature (p_0):

$$a_W = p \ p_0^{-1}. \tag{1}$$

Water activity is numerically equal to the equilibrium relative humidity (ERH=100 a_W). Sodium and chloride ions collect water molecules around them through ion hydration. This reduces the water activity in the food system; whereby, microbial growth decreases with less water. Further, salt causes water efflux through the semipermeable membrane of the microorganism due to osmotic pressure. Since cells should maintain a defined level of cytoplasmic water, the growth rates are reduces with NaCl addition. Bread shows a moisture content that typically ranges from 18 to 42% (ERH of 88-95%) (Kilcast and Ridder, 2007). With such moisture content (or rather ERH) bread is prone to microbial spoilage. The reduction the moisture content in a food product, e.g. by the addition of NaCl, extends the time it takes for mould or bacterial spoilage (Cauvain and Young, 2007). A further reason for the reducing effect of NaCl on yeast activity is that the yeast cell membrane changes in electro-chemical potential due to contact with Na^+ and Cl^- ions (Matz et al., 1992). Moreover, NaCl may also indirectly affect yeast leaving rates by lowering the oxygen solubility (Kilcast and Ridder, 2007). Thus, in yeast-leavened baked goods, yeast's leaving ability can be controlled and microorganism spoilage in bread can be reduced with the addition of NaCl.

Full or partial replacement of sodium chloride in food products will therefore lead to a loss in shelf life and stability of the products (Bidlas and Lambert, 2008) since microorganism growth is influenced by the nature of the solute (Sperber, 1983). Various salts show different capacities to depress the a_W value (Samapundo, 2010); and thus, NaCl replacers may influence the microbial stability and shelf life of food products. Various solutes showed different antimicrobial effects and even growth stimulating effects on bacteria and fungi (Institute of Food Research, 2005). Particularly in yeast-leavened products, mould spoilage is a serious and expensive problem (Suhr and Nielsen, 2004). Samapundo et al. (2010) analysed the effect of reduced NaCl on the growth of different moulds and on the microbial spoilage of white breads. The results reveal that no difference occurred in mould growth (*Penicillium roqueforti*) on white bread with 30% less NaCl (around 12 g NaCl kg^{-1} flour) compared to the standard white bread (around 18 g NaCl kg^{-1} flour). However, lower NaCl concentrations were not analysed, therefore when salt levels are further reduced, it may be necessary to include other preservative ingredients or reduce

the shelf life to maintain an adequate safety margin.

Technological effects

In various foods the addition of NaCl shows specific qualities which define manufacturing and end-product quality. In cheese products, the addition of NaCl shows a physical change in the protein structure which influences the water binding and the cheese texture. Further, NaCl controls the growth of the starter culture and their activity and various enzyme activities in cheese. NaCl also changes the syneresis of the curd, resulting in whey expulsion and in a decrease in moisture (Guinee and Fox, 1993). In meat and meat products NaCl illustrates a well-known preservative effect and a tenderising action by enhancing the water-holding capacity. Thus, myofibrils swell in high concentrations of NaCl (around 39 g kg^{-1} meat), increase the water binding properties of proteins and improve the meat texture by improve protein interlinking (Offer et al., 1984, Ruusunen et al., 2005). In detail, chloride ions penetrate the myofilaments, producing swollen proteins (Hamm, 1972) and sodium ions may form an ion cloud

around the filaments (Offer and Trinick, 1983). Furthermore, chloride is more strongly bound to the proteins compared with sodium ions; this may cause an increment in negative charges of proteins. Thus proteins hydrate better, repulse each other and swell (Hamm, 1972).

Since 25% up to 35% of the required sodium intake per day and person is taken up by cereal products (Figure 3) (Kilcast and Angus, 2007) cereal products are regarded as the sodium leading supplier in the human diet. Nevertheless, the traditional use of NaCl fulfils various important rheological, technological, sensory and preservatory properties in the manufacturing of foods and in particular of baked goods. Thus, reducing NaCl in cereal-based products will have consequences for food quality that must be taken into account. These consequences for cereal-based foods should be reviewed in detail to establish the necessary background knowledge to minimize sodium without shifting product quality. In following this background is briefly summarized.

The impact of NaCl on wheat protein properties

Protein alteration was also observed in baked goods and is regarded as the point of application of NaCl in dough system. Model studies for wheat sourdough systems using sodium chloride carried out changes in microstructure, dynamic rheology and swelling of wheat protein (Schober et al., 2003). In comparison with the control (without additives) wheat protein with 3% NaCl (solved in water) showed higher complex shear modulus (G*, dough stiffness), whereas the phase angle (δ, dough elasticity) was lower. The microstructure of the wheat protein pieces was analysed with confocal laser scanning microscopy (CLSM) in this study; the wheat protein structures with NaCl elicit a denser but partly dissolved fibrilla microstructure. Wheat protein swelling was reduced with NaCl addition, implicating that the water binding property of wheat protein with NaCl is decreased (Schober et al., 2003).

Zaidel et al. (2008) used response surface methodology (RSM) to analyse the effects of three factors (mixing time, NaCl and water level) on wheat protein formation during mixing. They observed an increase in wheat protein mixing time with

addition of 2% NaCl (based on wheat protein). Interestingly, the combination of high water content and NaCl also leads to an increase of wheat protein mixing time. Other works also illustrated changes in wheat protein mechanical behaviour with the addition of NaCl. Niman (1981) reported, that a smaller amount of NaCl (<2%, based on wheat protein) strengthens the protein network and thus assists in wheat protein development. Further, the study of Zaidel et al. (2008) illustrated an increase in the volume expansion of wheat protein while heating due to the addition of 2% NaCl (based on wheat protein) and a resulting, strengthen wheat protein network. Thewissen et al. (2011) analysed the foaming properties of wheat gliadin and found that poor foaming properties of gliadin at pH 2.0 progresses in the presence of 0.25 and 1.0% NaCl (based on gliadin). They suggested, that NaCl addition stabilises the cross-linking of the proteins. This effect may be based on changes in surface tension, which gradually declines as the NaCl concentration is increased to 2.0%. NaCl may cover positive charges on the protein chain at low pH, which results in decreased surface tension and enhanced foaming properties. The same theory has been proposed in other studies,

illustrating that NaCl addition at pH levels below or above the isoelectric point of proteins results in raised protein cross-linking due to charge masking (Dickinson, 1999, Foegeding et al., 2005).

The impact of NaCl on dough properties

Some papers have analysed the effect of NaCl on wheat dough using recording mixer as the Farinograph (Hlynka, 1962, Galal et al., 1978, Salovaara, 1982b, Tanaka et al., 1967) and Mixograph analyses (Lang et al., 1992, Danno and Hoseney, 1982). The Farinograph measurements showed that for dough consistency at 500 BU, NaCl addition produces a reduction in water absorption and an increase in dough development time (Hlynka, 1962). This effect is probably based on the charge shielding of proteins by NaCl (Figure 2) and the resulting improvement in protein cross-linking. Equivalent Farinograph results were observed by Galal et al. (1978), whereby only 1.5% NaCl (based on flour) was compared with the control (no additives). Takana et al. (1967) observed the effect of NaCl on Farinograph properties with three amounts of NaCl (0, 1, 3% based on flour) and received decreasing dough viscosity with rising NaCl amounts. However, other

Farinograph properties as well as further NaCl concentration were not being shown. Lang et al. (1992) measured the effect of different salts (NaCl, KCl, KNO_3, Na_2SO_4, $CaCl_2$ and $MgSO_4$) at two concentrations (1.3% and 2.6% based on flour) on Mixograph properties. All salts increased time to peak (dough development time) and peak height (dough consistency) compared with the control (without additives). A wider band width of NaCl concentrations (0, 0.5, 1, 2, 5, 10% NaCl)

were studied with Mixograph in the work of Danno and Hoseney (1982). The effects of sodium chloride increased the width and height of the mixogram curves as well as the mixing time.

Recent works (Angioloni and Dalla Rosa, 2005, Lynch et al., 2009, Larsson, 2002) studied the influence of NaCl on dough properties with dynamic rheometry. Using dynamic rheology with wheat dough, Larsson (2002) showed a significant increase in the storage modulus (G′, elastic part of the dough) when 2%

NaCl (based on flour) was included in the dough. Lynch et al. (2009) measured the influence of NaCl (0, 0.3, 0.6, 1.2% based on flour) with fundamental rheological properties within the linear visco-elastic region using an amplitude sweep. However, in these samples neither dough stiffness (complex modulus, G*) nor dough elasticity (tan δ) were significantly different (p<0.05) to the reference without NaCl (Lynch et al., 2009). Danno and Hoseney (1982) measured overmixed wheat dough and stated that dough which had lost its elastic character could be returned to normal elasticity with the addition of NaCl. While mixing dough, sodium and chloride ions affect water availability (Cauvain and Young, 2007) and thus restricted the amount of available water for wheat protein hydration. If less water is bound by the proteins the free water in the dough system increases, which leads to changes in dough rheology (Jekle and Becker, 2011).
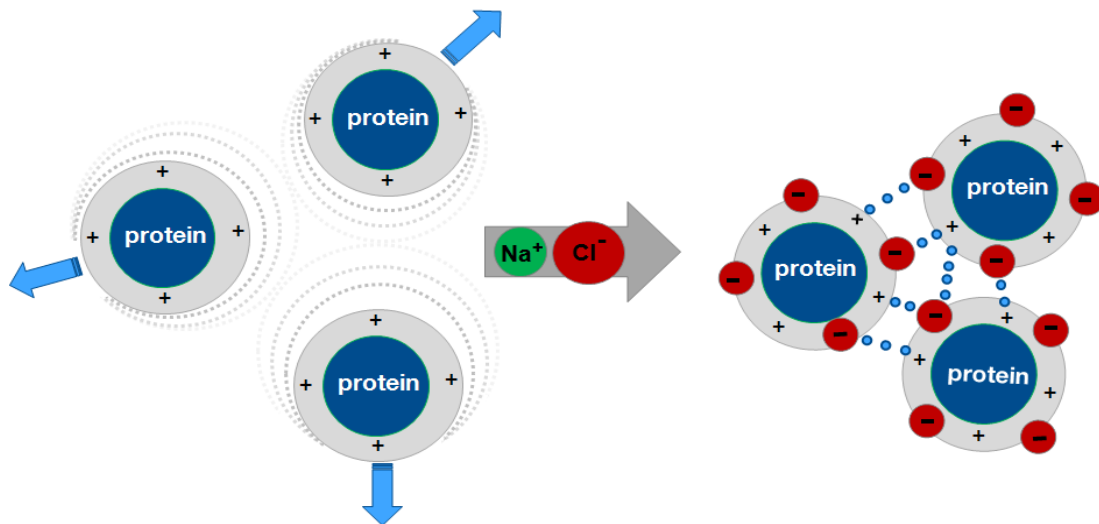
Figure 2: Schematic illustration of the influence of NaCl on the formation of wheat protein in flour-water solution. On the left: wheat protein features more positive charges in solution; therefore, the proteins repulse each other. On the right: wheat protein charge shielding by NaCl ions and the resulting improvement of the protein network formation.

Additionally, changes in dough strength due to NaCl addition were reported in Extensograph measurements (Preston, 1989, Tanaka et al., 1967, Lynch et al., 2009). NaCl was observed to increase both dough extensibility and resistance, whereby only two NaCl concentrations (1, 3%) were used (Tanaka et al., 1967). Preston (1989) analysed the effect of lyotropic neutral salts on Extensograph properties and observed similar effects to NaCl (nonchaotropic anion) on dough extensibility and resistance; whereby, chaotropic anions showed decreased dough extensibility. Another work also showed that decreased NaCl addition tends to reduce the dough resistance to extension (Lynch et al., 2009). However, only three NaCl levels (0.3, 0.6, 1.2% NaCl based on flour) were used and the differences in resistance were not significantly different from the reference (without additives). A further work analysed the influence of NaCl (0, 2.5, 3.5, 4.5 %) on dough thermo-mechanical properties and illustrated that the addition of NaCl to dough slowed down the heat-induced reactions (starch gelatinisation and protein coagulation) (Angioloni and Dalla Rosa, 2005). Therefore, not only the protein fraction of the dough is affected by NaCl, starch is also altered with NaCl addition. The impact of NaCl in delaying starch gelatinisation has been illustrated in several works (Chiotelli et al., 2002, Galal et al., 1978, Preston, 1989). This effect may be based on the lowered water activity in the starch-salt-water solution and thus, in

the increment of the energy required for chemical and physical reactions involving water (Kim and Cornillon, 2001, Seetharaman et al., 2004).

The impact of NaCl on yeast-leavened products

A strengthened or rather extensible wheat protein network is essential to achieve high bread volume with a fluffy crumb. The wheat protein network mainly contributes to the entrainment of gas bubbles in the dough system (Cauvain and Young, 2007, El Fawakhry et al., 2010). It is thought that growing gas cells are stabilised against coalescence and disproportionation by a primary wheat protein -starch matrix with a secondary liquescent lamella on its inner side where the gas cells are inserted (Gan et al., 1990). Gas holding depends therefore on protein quality and quantity (El Fawakhry et al., 2010). Baking studies have illustrated that although NaCl increases protein cross-linking and thus dough strengthening, the baking volume does not increase with raised NaCl addition. In detail, a decreasing bread volume was observed with a high amount of NaCl (Danno and Hoseney, 1982, Czuchajowska et al., 1989).

Decreasing bread volume may be a reason of increased osmotic pressure in the dough system. A quantitative estimation of the osmotic pressure (p_{osm}) in wheat dough due to NaCl addition may be realized by a simplification in calculating p_{osm} in a model yeast-water solution with added NaCl:

$$p_{osm} = z \cdot [NaCl] \cdot R \cdot T, \qquad (2)$$

where z is the amount of substance, R is the gas constant, and T is the temperature (in this case: 30 °C). Equivalent quantities of 15 g NaCl kg^{-1} flour in the model yeast-water solution-NaCl solution show an osmotic pressure of 2.2 MPa and feature negative impact on CO_2 production. Thus, this solution and higher NaCl amounts are hypertonic and consequently negative for yeast-leavening ability. In contrast, 5 g NaCl kg^{-1} flour illustrate an osmotic pressure of 0.75 MPa and this value is isotonic and thus rather optimal for yeast-leavening ability (Anremüller et al., 2008). In summary, low amounts of NaCl provide a stimulatory effect for yeast, and thus high NaCl amount provides a negative effect for the yeast-leavening ability and therefore lower bread volume.

Linko et al. (1984) have also analysed significantly decreasing wheat bread

volumes with increasing NaCl content. However, further studies from Linko et al. (1984) with barley flour illustrate a quite drastic increase in loaf volume, of 20%, when NaCl was raised to 2% (based on flour). This suggested that NaCl addition improves weak protein networks and strengthens normal protein quality to such an extent that loaf volume decrease. Another study of Farahnaky and Hill (2007) mentions, that sodium chloride has an impact on the brown colour of bread. Analysis of the Maillard reaction showed, that for some amino acids (alanine, phynylalanin and lysine) NaCl enhance the colour intensity (Kwak and Lim, 2004). Interestingly this effect was only shown with low levels of NaCl (1% NaCl); high levels of NaCl (10 % NaCl) inhibited colour intensity (Kwak and Lim, 2004).

Contrarily, the work of Lynch et al. (2009) illustrates no significant changes on a wide range of bread quality characteristics. Specific volume showed little difference and crumb hardness did not significantly alter at day zero of storage. However, only three NaCl levels (0.3, 0.6, 1.2% NaCl based on flour) were used for the analysis and compared with the reference (without NaCl). Further, it was mentioned, that bread without salt

was significantly harder after 120 h storage suggesting that NaCl might have an impact on bread staling behaviour (Lynch et al., 2009).

Changes in bread staling may be based on two facts: a) that the dough is too highly viscous to allow the bubbles to expand (Onyango C., 2008) and simultaneously b) the dough is insufficiently capable of retaining the gas, thus the gas bubbles may disperse during proofing and oven rise. Lost gas leads directly to a decrease in crumb porosity and in an increase in crumb firmness.

The gas retention effect, or rather foam stability of the dough, may be explained using the Gibbs-Marangoni effect:

$$Mg = -(d\gamma_S)/dT \; 1/\mu\alpha \; L \; \Delta T, \quad\quad (3)$$

were γ_S is the surface tension, α is the thermal diffusivity, μ is the dynamic viscosity, L the characteristic length, and ΔT is the temperature difference. The Gibbs-Marangoni effect defines the mass transfer along an interface between two fluids due to surface tension gradient. Foam stability caused by Gibbs-Marangoni effect increases due to a) NaCl, which generally raises the surface tension (Greenley, 1987) or b) protein changes, which change the surface tension. To

achieve optimal foam stability, the pH of the protein or rather dough should be near the isoelectric point at which the proteins are uncharged (Douglas and Srinivasa, 2010). If this is not the case, the gas retention capacity in dough decreases and loaf volume decreases too.

Sensory evaluation of wheat bread showed that bread with 1.2% NaCl was clearly distinguished from those without NaCl (Lynch et al., 2009). Furthermore, bread with 1.2% NaCl was described as having less "sour/acidic", "sour dough" and "yeasty" flavour. Another study tried to measure the optimum level of salt in French bread (Sosa et al., 2008). 100 consumers received seven samples of bread with different salt concentrations (0.6-4.6% NaCl based on flour). The panellists responded for each sample and rated the bread as "not-salty-enough", "okay" or "too-salty". The result was that the preferred sodium concentration was around 2.4% NaCl (based on flour), which is considerably higher than common bread which has an average NaCl content of around 1.8% (based on flour) (Sosa et al., 2008). Therefore, the reduction of NaCl results in less flavoursome bread, changes in processability and reduced quality. To

achieve similar bread quality and flavour it is necessary to use sodium replacers.

Sodium Replacers

For sodium reduction, several approaches are feasible in food products as shown in Figure 3. The ingredient combinations which operate as NaCl substitutes are based, on this occasion, on the substitute of sodium by potassium, ammonium, calcium, magnesium (Charlton et al., 2007) and lithium as chloride salts and also by anions like phosphate and glutamate. Salts such as lithium and ammonium chloride, are not suitable, although they provide a salt-like taste, on account of their low heat stability, and in the case of lithium, on account of the toxic effect.

Potassium chloride (KCl) is the most common choice as a possible sodium replacer (Rosett et al., 1995). Sensory analyses illustrate that 20% of NaCl may be replaced by KCl in wheat bread (Salovaara, 1982b, Braschi et al., 2009, Charlton et al., 2007). Further Salovaara (1982b) discussed that 10% of NaCl may be replaced with $MgCl_2$ without changes to flavour. Contrarily, it has been reported that the use of KCl often has a negative taste, by which wheat pastries are

described as metallic, bitter or chemical

(Salovaara, 1982a, Bartoshuk, 2000).

Figure 3: Strategies for improving saltiness perception while sodium reduction.

Substance	Substance	Most important information about the substances
Salts	KCl	Bitter or metallic off-flavour (Salovaara, 1982b, Braschi et al., 2009, Charlton et al., 2007, Salovaara, 1982a, Bartoshuk, 2000) (Bartoshuk, 2000)
	$MgCl_2$	More bitter or metallic off-flavour compared to KCl (Salovaara, 1982b)
	$CaCl_2$	Bitter, sour and sweet at low concentration (1 mM), however, at higher concentration (100 mM) $CaCl_2$ shows a more bitter and salty taste (Tordoff, 1996, Lawless et al., 2003b, Lawless et al., 2003a)
	KCl, $CaSO_4$, $CaCl_2$, $NaPO_4$, $NaSO_4$, NaJ	Negative impact on bread volume probably due to reduced yeast leavening ability (He et al., 1992, Danno and Hoseney, 1982).
	$NaSO_4$	Bitter in high concentrations and salty in low concentrations (Delwiche et al., 1999, Lawless et al., 2003a, Shallenberger, 1993)
Bitter blocker	AMP	Reduce bitterness (Ming et al., 1999)
	4-(2,2,3-trimethylcyclopentyl)-butyric acid	Blocks 18 of the bitter receptors (Slack et al., 2010)
	Sucrose	Commercially the most common sweetener (Kilcast and Angus, 2007)
	Lactose, Threhalose, Thaumatin	Decrease bitterness; however they are sweeteners and increase explicit sweet perception (Kilcast and Angus, 2007, Toshio et al., 1997, Shallenberger, 1993, Birch, 2000)
	Glycine, glycine monoethyl ester	Reduce the water activity in food; acting as a salt enhancer in sausages (Galabert et al., 2003, Omura et al., 2001)
Salt enhancer	Arginine, lysine	Stimulate the taste perceptions: salty and astringent (or harsh, acrid taste, irritating the throat) (Kilcast and Ridder, 2007, Guerrero et al., 1995).
	Lactate	Saltiness enhancer (Price, 1973, Kilcast and Angus, 2007, Takano and Kondou, 2002). Calcium lactate tastes sour and is therefore not widely used in the food industry (Tordoff, 1996). Naturally in sour dough available and may improve salty taste perception.
	Trehalose	Saltiness and flavour enhancer in meat and poultry products; decrease attributes like metallic, bitter and astringent (Toshio et al., 1997)
	Monosodium glutamate	Enhance the taste of other compounds and of saltiness (Kilcast and Ridder, 2007, Ball et al., 2002, Roininen et al., 1996).

Particularly with higher replacement of sodium with potassium salts (more than 30-50% substitution), the foods produced illustrated an off-flavour (Bartoshuk, 2000). The use of calcium chloride ($CaCl_2$) as a sodium replacer has been described as bitter, sour and sweet at low concentration (1 mM), however, at higher concentration (100 mM) $CaCl_2$ shows a more bitter and salty taste (Tordoff, 1996, Lawless et al., 2003b, Lawless et al., 2003a).

Further, the performed cations were also used as iodide-, sulphate and gluconate salts which cause sensory off flavour or a rubber-like texture in wheat bread (He, 1982, Takano and Kondou, 2002). Thus one work analysed the impact of various salts on the baking properties of different flours (He et al., 1992). All salts which may be used as a sodium replacer (KCl, $CaSO_4$, $CaCl_2$) decreased loaf volume. This is probably due to changes in yeast-leavening activity. They state the hypothesis, that factors other than hydrophobic interaction alter the baking quality of flours.

Also other salts ($NaPO_4$, $NaSO_4$, NaJ) showed decreasing effects on bread volume (Danno and Hoseney, 1982). Magnesium sulphate ($MgSO_4$) has been defined as bitter in high concentrations and salty in low concentrations (Delwiche et al., 1999, Lawless et al., 2003a, Shallenberger, 1993). Therefore, in low levels and in combination with other substances, $MgSO_4$ may be a suitable sodium replacer. The perception of bitterness due to several sodium replacers has been noticed since many years and many compounds have been proposed to inhibit bitter taste (Pangborn, 1989, Haga et al., 1984, Godshall, 1988). 4-(2,2,3-trimethylcyclopentyl)- butyric acid (Slack et al., 2010) has recently been discovered to block 18 of the bitter receptors.

It was shown, that sodium salts and bitter substances interact to reduce bitterness while saltiness is unaffected (Bartoshuk et al., 1988, Breslin, 1996). The following substances have been demonstrated to be effective in decreasing bitterness. Sweeteners such as sucrose (commercially the most common sweetener) (Kilcast and Angus, 2007), lactose (Kilcast and Angus, 2007), trehalose (Toshio et al., 1997) and thaumatin (a sweet protein) (Shallenberger, 1993, Birch, 2000) have been described in the literature as being effective in reducing bitterness. Thaumatin is characterised as a brown hydroscopic powder with a sweetness

level of 2000-3000 times that of sucrose (Shallenberger, 1993). Next to the ability to mask bitterness, thaumatin illustrates a more sugar-like profile and is therefore not useful for baked goods such as bread; however it could be helpful in sweet pastries. However, it is often used in toothpaste and breath fresheners since the active ingredients in these products presents an undesired, bitter or astringent note (Kilcast and Angus, 2007). 2,4-dihydroxybenzoic acid (DHB) has been described as a bitter inhibitor without affecting the sweetness (Kurtz and Fuller, 1997). Further, adenosine 5′-monophosphate (AMP) is described as well as a bitter blocker (Ming et al., 1999).

Additionally, novel substances are currently being used in food production to increase the salt perception of a salty tasting material without significant increasing the saltiness (Bertino et al., 1982) (Bonorden et al., 2003). The chemical structures of these salt boosters or salt enhancers are wide-ranging and include glycine and glycine monoethyl ester (Omura et al., 2001), which reduce the water activity in food as well as acting as a salt enhancer in sausages (Galabert et al., 2003). Two other kinds of salt booster are L-lysine and L-arginine. They are obtained

from fermented starch and stimulate the taste perceptions: salty and astringent (or harsh, acrid taste, irritating the throat) (Kilcast and Ridder, 2007, Guerrero et al., 1995). Lactates (potassium, sodium and calcium lactate) are also described as saltiness enhancers (Price, 1973, Kilcast and Angus, 2007, Takano and Kondou, 2002) and are generally used in the meat and poultry industry as preserving agents. Lactate salts as calcium lactate presents a sour component (Tordoff, 1996) and are therefore may be one of the reasons why sourdough breads illustrate better odour and improved taste compared to bread without sourdough (Marklinder et al., 1996, Hansen and Schieberle, Jekle et al., 2010). This effect was illustrated in sourdough fermented wheat bread, since it has a higher sensory score for the overall flavour as well as for salty perception compared to non-fermented wheat bread (Rizzello et al., 2010). Some dipeptide derived from L-ornithine has also been argued to increase saltiness (Tada et al., 1984). On the contrary, in 1987 the saltiness perception by ornithine peptides have been re-examined as the previously expected saltiness of Orn-Tau-HCl result from NaCl residue present as an artefact of the process of the preparation (Huynh Ba and Philippossian,

1987). Further, trehalose, a non-reducing disaccharide known as a saltiness and flavour enhancer in meat and poultry products, may be used to decrease attributes like metallic, bitter and astringent (Toshio et al., 1997). Monosodium glutamate is commonly used in several Asian cuisines due to the umami taste, which does not have a pleasant taste by itself but which enhances the taste of other compounds when present at low levels (Kilcast and Ridder, 2007). Several studies have shown that monosodium glutamate enhances saltiness perception (Ball et al., 2002, Roininen et al., 1996, Kilcast and Ridder, 2007).

Reducing the overall sodium intake is also possible by taste adaptation. Taste adaptation is defined as the customisation of the preferred sodium level to a certain concentration (Kroeze, 1990). Several studies have discussed the positive aspects of a general low-sodium diet with the theory that as a result of a low-sodium diet, individuals adjust their salt perception and perceive food with high NaCl levels as less preferred and less pleasant (Beauchamp et al., 1987, Bertino et al., 1982). This theory is based on the fact, that adults with a very low-sodium diet are able to detect lower levels of NaCl in water compared to adults with a higher sodium intake (Bertino et al., 1982). Furthermore, herbs and spices have customarily been used as salt substitutes, such as black pepper, garlic, onion, ginger, dill seed (Kilcast and Angus, 2007) and may superimpose the missing salty perception.

Obviously NaCl cannot be substituted with a single substance. Rather the possible substitutes should be combined in such a way that the taste as well as the quality of the baked goods is preserved to a great extent.

Conclusion

At this time, less favourable sodium-free salt alternatives are on the market. To reduce the sodium level in baked goods it is conductive to receive at first detailed, systematically analysed, basic knowledge about the alteration in flour-water systems and their thermal treated end-products by NaCl. After establishing this background, sodium substitutes or sodium mixtures may be developed to receive comparable rheological, technological and sensory qualities in the product.

Since the previous studies which are discussed in the current work use less NaCl levels and little comprehensive analysis about the complete manufacturing process (dough, proofing, end-product), future experiments should be preferentially be in the form of novel rheological tests (dynamic rheology, stickiness) which help to provide important data on dough alteration with a wide spectrum of NaCl amounts. Since bread volume depends on the ability of gas pores to grow fundamental data about yeast leavening ability, the gas production and the possibility of containing gas in the dough as a function of decreasing NaCl level additionally may provide a deeper insight into an end-product with less sodium chloride. An overall impact of numerous NaCl levels on the end-product's quality (crumb texture, staling rate and retrogradation) has not yet be done. A global consideration of the detailed effect of a comprehensive range of NaCl on the main flour component (starch and protein) is requested. With a wide-ranging knowledge sodium reduction while maintain processing and product quality is less critical.

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2.3 Impact of sodium chloride on wheat flour dough and baked goods. I. Rheological attributes

Research Article

○SCI

Impact of sodium chloride on wheat flour dough for yeast-leavened products. I. Rheological attributes

Margit Beck,* Mario Jekle and Thomas Becker

Abstract

BACKGROUND: The rheological properties of wheat dough for yeast-leavened products were tested at different levels of sodium chloride (NaCl) addition ranging from 0 to 40 g NaCl kg^{-1} wheat flour. Rheological tests carried out to make this evaluation included (1) empirical rheological methods of the Farinograph, load extension and a dough stickiness test and (2) fundamental rheological methods of creep recovery and dynamic rheometry. Modifications to the gluten matrix microstructure by NaCl were examined by confocal laser-scanning microscopy.

RESULTS: Highly significant ($P \leq 0.001$) differences due to NaCl addition could be determined in particular by the stickiness test as well as by examination of the creep test with the Burger model. Rheological changes measured in the creep test probably depend on protein charge shielding due to NaCl interaction, resulting in an improvement in gluten network formation. An increase in dough stickiness was measured when using NaCl.

CONCLUSION: The present result for stickiness is contrary to the common subjective results. Therefore the theory proposed here for increased stickiness suggests that it is based on more non-protein-bound water in the dough system due to NaCl interaction and thus more viscous dough behaviour, which leads to higher stickiness as measured with the stickiness test. This may also suggest that the objectively measured 'stickiness' in this case does not properly indicate the subjectively measured stickiness it was designed to represent.
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Keywords: dough extensibility; stickiness; CLSM; Burger model

INTRODUCTION

The traditional use of sodium chloride (NaCl) produces various important rheological, technological and sensory changes during the manufacture of baked goods. NaCl is important for controlled proving,[1,2] prolonged shelf life, improved gluten structure and better processability of yeast-leavened products. It is well known that NaCl increases dough stability, mixing time, extensibility and resistance and decreases stickiness because of changes in the gluten network.[3–8] These effects lead to better processability of wheat dough and changes in end-product quality, since some authors have demonstrated decreasing bread volume with increasing NaCl addition.[9,10] Moreover, NaCl is important for sensory perception of baked goods.[11] However, sensory perception is not only determined by salty taste. NaCl also induces other sensory perceptions such as sweet taste and masks metallic or bitter taste.[11] Nevertheless, the use of NaCl in food production is controversial. Increased intake of sodium is considered to be associated with hypertension and increased possibility of cardiovascular disease. Cardiovascular disease (apoplexy, heart attack, heart failure) based on hypertension is thought to be the most common cause of death in industrial countries.[12] Therefore NaCl has been classified as an unwanted constituent of food. Following this governmental classification, nutritive value profiles are generated defining maximum concentrations of food additives such as sodium or NaCl, as about 35% of sodium intake is due

to baked goods.[13] Baked goods containing more than 16 g NaCl kg^{-1} flour are therefore critical according to regulations such as the traffic light system.[14] Therefore determining the permissible reduction in the level of NaCl that still allows for adequate rheological properties of dough and yields high-quality bread remains a formidable challenge that warrants further investigation.

Wheat dough is generally regarded as a complex material composed of a protein network in which starch granules and insoluble flour particles are dispersed. Thus wheat starch granules play a more complex role in dough than an inert filler.[15] Concerning wheat proteins, the unique capability of gluten to build up a network structure is well known.[16–18] The appearance of wheat dough viscoelastic properties is largely attributable to gluten molecules. It is widely accepted that glutenin contributes mainly to gluten's elastic properties while gliadin contributes primarily to its viscous properties.[19] Owing to protein network formation, wheat dough is able to retain gas, producing an aerated bread texture.[20] Variations in processing conditions such as water

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585

content, mixing procedure and time as well as in ingredients such as NaCl have large effects on measurable dough rheological properties. To describe the impact of various ingredients in dough, different rheological approaches are typically used. Empirical dough rheological properties can be tested on the one hand with Z-blade mixer recordings (e.g. Farinograph or Mixograph) and on the other hand with load extension tests (e.g. Extensograph or Alveograph). While these machines are used for quality control in the bakery and milling industries, they conform only slightly to the requirements of fundamental rheological measurement systems of controlled stress and strain conditions.[21] To obtain dough characteristic parameters, it is beneficial to use rheological measurements under conditions that do not largely affect or destroy the structure of the dough system. For this purpose, oscillatory measurements with small amplitudes are appropriate.

Some older studies describe the effect of NaCl addition to wheat dough by using Farinograph or Mixograph analyses.[4,7,22,23] These Farinograph studies show that NaCl decreases water absorption and lengthens the mixing time of wheat dough. Moreover, NaCl generates a strengthening effect on wheat dough due to reinforcement of the gluten network. Other publications show, by means of fundamental rheological measurements, e.g. using a rheometer,[24,25] an increase in structural protein disorganisation and higher dough firmness in fundamental rheological behaviour during a temperature sweep. Larsson[26] found a significant increase in the shear storage modulus G' when 10–20 g NaCl kg^{-1} wheat flour was added. In contrast, other authors reported a decrease in G' with increasing NaCl addition.[25,27] An analysis of the effect of NaCl in wheat dough using an Extensograph showed increased dough resistance and extensibility due to the addition of 10 and 30 g NaCl kg^{-1} wheat flour respectively.[7] Recent Extensograph and Kieffer rig studies found no significant differences between 0 and 12 g NaCl kg^{-1} wheat flour in wheat dough.[25] In summary, our knowledge about ingredients (e.g. NaCl) in dough systems, while substantial, is sometimes contradictory and leaves gaps that need to be filled. Likewise, it is not entirely clear which rheological methods for the analysis of cereal-based dough are best suited and sensitive enough to distinguish subtle differences in dough properties when incremental changes in the level of NaCl are made.

The aim of the present work, therefore, is to provide a detailed analysis of the effects of a wide range of NaCl levels (40–0 g NaCl kg^{-1} wheat flour) on a variety of rheological properties of wheat dough systems as well as on the microscopic structure of proteins. This study will compare fundamental dough properties against rheological measurements to gain an improved understanding of the rheology.

MATERIALS AND METHODS
Materials
For all measurements, wheat flour type 550 harvested in 2008 and obtained from Schapfenmühle (Ulm, Germany) was used. Protein content, moisture content, water absorption, extensibility, pasting properties, α-amylase activity and ash content were determined according to the general principles of the International Association for Cereal Science and Technology (ICC)[28] by methods 105/2, 110/1, 115/1, 114/1, 126/1, 107/1 and 104/1 respectively. Rhodamine B dye was purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany). NaCl was obtained from Merck KGaA (Darmstadt, Germany).

Farinograph measurements
Farinograph measurements were executed in a 300 g Farinograph bowl (Brabender, Duisburg, Germany) at a mixing speed of 63 rpm according to ICC method 115/1.[28] All farinograms were recorded with 300 g of wheat flour (corrected to 140 g kg^{-1} moisture) and at the same absorption level as that in the control experiments (0 g NaCl kg^{-1} flour), where 232 mL of water was required to reach 500 Farinograph units (FU; 1 FU $\equiv$ 9.8 mN m). NaCl was dissolved in the water and then added at levels of 0–40 g NaCl kg^{-1} flour. The Farinograph measurement parameters included water absorption ($W_{\text{Abs}}^{\text{F}}$, %), ICC degree of softening (D_{S}^{F}, mN m) and dough stability (S^{F}, min).

Dough formulation for dough extensibility, stickiness and rheometer measurements
The formula for wheat dough comprised 30 g of wheat flour (corrected to 140 g kg^{-1} moisture) and 23.2 mL of water. NaCl was added at levels of 0–40 g kg^{-1} flour. The dough was kneaded for 5 min in a 50 g Farinograph bowl at a mixing speed of 63 rpm, then proved for 20 min at 30 °C in a closed plastic cup.

Dough extensibility
Extensibility measurements of wheat dough were carried out using a TA-XT2 texture profile analyser (TPA) (Stable Micro Systems, Godalming, UK) and an SMS/Kieffer rig (Stable Micro Systems). Mode was measure force in tension, option was return to start, pre-test speed was 2 mm s^{-1}, test speed was 3.3 mm s^{-1}, post-test speed was 10 mm s^{-1}, distance was 75 mm and trigger force was 0.05 N. After preparation the dough was placed in a closed plastic cup and rested in a clamping tool for 20 min at 30 °C, then removed from the cup and rested for a further 40 min at 30 °C and 80% relative air humidity. The output quantities were dough resistance ($R_{\text{max}}^{\text{k}}$, N) (maximum height of curve = maximum resistance) and dough extensibility (E^{k}, mm) (length of curve at maximum resistance = extensibility).

Stickiness measurement
Dough machinability was determined using a TA-XT2 TPA (Stable Micro Systems) and a dough stickiness rig (Stable Micro Systems) as described by Chen and Hoseney.[29,30] A Chen and Hoseney cell with a cylindrical probe of 25 mm diameter was used. Pre-test speed and test speed were 0.5 mm s^{-1}, post-test speed was 10 mm s^{-1}, applied force was 40 g, return distance was 4 mm, contact time was 0.1 s, trigger force was 0.05 N and way (before test) was 2 mm. The force required to separate the probe from the dough surface was recorded. Double determinations of each single dough with ten single measurements were performed. The TPA profile recorded dough stickiness (D^{S}, g) and dough strength (cohesiveness) (D^{C}, mm).

Rheometer setting and procedure
An AR-G2 rheometer (TA instruments, New Castle, DE, USA) with a smart swap Peltier plate temperature system, 40 mm parallel plates and a plate distance of 2 mm was used for all tests. The temperature was maintained at 30 °C throughout. The wheat dough was clamped between the two serrated parallel plates. Paraffin oil was used on the lateral surface of the dough to ensure that no evaporation or drying took place during each procedure. A thermal cover was used to decrease the thermal gradient of the patterns. First a creep recovery step was carried out and then dynamic rheometry. At least a triplicate determination for each NaCl level was done.
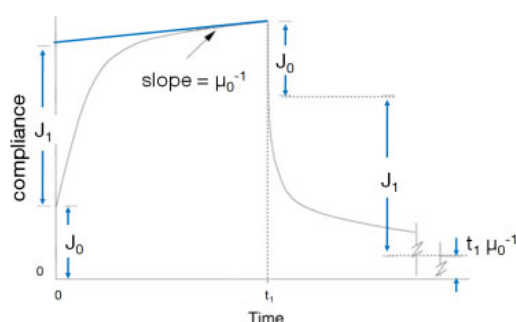
Figure 1. Schematic illustration of Burger model for analysing creep curves.

Creep recovery test

The procedure used a conditioning step of equilibration at 30°C for 10 min to allow relaxation after sample handling. A constant stress (σ) of 250 Pa at 30°C was applied for 60 s and then removed (applied load phase). This stress was used according to the work of Van Bockstaele et al.,[31] who found the best correlation of creep recovery measurements with bread baking quality at a stress of 250 Pa. After removing the torque, the material was allowed to recovered for 180 s, a time long enough for the steady state to be reached. The creep measurement parameters included the time- and stress-dependent recoverable shear deformation (compliance). Creep data may be described by a creep compliance function

$$J = f(t) = \gamma/\sigma \qquad (1)$$

where J is the compliance (Pa^{-1}), γ is the strain and σ is the constant stress applied during creep (Pa).

Creep behaviour can be analysed using the Burger model, which is a four-element model consisting of springs and dashpots in the form of Maxwell and Kelvin components.[32] In the Burger model the mathematical expression of mechanical analogues (springs, dashpots) clarifies the viscoelastic behaviour of the sample. The Burger model was originally applied to the modelling of linear viscoelastic properties; however, it can also be an effective tool for examining creep recovery curves recorded outside the linear viscoelastic range,[33,34] though this remains an empirical point of view. Subsequently, the four-parameter Burger model, described more comprehensively by Steffe,[32] was fitted to the data obtained from the creep test. The model was used to define changes in sample strain (σ) as a function of creep time (t):

$$\gamma = f(t) = \sigma_0/G_0 + (\sigma_0/G_1)[1 - \exp(-t/\lambda_{ret}) + \sigma_0 t/\mu_0] \qquad (2)$$

The Burger model can be written in terms of creep compliance by dividing by a constant stress and considering the retardation time $\lambda_{ret} = \mu/G$:

$$\gamma/\sigma_0 = f(t) = 1/G_0 + (1/G_1)[1 - \exp(-t/\lambda_{ret}) + t/\mu_0] \qquad (3)$$

In the creep phase, where the sample is able to flow after being subjected to a constant shear stress (σ_0), the change in stress with time is zero ($d\sigma/dt = 0$).[32] The outcome can be also written as a function of creep compliance. The total strain ($J(t)$, Pa^{-1}), which consists of elastic (J_0), viscous flow (t/η_0) and viscoelastic ($J_1[1 - \exp(-t/\lambda)]$) contributions, is shown in Fig. 1 and is given by

the expression

$$J(t) = J_0 + t/\eta_0 + J_1[1 - \exp(-t/\lambda)] \qquad (4)$$

Here J_0 is the instantaneous elastic modulus (Pa^{-1}), J_1 is the retarded elastic modulus (Pa^{-1}), η_0 is the coefficient of viscosity associated with viscous flow (Pa s) and λ is the retardation time (s) taken for the strain in the dough that follows the Kelvin model to reduce to e^{-1} of its original equilibrium value after removal of the stress:

$$\lambda = \eta/G \qquad (5)$$

where η is the Newtonian viscosity (Pa s) and G is the shear modulus (Pa).

The parameters J_0, J_1, η_0 and λ were obtained by fitting the experimental data to the Burger model with the software Rheology Advantage Data Analysis (TA instruments).

Dynamic rheometry

The dynamic rheometry changed the frequency from 0.1 to 20 Hz at constant strain amplitude (0.1% strain, 30°C). Preliminary tests indicated that the deformation was well within the linear viscoelastic region. The dynamic rheological properties of samples were assessed in terms of the shear storage modulus (G', Pa), the shear loss modulus (G'', Pa), the complex shear modulus ($|G^*| = (G'^2 + G''^2)^{1/2}$, Pa) and the loss factor (tan $\delta = G''/G'$).

Experimental data as a function of frequency were further described by the power law equations[35]

$$G'(\omega) = K'\omega^{n'} \qquad (6)$$
$$G''(\omega) = K''\omega^{n''} \qquad (7)$$

where G' is the shear storage modulus (Pa), G'' is the shear loss modulus (Pa), ω is the angular frequency (s^{-1}), K' and K'' are the flow consistency indices (Pa s^n) (intercepts of the power law model) and n' and n'' are the flow behaviour indices (the corresponding slopes).

Dough preparation for confocal laser-scanning microscopy (CSLM)

The dough for CLSM was prepared as described for rheological measurements in a 50 g Farinograph at a mixing speed of 63 rpm. Rhodamine B dye was added to the formula at a concentration of 0.001 g per 100 mL water. The dye was dissolved in distilled water before mixing to ensure homogeneous distribution. After mixing, the dough was proved for 60 min at 30°C. Dough handling followed the method of Baier-Schenk et al.[36] A small piece (~0.6 g) was taken from the inner part of the dough and transferred to a specimen shape (diameter 18 mm, height 0.3 mm) before application of a glass coverslip. A TI Inverted Research confocal laser scanning microscope (Nikon, Düsseldorf; Germany) with a 60× oil immersion objective was used. The formation of protein crosslinks in the wheat dough was monitored as a fluorescence image ($\lambda_{exc} = 543$ nm, $\lambda_{em} = 590$ nm) with constant z-position. Each image (1714 μm × 1542 μm) was constructed from 12 single images to provide an enlarged image section of the protein structure.

Statistical analysis

Statistical examinations were carried out by one-way analysis of variance to establish which samples differed from the control (0 g NaCl kg^{-1} flour). Data were analysed with the software Statgraphics Centurion Version 15.1.02 (Statpoint Technologies Inc., Warrenton, VA, USA) at the 5% significance level.

Table 1. Farinograph results of wheat dough with increasing amounts of sodium chloride

NaCl [g kg flour]	Water absorption W^F_{Abs} [%]	Dough stability S^F [min]	Degree of softening D^F_S ICC (mN m)
0	58.7 ± 0.8	3.73 ± 0.43	6.87 ± 1.00
5	58.3 ± 0.6	5.13 ± 1.31	6.05 ± 1.81
10	58.2 ± 0.6	7.00* ± 4.71	5.85 ± 1.80
15	57.9 ± 0.7	12.53** ± 3.71	4.15* ± 1.34
20	58.1 ± 0.7	10.50* ± 2.94	4.69 ± 2.53
25	57.9 ± 0.9	16.13** ± 2.35	3.54* ± 1.38
30	58.0 ± 0.8	17.47** ± 2.32	3.10** ± 1.74
35	57.2* ± 1.1	18.47** ± 0.28	3.10** ± 0.79
40	57.5* ± 0.8	18.47** ± 0.47	2.2** ± 0.31

Results are shown as mean ±95% confidence interval ($n = 3$). Within each column, means significantly different from the 0 g kg^{-1} value are marked: * $P \leq 0.05$; ** $P \leq 0.01$.

Table 2. Biaxial extension measurements as well as dough stickiness and dough strength (cohesiveness) measurements of wheat dough with increasing amounts of sodium chloride. The same amount of water was used for all tests

NaCl [g kg flour]	Resistance (R^k_{max}) [N]	Extensibility (E^k) [mm]	Dough stickiness (D^S) 10^3 [N]	Dough strength (D^C) [mm]
0	0.25 ± 0.01	52.8 ± 2.3	0.00 ± 0.00	3.17 ± 0.4
5	0.28 ± 0.01	50.0 ± 4.0	0.58** ± 0.00	2.01** ± 0.1
10	0.30 ± 0.03	53.1 ± 3.1	0.65 ± 0.00	2.25** ± 0.2
15	0.35** ± 0.03	59.1 ± 4.9	0.65** ± 0.00	2.09** ± 0.2
20	0.33** ± 0.05	67.3** ± 4.0	0.63 ± 0.00	2.32** ± 0.5
25	0.36** ± 0.04	64.2* ± 4.6	0.65* ± 0.00	1.98** ± 0.1
30	0.34** ± 0.03	63.2** ± 3.4	0.67 ± 0.00	2.42** ± 0.3
35	0.33** ± 0.03	65.4** ± 5.8	0.72 ± 0.00	2.38** ± 0.2
40	0.38** ± 0.03	64.1** ± 4.2	0.69** ± 0.00	2.09** ± 0.1

Results are shown as mean ±95% confidence interval ($n = 20$). Within each column, means significantly different from the 0 g kg^{-1} value are marked: * $P \leq 0.05$; ** $P \leq 0.01$.

RESULTS AND DISCUSSION

Physicochemical characteristics of wheat flour

The protein content of the wheat flour used was 12.50 ± 0.02 g kg^{-1} flour, its ash content was 0.59 ± 0.02 g kg^{-1} flour, its water (moisture) content was 13.42 ± 0.11 g kg^{-1} flour and its water absorption was 57.0 ± 1.2 g kg^{-1} flour (values are mean ± standard deviation, $n = 3$). The Extensograph studies showed an area (energy) of 132, 149 and 138 cm^2, a resistance to extension of 274, 358 and 385 Brabender units (BU), an extensibility maximum of 207, 203 and 209 mm, a peak maximum of 495, 627 and 618 BU, an inverse extensibility/resistance ratio of 2.4, 3.1 and 3.1 and an inverse extensibility/maximum ratio of 2.4, 3.1 and 3.1 (mean values after 45, 90 and 135 min respectively). The Extensograph results indicated a strong flour with a large proving tolerance, resulting in well-aerated, voluminous baking products.

Farinograph measurements

The Farinograph studies indicated that decreasing NaCl concentration (40–0 g kg^{-1} flour) increased water absorption (W^F_{Abs}) (Table 1). However, the effect was significant ($P \leq 0.05$) only at ≥35 g NaCl kg^{-1} flour. The tendency of increasing W^F_{Abs} confirms the assumption of decreasing protein hydration capacity due to the competition of sodium and chloride ions and water molecules on the protein surface.[4] It is thought that sodium as well as chloride ions occupy the protein side chains overlaid at the beginning by bound water and therefore the flour's water absorption decreases. Hence NaCl addition may develop a particular, more interconnected protein structure, resulting in more stable and flexible and less sticky dough. This is also indicated by the decrease in dough stability (S^F) with reduced NaCl (18.47 min for the dough made with 40 g NaCl kg^{-1} flour *versus* 3.73 min for the reference dough without salt), with levels of ≥15 g NaCl kg^{-1} flour significantly ($P \leq 0.05$) affecting dough stability. Similar results were reported previously.[5,22] The effect of decreasing S^F is based on charge shielding by sodium and chloride ions, allowing the protein side chains to approach each other, which leads to improved gluten network formation together with lower S^F and higher degree of softening (D^F_S).[23] Furthermore, Danno and Hoseney[23] reported that the effect of even overmixed wheat dough can be reversed by NaCl. Overmixed dough that has lost its elastic character and become stickier can be restored to a more elastic and less sticky

dough by subsequent NaCl addition. Stickiness in the work of Danno and Hoseney[23] was analysed manually based on subjective measurement. In order to make stickiness easier to measure and provide objective numbers, the stickiness measurement device and technique of Chen and Hoseney[29,30] were developed.

Stickiness measurement

Sticky dough causes problems in bakery production since it often adheres to proving baskets, thus hindering continuous production flow.[37] Therefore the effect of decreasing NaCl concentration on the stickiness (D^S) and strength (cohesiveness, D^C) of wheat dough was determined (Table 2). Decreasing NaCl concentration (40–0 g kg^{-1} flour) decreased dough stickiness. Owing to the high statistical spread of the stickiness data, differences from the control (0 g NaCl kg^{-1} flour) were significant above 5 g NaCl kg^{-1} flour addition. Therefore dough strength showed highly significant ($P \leq 0.05$) differences from the control for all NaCl concentrations, indicating that D^C is a good parameter to analyse changes in wheat dough due to variations in NaCl addition.

However, it is well known, based on the practical experience of bread bakers, that (a) NaCl strengthens dough and makes it tighter and (b) dough without NaCl is subjectively stickier and more liquid-like. While the results of the present study are clearly contrary to the expectations of most cereal scientists, there are several possible reasons why these unexpected results may have occurred: (a) the commonly accepted notion that more salt reduces stickiness, as noted by Danno and Hoseney,[23] was based on subjective evaluation and (b) it may be that for many other tests the testing device of Chen and Hoseney[29,30] correlates well with subjectively evaluated stickiness. However, in this particular case the correlation is contrary to expectation.

A hypothesis for this contrary result may be as follows. Colloid titration measurements indicated that gluten proteins show more positively charged amino acids than negatively charged amino acids.[38] Thus in normal wheat dough (pH ≈ 6) the gluten surface molecules have a positive charge and repulse each other. Owing to the hydrophilic character of proteins, the protein chains hydrate faster and more intensively. A low level of NaCl (0.05 mol L^{-1}) neutralises the charge repulsion of amino acids on the surface of gluten proteins, and the proteins act as neutral dipoles.[39]

This shielding effect will reduce electrostatic repulsions between gluten molecules and thus favour protein network formation. Based on these facts, gluten molecules in the presence of NaCl do not hydrate as much as they do without NaCl. Hence there is water in wheat dough that is not bound by proteins, and this seems to be responsible for the increased stickiness. As in previous studies,[37,40,41] dough stickiness was markedly influenced by water content. With firmer dough (due to NaCl addition) the contact area between the dough and the surface of the plunger (of the stickiness assembling) is less compared to less viscous dough. Thus firmer dough will separate easily from the plunger surface. If the dough is rather viscous owing to the presence of non-protein-bound water, it will flow and not easily overcome the adhesive force,[42,43] thus increasing in stickiness. In summary, a higher amount of NaCl increases the contact force between the plunger and the dough surface owing to the altered water circumstances. Thus unbound water in the dough results in higher stickiness.

Dough extensibility

Load extension tests on flour dough are widely used by cereal scientists. The Kieffer dough and gluten extensibility rig is a small-scale version of the Brabender Extensograph, and the experimental data can be expressed in terms of stress and strain. Grausgruber et al.[44] found significant ($P \leq 0.001$) relationships between micro-Extensograph (SMS/Kieffer rig) and standard Extensograph (Brabender) results.

The results of biaxial extension tests performed on wheat dough with increasing amounts of NaCl ($0-40$ g kg^{-1} flour) are presented in Table 2. Overall, wheat dough showed low extensibility ($E^k < 65$ mm) and low resistance ($R^k_{max} < 0.40$ N). Furthermore, increases in dough resistance and extensibility were observed with decreasing NaCl concentration from 20 to 0 g kg^{-1} flour owing to reduced repulsion of gluten molecules and thus less protein network formation. Thus R^k_{max} and E^k were significantly ($P \leq 0.05$) affected by NaCl levels above 15 g kg^{-1} flour. Recent Extensograph studies with $12-0$ g NaCl kg^{-1} flour displayed similar results, with the extensibility and resistance of wheat dough decreasing, though these results were not significantly ($P \geq 0.05$) affected by NaCl.[25] Without NaCl, gluten within the dough complex is expected to be very weak and thus the protein network retains less CO_2 (produced by yeast fermentation) compared with dough from 20 g NaCl kg^{-1} flour. A better tolerance of dough to proving can be predicted owing to an improved gluten network when salt is added. Thus dough without NaCl lacks stability, based on few elastic components, resulting in a viscous dough that does not retain its shape completely. Improved gluten network formation due to less shielding of charged gluten molecules is achieved by increasing NaCl addition. Thus the interaction between polymer crosslinks becomes stronger, leading to increased dough resistance and extensibility.[45]

Higher levels of NaCl ($20-40$ g kg^{-1} flour) led to further increases (but not to the same extent as $0-20$ g NaCl kg^{-1} flour) in dough resistance and extensibility. At this stage the interaction between polymer crosslinks becomes stronger, leading to increased dough strength, maximum resistance to extension and restored force after deformation.

CLSM images

Following the study of the rheological changes due to linkages in wheat dough, microscopic images were obtained to clarify the
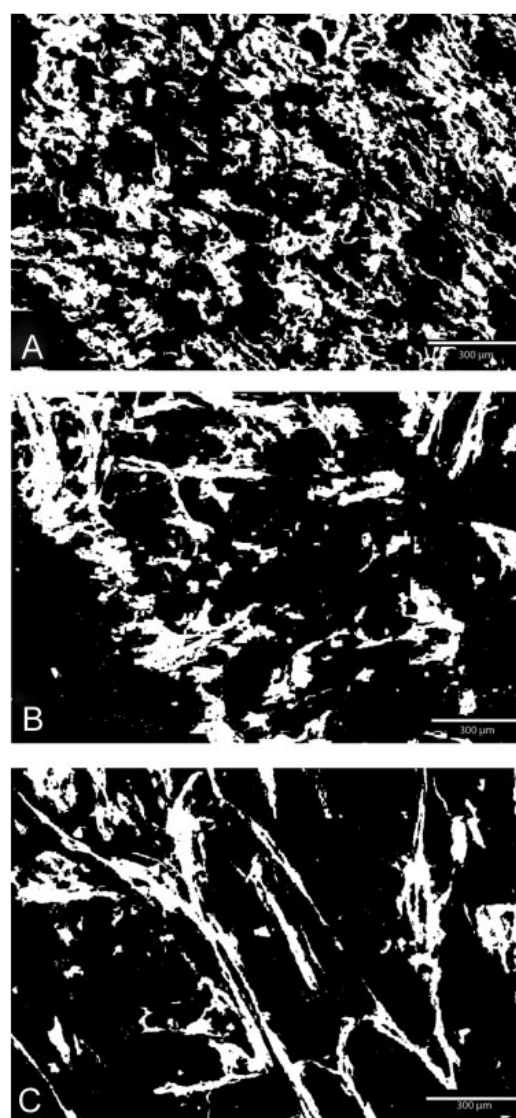

Figure 2. Binary 2D CLSM images of wheat dough with (A) 0, (B) 20 and (C) 40 g NaCl kg^{-1} flour stained with rhodamine B after 60 min of proving at 30 °C. Each image (1714 µm × 1542 µm) was constructed from 12 single images to provide an enlarged image section of the protein structure.

extent of the linking of wheat proteins. The protein structure was investigated using CLSM. The resulting images are shown in Fig. 2, where white structures represent the wheat proteins stained with rhodamine B dye. Different amounts of NaCl (20 and 40 g kg^{-1} flour; Figs 2B and 2C respectively) were added for comparison with the control sample (0 g NaCl kg^{-1} flour; Fig. 2A). Less linkages between proteins and elongated protein structures were found in the dough with 20 g NaCl kg^{-1} flour compared to 40 g NaCl kg^{-1} flour. Further reduction of NaCl changed the protein structure from elongated protein strands to very less connected protein particles.

M Beck, M Jekle, T Becker

Table 3. Creep results of wheat dough with increasing amounts of sodium chloride (250 Pa, 500 FU, 30 °C), analysed by Burger model

NaCl (g kg^{-1} flour)	Instantaneous elastic modulus J_0 (10^{-6} Pa^{-1})	Retarded elastic modulus J_1 (10^{-3} Pa^{-1})	Zero shear viscosity η_0 (10^3 Pa s)	Retardation time λ (s)
0	0.2 ± 0.1	2.2 ± 0.5	28.8 ± 2.3	13.3 ± 1.6
5	1.6 ± 0.6	3.6 ± 0.3*	14.4 ± 1.6**	13.6 ± 1.9
10	1.2 ± 1.4	3.3 ± 0.4	14.8 ± 3.1**	13.3 ± 0.1
15	0.5 ± 1.5	3.2 ± 0.4*	17.5 ± 4.3**	16.1 ± 1.8*
20	1.2 ± 0.6	2.8 ± 0.3	18.4 ± 2.1**	15.4 ± 0.8
25	0.8 ± 1.9	2.8 ± 0.3	17.6 ± 0.6**	16.0 ± 0.5*
30	1.7 ± 1.9	3.4 ± 0.3*	17.5 ± 1.3**	16.0 ± 0.5*
35	2.5 ± 4.3	3.3 ± 1.0*	14.5 ± 4.2**	15.6 ± 1.6
40	4.0 ± 3.4*	3.4 ± 0.6	6.2 ± 2.7**	19.5 ± 1.2**

Results are shown as mean ±95% confidence interval ($n = 3$). Within each column, means significantly different from the 0 g kg^{-1} value are marked: * $P \leq 0.05$; ** $P \leq 0.01$.

Creep recovery test

The effect of varying amounts of NaCl on the creep behaviour of wheat dough analysed by the Burger model is shown in Table 3. The Newtonian viscosity (η_0) was higher and the retardation time (λ) and instantaneous elastic modulus (J_0) were lower for dough without NaCl than for dough with NaCl. The Farinograph analyses (Table 1) and extensibility tests (Table 2) showed an decrease in protein network formation due to NaCl reduction.

The retarded elastic modulus ($J_1 \approx 10^{-3}$ Pa^{-1}) was much higher than the instantaneous elastic modulus ($J_0 \approx 10^{-6}$ Pa^{-1}). Hence viscoelastic deformation was more important in total creep deformation than instantaneous elastic deformation.

With decreasing NaCl (40–0 g kg^{-1} flour) addition, J_0 dereased. Since the instantaneous elastic modulus reflects the extent of bonding between gluten proteins, this increase in elasticity can be attributed to the shielding of protein charges due to NaCl addition.

The coefficient of viscosity associated with viscous flow, referred to as zero shear viscosity (η_0), which is the viscosity at the steady state (the terminal region of the creep phase), was highest for pure wheat dough (0 g NaCl kg^{-1} flour). Sivaramakrishnan et al.[46] explained this result as the effect of free-moving particles in an

aqueous suspension such as wheat dough without NaCl due to less crosslinking of proteins, resulting in higher flowability. Thus η_0 increased significantly ($P \leq 0.01$) with decreasing NaCl (40–0 g kg^{-1} flour) addition. Since the crosslinking of proteins decreased with NaCl addition, the mobility of particles is increased.

The retardation time (λ) characterises the viscoelastic response of a material and can be thought of as the time required for a macromolecule to be stretched out after deformation.[32] A short relaxation time indicates that the relaxation process will be fast. Thus, with decreasing NaCl addition, λ of wheat dough decreased owing to greater dough elasticity.

Dynamic rheometry

The combination of creep recovery measurements and dynamic rheometry measurements provides a complete spectrum of relaxation times from low to high frequency. As an example, the effect of NaCl on the evolution of shear storage modulus (G'), shear loss modulus (G''), complex shear modulus ($|G^*|$) and loss factor (tan δ) values at a frequency of 10 Hz is shown in Table 4. Overall, the shear storage modulus was higher than the shear loss (viscous) modulus, indicating that all dough samples presented a firm, elastic-like behaviour. In wheat dough, both G' and G'' decreased with NaCl reduction for all ranges of frequency studied. These results were in accordance with Larsson,[26] who found that increasing NaCl (10–20 g kg^{-1} flour) concentration in wheat dough resulted in a small but significant increase in G'. Increased values of G' and G'', and thus a higher aggregation tendency of proteins, indicated an improved elastic modulus as well as an improved viscous modulus. The complex shear modulus ($|G^*|$) and loss factor (tan δ) of wheat dough with 10–0 g NaCl kg^{-1} flour decreased owing to protein charge shielding by sodium and chloride ions. However, other authors observed no change in tan δ with NaCl concentration from 12 to 0 g kg^{-1} flour.[25,47]

The dynamic rheometry results were analysed systematically using flow theory (Eqns (6) and (7)). Table 4 presents the parameters of the power law equations describing the dependence of moduli on oscillation frequency. K can be used as the consistency coefficient. It describes the overall range of viscosities across the part of the flow curve that is being modelled. The lowest values of K' and K'' were obtained for the dough without NaCl, while K' and K'' increased with increasing NaCl addition. Thus the viscosity

Table 4. Effect of NaCl addition to wheat flour on dynamic rheometry measurements (30 °C, 10 Hz) and on data analysed by power law equations

NaCl (g kg^{-1} flour)	Dynamic rheometry measurements				Power law constants			
	Complex shear modulus $|G^*|$ (Pa)	Loss factor tan δ	Shear storage modulus G' (Pa)	Shear loss modulus G'' (Pa)	Flow consistency index of G' k' (Pa s$^{n'}$)	Flow behaviour of G' n'	Flow consistency index of G'' k'' (Pa s$^{n''}$)	Flow behaviour of G'' n''
0	3255 ± 256	0.425 ± 0.018	2997 ± 260	1270 ± 42	1271 ± 44	0.218 ± 0.003	499 ± 75	0.240 ± 0.001
5	3780 ± 446	0.413 ± 0.001	3502 ± 427	1423 ± 133	1296 ± 197	0.210 ± 0.006	489 ± 65	0.233 ± 0.007
10	3826 ± 102	0.417 ± 0.006	3532 ± 86	1471 ± 58	1516 ± 163	0.206 ± 0.008	533 ± 22	0.240 ± 0.015
15	3861 ± 168	0.417 ± 0.010	3564 ± 157*	1486 ± 69	1506 ± 39	0.205 ± 0.007	566 ± 45*	0.230 ± 0.015
20	3847 ± 51	0.412 ± 0.000	3748 ± 48	1466 ± 19	1473 ± 30	0.209 ± 0.005	531 ± 17	0.242 ± 0.009
25	4327 ± 132*	0.412 ± 0.009	4001 ± 121**	1647 ± 61**	1699 ± 116**	0.209 ± 0.014**	591 ± 28**	0.241 ± 0.003
30	4379 ± 296*	0.402 ± 0.008**	4063 ± 277**	1633 ± 107	1804 ± 211**	0.200 ± 0.014**	626 ± 66**	0.235 ± 0.008
35	4292 ± 191	0.392 ± 0.000*	3996 ± 178**	1565 ± 71	1700 ± 12**	0.207 ± 0.011	573 ± 2*	0.236 ± 0.009
40	4011 ± 311	0.398 ± 0.002*	3793 ± 335*	1429 ± 259	1537 ± 186	0.207 ± 0.009	537 ± 76	0.230 ± 0.004

Results are shown as mean ±95% confidence interval ($n = 3$). Within each column, means significantly different from the 0 g kg^{-1} value are marked: * $P \leq 0.05$; ** $P \leq 0.01$.

increased owing to NaCl addition. However, this effect was only significant ($P \leq 0.05$) for K' and K'' at ≥ 25 and ≥ 15 g NaCl kg^{-1} flour respectively.

The exponent n is known as the power law index (or sometimes the rate index). Values of n closer to zero indicate protein network formation.[35] In wheat dough a mixture of highly crosslinked material and non-crosslinked material is present. A lower n value indicates that the dough comprises more crosslinked material. In the present study, n values ranged between 0.200 and 0.242, indicating the existence of a protein network.[35] A significant ($P \leq 0.05$) increase in n' and n'' due to NaCl addition was obtained, suggesting that more protein particles were crosslinked and network formation decreased as a result of NaCl reduction.

CONCLUSIONS

Wheat dough manufacture by mixing flour and water is a challenging process, and with the addition of ingredients such as NaCl it becomes even more complex. The present research demonstrates comprehensively the effect of NaCl on the rheological behaviour of wheat dough as well as giving an overview of to what extent rheological methods show significant differences among varying NaCl concentrations.

1. The addition of NaCl causes rheological modification of dough primarily by influencing the structure and formation of the gluten matrix, as visualised by CLSM. These effects are due to the neutralisation of charge repulsion emanating from charged amino acid moieties on the surface of gluten proteins. This charge neutralisation is caused by sodium and chloride ions. It has to be borne in mind that the elucidation of differences in wheat dough with NaCl addition of <5 g kg^{-1} flour by rheological methods is complex. Not every rheological method showed significant differences when compared with the control (0 g NaCl kg^{-1} flour). This insight is in agreement with Lynch et al.[25]

2. When adding <5 g NaCl kg^{-1} flour, highly significant ($P \leq 0.001$) differences from the control could be shown only in dough strength (D^C, measured by the stickiness test) and zero shear viscosity (η_0, analysed by the Burger model). Thus rheological consideration of dough with different levels of NaCl is complex but of great value. Using the Burger rheological model, the complexities of wheat dough systems can be analysed systematically to great effect.

3. A conclusion from this study is that NaCl increases dough stickiness as measured by the method of Chen and Hoseney.[29,30] This contradicts the accepted norm of bakery engineers that NaCl decreases dough stickiness, confirmed in earlier work by Danno and Hoseney.[23] The subjective method reflects the experience of innumerable bakery engineers and dough scientists, past and present. Therefore the 'greater stickiness' found using the objective method is not 'stickiness' per se. It is an artefact of the objective method, even if it is a valuable artefact. This method was designed to be of value only so long as it represented subjectively measured stickiness, universally known to bakers of bread and similar products, bakery scientists, rheologists and others.

This paper presents for the first time a comprehensive study of the effect of NaCl on wheat dough systems by means of a number of rheological measurements over a wide range of NaCl levels to obtain an improved understanding of wheat dough rheology. Moreover, the results obtained from this study provide a valuable basis for designing functionally effective sodium replacers as well as explaining modifications to dough rheology caused by a reduction in sodium content.

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2.4 Impact of sodium chloride on wheat flour dough and baked goods. II. Baking quality parameters and their relationships

Impact of sodium chloride on wheat flour dough for yeast-leavened products. II. Baking quality parameters and their relationship

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Abstract

BACKGROUND: The traditional use of sodium chloride (NaCl) fulfills various important rheological, technological and sensory properties in the manufacturing of yeast-leavened products. However, the use of NaCl in food production has been discussed controversially since a high intake of sodium seems to be associated with hypertension. This study investigates the baking quality parameters of wheat breads containing various levels of NaCl ($0-40$ g NaCl kg^{-1} flour).

RESULTS: Crumb firmness and rate of bread staling decreased with decreasing NaCl levels. A slight increase in loaf volume was observed based on the increased yeast leavening ability resulting from additional NaCl. Higher crumb retrogradation (measured by differential scanning calorimetry) was observed with low NaCl levels.

CONCLUSION: The retrogradation effect is based on the theory that NaCl probably leads to Na^+ inclusion in starch molecules during storage and thus reduces retrogradation. Further, significant ($P \leq 0.05$) linear relationships ($r \geq 0.829$) between Rheofermentometer results, bread volume and crumb firmness were found, suggesting a predictability of bread quality by measurement of gas release.
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Keywords: staling rate; gas release; DSC; retrogradation; Rheofermentometer

ABBREVIATIONS

a	initial crumb firmness (Pa)
A_w	water activity ($-$)
b	crumb firming rate ($-$)
C^F	crumb firmness (N)
C^S	crumb springiness ($-$)
ES	extent of staling (N)
h	height at the end of the test (mm)
H_m	maximum height (mm)
H'_m	maximum height of gas release curve (mm)
$\triangle H$	gelatinization enthalpy of dry sample (J g^{-1})
NaCl	sodium chloride
R_C	retention coefficient of dough (%)
T_1	time at maximum dough height (min)
T'_1	time to reach H'_m (min)
T_{max}	peak maximum ($^\circ$C)
T_x	time of gas release (min)
$T_{2\,NaCl}$	time when H_m of 60 mm is reached (20 g NaCl kg^{-1} flour)
V_B	bread volume (cm^3 g^{-1} flour)
V_T	total volume of CO_2 (mL)
V_L	volume loss of CO_2 (mL)
V_R	volume retention of CO_2 (mL)

INTRODUCTION

Sodium chloride (NaCl) is essential for the survival of human beings, and an intake of abound 1.5 g NaCl per day per person is necessary to maintain important bodily functions. However, the mean daily NaCl intake (estimation from total urinary sodium) in the UK, as well as in other countries, increased during the last decades up to 11.0 g for men and 8.1 g woman.[1] High sodium intake is assumed to be associated with high blood pressure and increase the risk of cardiovascular disease, and is therefore under critical observation by the European community.[2] Since baked goods are considered to deliver around 35%[3] of the daily sodium intake, it is important to reduce sodium content.

However, the traditional use of sodium chloride in baked goods has important sensory, rheological and technological effects in wheat breads, and it is therefore a challenge to reduce this substance. Primarily, the saltiness of NaCl is most important for the sensory impression of baked foods.[4,5] However, sensory perception is not determined by the salty taste alone, since NaCl also enhances other sensory impressions, e.g. sweet taste, and masks metallic or bitter tastes.[4] Further, empirical dough rheological properties such as farinograph studies have determined that NaCl increases dough development time,[6,7] while extensograph measurements show an increase in extensibility and resistance.[7–10] These effects result from improved processability

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of the wheat dough and enhancement of the gluten structure. In addition to these sensory and rheological effects, NaCl also affects the leavening rate of yeast cells, prolongs shelf life and boosts quality.[4] Concerning yeast leaving rates, several controversial studies are discussed. Oda and Tonomura[11] report that yeast cells have a higher leavening ability with 30 g NaCl kg^{-1} flour compared to dough without NaCl, whereby the cell growth decreases with increasing NaCl. In contrast, other studies show reduced CO_2 production by yeast cells or lower yeast activity with increasing NaCl concentration in wheat dough.[12–15]

The addition of ingredients such as NaCl, sugar or fat always induces alteration in loaf volume and thus in bread crumb texture.[16] Some studies only analyze loaf volume in relation to increasing NaCl addition, with the volume decreasing as the NaCl level rises.[16,17] However, so far no studies regarding crumb texture as well as bread quality alteration during storage (bread staling) and subjected to wide ranges of NaCl addition have been published. Bread staling is an important but complex phenomenon in which multiple chemical changes occur. It is also responsible for huge economic losses to both the baking industry as well as the consumer. Several works show that bread staling is closely associated with starch changes during storage.[18,19] Thus the effect of NaCl on starch gelatinization in starch–water model systems has been studied by many researchers using differential scanning calorimetry (DSC).[20–22] However, little work on starch recrystallization (retrogradation) in wheat bread as a result of NaCl has been done so far. It is obvious that the topic of NaCl-induced starch changes was studied solely for model starch systems. The impact of NaCl on retrogradation in complex dough studies has hardly been examined. Thus, to evaluate starch recrystallization due to NaCl addition in yeast-leavened products such as bread would increase scientific understanding of reduced sodium breads.

Past works only deal with the influence of NaCl on wheat dough volume analyzed in the Rheofermentometer and with added lower amounts (0–12 g NaCl kg^{-1} flour) of NaCl than usual.[12] Others measure wheat bread volume with NaCl addition[16,17] but do not determine the staling rate of the breads. Thus a complete bread quality analysis with varying amounts of NaCl is lacking. The objective of this study was to evaluate the broad effect of various NaCl contents (0–40 g kg^{-1} flour) on wheat bread quality during storage, e.g. extent of staling, rate of staling, and changes in starch retrogradation. Further, a relationship between bread quality parameters and wheat dough properties during proofing was determined. Only with proper scientific data regarding sodium functionality may bakers gain a wider insight into end-product alteration which will help in the processing of sodium-reduced baked goods.

MATERIALS AND METHODS
Materials
For all measurements, wheat flour type 550 harvested in 2008 from Schapfenmühle (Ulm, Germany) was used. Protein content, moisture content, water absorption, extensibility, pasting properties, α-amylase activity, and ash values were determined according to the general principles of the International Association for Cereal Science and Technology (ICC) 105/2, 110/1, 115/1, 114/1, 126/1, 107/1, and 104/1 (23), respectively. For the baking test, dry yeast Fermipan red (DSM Bakery ingredients, Heerlen, Holland) was used. NaCl was obtained from Merck KGaA (Darmstadt, Germany).

Dough development and gaseous release characteristics
The formula comprised 300 g wheat flour (corrected to 14% moisture), 175 mL water and 3 g dried yeast. NaCl was added at levels of 5 g (0–40 g kg^{-1} flour). Dough development and gaseous release procedure followed the guidelines of the Chopin Group. A Rheofermentometer (Chopin, Tripette & Renaud, Villeneuve-la-Garenne, France) was used to study the dough development and gas release of dough loaded with a 1 kg weight. The dough was kneaded for 300 s in a 300 g Farinograph (Brabender, Duisburg, Germany) at a mixing speed of 63 rpm. All NaCl levels were replicated thrice.

Bread-making procedure
For baking, wheat flour (corrected to 14% moisture), the respective amount of NaCl, 583 mL water, and 10 g dry yeast were used per kilogram of flour. Although up to 40 g NaCl kg^{-1} flour is uncommon in recipes, this amount was studied to achieve a broad scientific knowledge about the effect of various NaCl levels in yeast-leavened products. The dough was mixed for 60 s at 100 rpm and 360 s at 200 rpm in a laboratory-scale spiral mixer (Diosna, Dierks & Söhne GmbH, Osnabrück, Germany). Dough rested for 15 min and was separated into 550 g pieces. The pieces were molded, inserted in a pan, proofed for 60 min (30 °C; 80% air relative humidity), and baked at 220 °C for 30 min in a multiple-hearth oven (Matador MD 120, Werner & Pfleiderer, Dinkelsbühl, Germany). This standard baking test was performed for each NaCl level with multiple pieces of dough (= four loaves). Each level was repeated thrice (a mean value of 12 loaves).

Bread analysis
Bread volume was measured 2 h after baking using a laser-based volumeter BVM (TexVol Instruments AB, Viken, Sweden). After cooling, the loaves were sealed in PM-LDPE bags with an inert gas atmosphere (80% N_2 and 20% CO_2), to protect them from mold, and stored at 18 ± 3 °C. Crumb quality was measured after 24, 96 and 168 h with a Texture Profile Analyzer (TPA) type TA.XT2 (Stable Micro Systems, Godalming, UK) according to AACC method 74-09. Slices of 25 mm thickness were penetrated with a plunger (diameter 36 mm). The pre-test speed was 1 mm s^{-1}, test speed 1.7 mm s^{-1}, and post-test speed 10 mm s^{-1}. Distance was 10 mm, strain 40% compression, trigger force 0.05 N, distance (before test) 2 mm, and waiting time between first and second compression cycles 5 s. At each measurement all loaves were sliced into six slices. Each measurement was repeated three times (the mean value consisted of 18 slices). TPA software recorded the following parameters: crumb firmness (C^F) (newtons) and crumb springiness (C^S) (−). The extent of staling (ES) (newtons) was calculated as the difference between final (168 h storage) and initial crumb firmness (24 h storage).

Differential scanning calorimetry
A DSC 6 (PerkinElmer GmbH, Überlingen, Germany) with DSC pan part no. BO 14–3004 and cover 0.15 mm (PerkinElmer) was used in the experimental work to evaluate dynamic thermal behavior. After 96 h storage the bread was weighed ($\sim$25 mg) into the pan, which was then sealed. Heating scans were run on the samples in pans from 25 to 90 °C against an empty pan (reference) with a heating rate of 10 °C min^{-1}. Gelatinization enthalpy (ΔH) was calculated from the area under the achieved curve and was expressed in joules per gram dry weight. All results were the average of six experiments.

Statistical analysis

Statistical evaluation was realized by one-way analysis of variance (ANOVA) to establish which samples provided differences amongst the control (0 g NaCl kg^{-1} flour). Data were analyzed with the Statgraphics Centurion software (version 15.1.02, Statpoint Technologies Inc., Warrenton, VA, USA) at 5% significance level. To explore relationships between variables a simple linear regression analysis was calculated using Statgraphics Centurion. The correlation coefficient r was used for illustration of the degree of correlation.

RESULTS AND DISCUSSION

Physicochemical characteristics of wheat flour

The amylograph results show a pasting maximum of 665.0 ± 32.2 AE and a pasting temperature of $86.1 \pm 4.1\,^{\circ}$C; this indicates a low enzyme activity. The decrease of 341.2 ± 6 s indicates low α-amylase activity. Furthermore, the protein content was 125 ± 2 g kg^{-1} flour, ash content 5.9 ± 0.2 g kg^{-1} flour, moisture content 13.4 ± 0.1%, and water absorption 57.0 ± 1.2% (mean values $\pm$ SD, $n = 3$). Extensograph results show an area of 132.0 ± 8.1, 149.2 ± 10.1, 138.3 ± 9.0 cm^2; resistance to extension of 274.2 ± 8.1, 358.2 ± 5.1, 385.5 ± 12.1 BE; extensibility maximum of 206.5 ± 12.1, 203.2 ± 8.5, 208.5 ± 10.1 mm; maximum of 495.4 ± 13.1, 626.8 ± 10.1, 618.3 ± 8.1 BE; ratio extensibility resistance^{-1} of 2.41 ± 0.1, 3.11 ± 0.5, 3.11 ± 0.2; and ratio extensibility maximum^{-1} of 2.40 ± 0.2, 3.12 ± 0.3, 3.11 ± 0.1 (mean values after 45 min, 90 min and 135 min, respectively). The Extensograph results approved the strong flour characteristics with a large proving tolerance, resulting in well-aerated, baking products with a good volume.

Loaf volume of wheat bread

The effect of different levels of NaCl on wheat loaf volume and crumb characteristics was determined (Table 1). Reducing NaCl leads to an increase in loaf volume (V_B). As shown by comparison of the respective sample with 40 and 5 g NaCl kg^{-1} flour, V_B increased significantly ($P \leq 0.01$) by approximately 55% from 3.6 cm^3 g^{-1} flour to a final level of 5.6 cm^3 g^{-1} flour. Less difference can be observed between the loaf volumes with NaCl levels of >20 and 5 g NaCl kg^{-1} flour. Thus reducing NaCl to these levels does not drastically alter V_B. V_B showed the highest values with 5 g NaCl kg^{-1} flour, suggesting that this amount of NaCl produces a possible optimum between gluten strength and gas retention in wheat dough. Further, it has been discussed that at low concentrations NaCl provides a stimulatory effect on yeast leavening ability,[14] whereby 5 g NaCl kg^{-1} flour also illustrated an inflection point, since V_B decreased with higher levels.

High amounts of NaCl lead to a decrease in V_B, due to two factors. On the one hand, the protein network was strengthened by NaCl to the extent that the inner cell gas pressure was comparably too low to expand the dough further. In wheat dough, (pH $\sim$6.0) gluten surface molecules illustrate a positive charge and repulse each other. Low levels of NaCl (0.05 mol L^{-1}) neutralize the charge repulsion of amino acids on the surface of gluten proteins, and gluten acts as a neutral dipole.[7,25] This shielding effect may reduce electrostatic repulsion of gluten particles and thus favor a protein network development. On the other hand, yeast activity becomes increasingly inhibited due to osmotic stress and alteration in the yeast cell membrane with rising NaCl concentration. Therefore yeast gas production is reduced and V_B decreases. These results are in accordance with Holmes et al.,[16] who illustrated high V_B with low levels of NaCl and decreasing V_B at elevated levels of NaCl.

Crumb quality characteristics

Crumb firmness (24 h storage) decreased with reduced NaCl amounts. Thus the C^F of bread with 0 g NaCl kg^{-1} flour illustrated a decrease in crumb firmness by 40% in comparison to breads with high amounts of NaCl (40 g NaCl kg^{-1} flour) (Table 1). However, few significant changes in crumb springiness were observed. Further, no significant change in crumb firmness, or rather springiness, was observed in breads with a common level of NaCl (20 g NaCl kg^{-1} flour) and no added NaCl.

Higher loaf volumes showed less crumb firmness due to lower NaCl as a result of an increase in the diameter of the gas bubbles. With a rise in NaCl, bread volume, air bubbles, and thus crumb density decreased, thereby increasing crumb resistance to compression. This correlation is shown in Fig. 1, which indicates the extent of staling (ES; measured by the difference between final ($C^F_{168\,h}$ or $C^F_{96\,h}$) and initial crumb firmness ($C^F_{24\,h}$) as a function of

Table 1. Bread volume and crumb characteristics (crumb firmness and crumb springiness) of wheat bread with different amounts of sodium chloride (0–40 g NaCl kg^{-1} flour)

NaCl (g kg^{-1} flour)	Bread volume V_B (cm^3 g^{-1} flour)	Crumb springiness			Crumb firmness		
		24 h storage $C^S_{24\,h}$ (—)	96 h storage $C^S_{96\,h}$ (—)	168 h storage $C^S_{168\,h}$ (—)	24 h storage $C^F_{24\,h}$ (N)	96 h storage $C^F_{96\,h}$ (N)	168 h storage $C^F_{168\,h}$ (N)
0.0	5.6 ± 0.2	0.9 ± 0.052	0.9 ± 0.049	0.9 ± 0.007	6.6 ± 0.4	12.5 ± 1.8	11.3 ± 0.5
5.0	$5.8^{**} \pm 0.0$	0.9 ± 0.017	$1.0^* \pm 0.001$	0.9 ± 0.004	5.8 ± 0.2	11.0 ± 0.4	10.7 ± 0.1
10	5.8 ± 0.2	1.0 ± 0.003	$1.0^* \pm 0.003$	0.9 ± 0.004	5.7 ± 0.4	12.0 ± 0.4	11.4 ± 0.3
15	5.5 ± 0.1	$1.0^* \pm 0.013$	0.9 ± 0.011	0.9 ± 0.003	6.8 ± 0.4	12.8 ± 1.4	13.3 ± 0.5
20	5.7 ± 0.1	1.0 ± 0.006	$0.9^* \pm 0.004$	0.9 ± 0.002	6.2 ± 0.2	12.8 ± 0.4	13.3 ± 0.2
25	$5.1^{**} \pm 0.1$	0.9 ± 0.015	0.9 ± 0.003	$0.9^* \pm 0.019$	8.7 ± 0.2	16.2 ± 1.5	20.0 ± 0.2
30	$4.6^{**} \pm 0.1$	0.9 ± 0.012	0.9 ± 0.006	0.9 ± 0.003	$10.4^{**} \pm 0.5$	$19.1^{**} \pm 0.9$	$26.5^{**} \pm 0.4$
35	$4.2^{**} \pm 0.1$	0.9 ± 0.010	0.9 ± 0.002	0.9 ± 0.003	$13.5^{**} \pm 0.4$	$23.9^{**} \pm 1.0$	$34.1^{**} \pm 1.1$
40	$3.6^{**} \pm 0.2$	$0.9^{**} \pm 0.009$	0.9 ± 0.002	0.9 ± 0.006	$16.4^{**} \pm 0.8$	$29.6^{**} \pm 3.3$	$38.7^{**} \pm 1.9$

Results are shown as mean $\overline{X} \pm 95$% confidence interval, $n = 3$. From 0 g NaCl kg^{-1} flour NaCl significant different values are marked ($^* P < 0.05$; $^{**} P < 0.01$). V^B, bread volume; C^S, crumb springiness; C^F, crumb firmness.
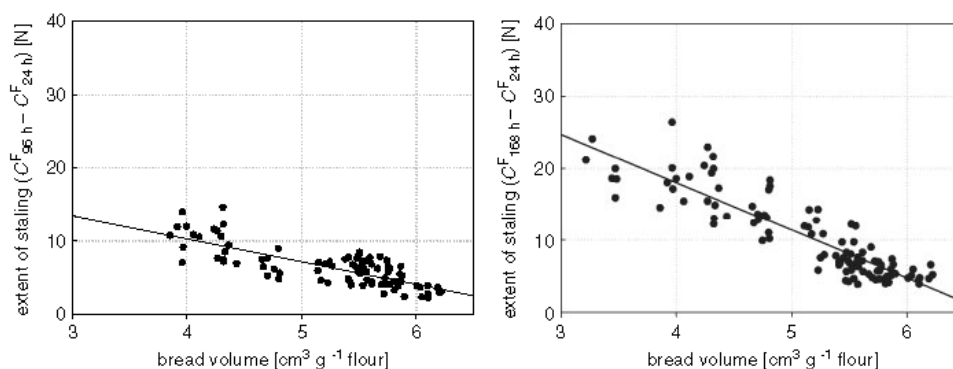
Figure	Number of data	Regression line	Correlation coefficient	Significance
Left	108	$C^F_{96\,h} - C^F_{24\,h} = 22 - 2.9 * V_B$	−0.792	$P \leq 0.05$
Right	100	$C^F_{168\,h} - C^F_{24\,h} = 45 - 6.8 * V_B$	−0.900	$P \leq 0.05$

Figure 1. The figures plot the extent of staling of various crumbs (measured by the difference between final and initial crumb firmness moduli) against loaf specific volume for bread with different NaCl levels. Left: extent of staling after four days ($C^F_{96\,h} - C^F_{24\,h}$). Right: extent of staling after seven days ($C^F_{168\,h} - C^F_{24\,h}$). The table shows the correlation coefficients and equations of the regression lines.
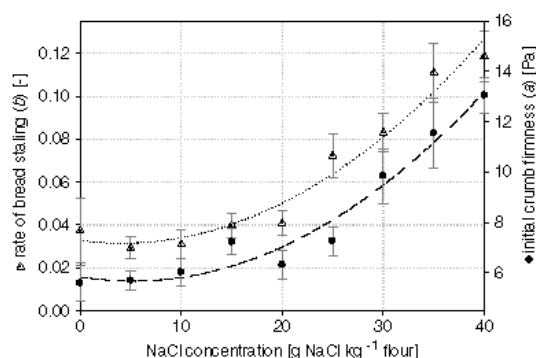

Figure 2. Staling kinetic parameters during storage of adjusted textural characteristics fitted to linear regression equations. Value a (Pa) corresponded to the initial crumb firmness and value b (—) was related to the firming rate of the bread crumb.

NaCl. The respective regression lines and correlation coefficients are shown in Fig. 1, illustrating that the correlation between the extent of staling and bread volume is significant ($P \leq 0.05$) for both regression lines.

Regarding bread storage over a period of 168 h, C^F further increased due to NaCl addition, at 40 g NaCl 100^{-1} g flour by 236%, in comparison to crumb firmness after 24 h. Therefore a higher rate of crumb staling appears in correlation with high amounts of NaCl (Fig. 2). To analyze the rate of bread firming, the juncture crumb firmness (C^F) over storage time, 24–186 h, was fitted to several regressions models. The model with a high fit ($r > 0.800$) was a linear model $C^F = a + bx$. Parameter a corresponds to the initial crumb firmness and parameter b to the firming rate of the bread crumb.[26]

A small decrease in initial crumb firmness (a) was observed by reducing NaCl from 20 to 0 g NaCl kg^{-1} flour. Upon diminishing the NaCl level from 40 to <25 g NaCl kg^{-1} flour, the initial crumb firmness decreased by 60%. The same tendency was recognized when analyzing the crumb firming rate (b), since reducing NaCl from 40 to 20 g NaCl kg^{-1} flour decreased the initial crumb firmness by 46% (Fig. 2). Both values (a and b) remain nearly constant between the NaCl levels of 0 and 20 g NaCl kg^{-1} flour.

Bread staling is regarded as a complex phenomenon and still remains unclear and not fully understood. However, it is associated with a range of changes in the crumb that occur during aging. Thus various mechanisms have been proposed: (a) gluten–gluten interactions;[27,28] (b) starch–starch interactions,[29] or (c) gluten–starch interactions.[28] Consequently, increasing crumb firmness as well as rising rate of firming due to NaCl addition might be a phenomenon of changed water availability in the dough system, since dissociated NaCl attracts water molecules by ion hydration. Rogers et al.[30] found an inverse relation between the rate of firming and bread moisture, meaning the higher the bread moisture, the slower the firming rate. Regarding the function of water in bread, water is considered to act as a plasticizer. Thus a decrease in water availability or in the binding of water to Na$^+$ and Cl$^-$ ions as hydrate envelopes may accelerate the formation of cross-links between starch as well as within starch and protein, and thus increase firming.[31] This means the available water amount and crumb firmness are closely related, resulting in the great effect NaCl had on the rate of firming, especially in higher amounts (>2 g NaCl 100^{-1} g flour).

Differential scanning calorimetry

As seen in Table 2, changes in NaCl concentration affected the extent of melting enthalpy in wheat bread systems after 96 h storage, where crumb-melting enthalpy after storage is referred to as the amount of recrystallized starch. Retrogradation of starch is defined as transition of gelatinized starch molecules from an

Impact of sodium chloride on wheat flour dough. II

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Table 2. Effect of sodium chloride (0–4 g NaCl 100 g^{-1} flour) in DSC results

NaCl (g kg^{-1} flour)	96 h storage	
	Peak maximum T_{max} (°C)	Enthalpy ΔH (J g^{-1})
0.0	61.90 ± 1.01	1.60 ± 0.03
5.0	62.43 ± 0.89	1.35** ± 0.07
10	62.68 ± 0.47	1.19** ± 0.16
15	62.90 ± 1.03	0.97** ± 0.06
20	63.00 ± 0.38	0.94** ± 0.11
25	64.25* ± 0.41	0.69** ± 0.07
30	64.63* ± 2.22	0.68** ± 0.06
35	64.60* ± 0.85	0.63** ± 0.05
40	64.63* ± 1.60	0.46** ± 0.06

Results are shown as mean $\overline{X}$ ± 95% confidence interval, $n = 6$. From 0 g NaCl 100 g^{-1} flour NaCl significant different values are marked (* $P < 0.05$; ** $P < 0.01$).

initially amorphous state to a more ordered or rather crystalline state. This has an impact on the texture and accordingly staling. Several papers deal with this theory, with starch retrogradation as the major influential factor in bread staling.[32–34]

Analyzing wheat bread with different NaCl amounts by DSC, it was found that the melting enthalpy (ΔH) increased nearly linearly and highly significantly ($P \leq 0.01$) by 348%, with NaCl concentration from 40 to 0 g NaCl kg^{-1} flour. Changes in NaCl concentration also affected the temperatures associated with the melting and peak maximum (T_{max}) of the melting curve. After four days' storage T_{max} decreased by 4%, whereby T_{max} showed significantly ($P \leq 0.05$) different values only from 25 to 40 g NaCl kg^{-1} flour. Other authors have studied starch–water solutions with different NaCl amounts. However, they achieved similar results, since the enthalpy values after storage decreased with rising NaCl amounts.[35,36]

Numerous publications have discussed the effect of NaCl on starch gelatinization and diverse explanations have been proposed. Commentaries have referred on the ion influence (a) on water, (b) on starch, and (c) on the interaction between water and starch. Another more detailed work on different starches suggested that (a) starch acts as a weak acid ion exchanger, capable of replacing some protons of starch alcoholic groups with cations (Na$^+$) out of salt (here NaCl), (b) cations stabilize the starch granule structure by generating a negative Donnan potential, which prevents the penetration of the anions, and (c) the anions (Cl$^-$) of the salt used (here NaCl) induce gelatinization due to the splitting of hydrogen bonds.[37,38] Although various mechanisms of starch gelatinization were proposed, the effect of NaCl on the retrogradation of starch is still complex. The most likely theory is based upon the gelatinization mechanism of Oosten.[37] Here, NaCl prevents the reorganization of starch chains and thus slow retrogradation. Owing to a Donnan potential – an ion concentration gradient of starch–water solution – sodium ions showed a tendency to penetrate starch particles. As a result, sodium ions replace hydrogen ions inside the starch molecules and the hydrogen ions move to the water phase, lowering the pH of the solution. Entrapment of the larger Na$^+$ ions instead of smaller H$^+$ ions leads to less recrystallization of the starch molecules and thus to decreased melting enthalpy (ΔH).

Dough development and gaseous release characteristics

The Rheofermentometer method provides information on gas production and the ability of dough to retain gas, allowing evaluation of the fermentative capacity of flours and yeast activity. Dough development and gaseous release are illustrated in Table 3.

The height of maximum dough development (H_m) and the height at the end of the test (h) increased significantly, by 182%, while decreasing NaCl from 40 to 20 g NaCl kg^{-1} flour. These results were in accordance with some earlier works in which decreasing values of H_m were determined with the addition of NaCl.[39,40] In parallel, the time to reach the maximum dough height T_1 was less for dough without NaCl, as this dough was not able to retain the produced gas.

The total volume of CO$_2$ (V_T) increased significantly ($P \leq 0.05$) with the reduction of NaCl from 40 to 0 g NaCl kg^{-1} flour. Comparison of the control (without NaCl) and 40 g NaCl kg^{-1} flour showed that 70% more 323% CO$_2$ was produced. However, smaller differences between 15 and 0 g NaCl kg^{-1} flour were observed. This may be explained by another aspect: if all dough should achieve the same height, how long would the fermentation take? This is expressed as the time to reach a dough height of 60 mm, since the dough with 20 g NaCl kg^{-1} flour displays a height of 60 mm ($T_{20\,g\,NaCl}$). By extrapolation of the received dough development curve it was possible to provide data on $T_{20\,g\,NaCl}$. Thus, without NaCl, the dough would have to proof for 45 min to reach a height of 60 mm, while 40 g NaCl kg^{-1} flour dough should proof in 118 min. The same tendency was recognized in the retention volume of CO$_2$ (V_R), since reducing NaCl increases the rate of fermentation and baker's yeast produces more CO$_2$. This effect is based on reduced osmotic pressure that alters the yeast semi-permeable membrane.[41] Higher NaCl concentrations outside the yeast cells and relatively low solute concentration inside causes water to be drawn out, which limits the yeast's leavening ability. Oda and Tonomura demonstrated the effect of osmotic pressure on yeast when they recognized a suppressive effect of NaCl on the growth of baker's yeast cells (*Saccharomyces cerevisiae*) due to a prolongation of the lag phase in the microorganism.[11] Additionally, some reviews have discussed a mechanism in some yeast species that tolerate high NaCl concentration and thus tolerate lower A_w values. First, yeast species produce high concentrations of intracellular compatible solutes like glycerol, arabitol, mannitol, and erythritol that lower the external osmotic pressure. Further, a change in the semi-permeable cell wall by sodium and chloride ions also affects the leavening rate, since the yeast membrane transporters play a crucial role in the functions of salt tolerance. Regarding salt-tolerant yeast strains beyond intracellular compatible solutes, more active Na$^+$/K$^+$-ATPase systems are determined that maintain a lower intracellular concentration of sodium ions[42] and thus offer salt-tolerant yeasts.

Another reason for reduced leavening ability deals with the effect of chloride ions on a reduced oxygen tension and interference with the action of enzymes.[3,14]

The amount of lost CO$_2$ (V_L) in dough without NaCl increased significantly ($P < 0.01$) due to NaCl addition as a consequence of a strengthened dough system.[7] In normal wheat dough (pH ∼6.0) gluten surface molecules exhibit a positive charge and thus repulse each other. Low levels of NaCl (0.05 mol L^{-1}) neutralized the charge repulsing the amino acids on the surface of the gluten proteins and the protein acted as a neutral dipole.[25] This shielding effect will reduce electrostatic repulsions between gluten molecules and thus favor a protein network formation with high gas-holding capacity. The gas production of baker's yeast causes a permanent

303

69

Table 3. Dough development and gas release characteristics measured by Rheofermentometer of wheat bread as a function of sodium chloride ($0-4$ g NaCl $100\,g^{-1}$ flour)

	Dough development				Gas release				
NaCl	Max. dough height	Time at max. dough height	Height at end of test	Time when H_m of 60 mm (2 g NaCl) is reached[a]	Max. height of gas release curve	Time to reach H'_m	Total volume	Volume lost	Retention volume
(g kg^{-1} flour)	H_m (mm)	T_1 (mm)	h (mm)	$T_{2\,g\,NaCl}$ (min)	H'_m (mm)	T'_1 (min)	V_T (mL)	V_L (mL)	V_R (mL)
0.0	74 ± 2.5	96.0 ± 2.1	66 ± 0.9	$46^* \pm 1.5$	100 ± 0.3	88.5 ± 44.5	1591 ± 36	85.0 ± 9.9	1506 ± 46
5.0	$82.0^{**} \pm 2.3$	$118.0^{**} \pm 2.1$	$82.0^{**} \pm 3.3$	$38^* \pm 7.1$	104 ± 6.2	100.5 ± 33.8	1615 ± 109	$41.0^* \pm 7.8$	1574 ± 117
10	$86.0^{**} \pm 3.4$	$119.0^{**} \pm 1.1$	$85.0^{**} \pm 3.8$	$50^* \pm 3.2$	102 ± 6.2	117.8 ± 3.2	1576 ± 73	$29.5^{**} \pm 0.7$	1547 ± 74
15	76 ± 5.6	$118.0^{**} \pm 1.5$	$76.0^{**} \pm 5.2$	56 ± 0.8	93 ± 6.3	115.0 ± 8.7	$1341^* \pm 88$	$40.7^* \pm 28.7$	$1300.0^* \pm 102$
20	73 ± 2.2	$118.0^{**} \pm 2.6$	$72.0^* \pm 2.2$	60 ± 0.0	91 ± 9.1	119.0 ± 1.7	$1308^* \pm 182$	$42.3^* \pm 36.7$	1266 ± 192
25	$60.0^{**} \pm 0.2$	$120.0^{**} \pm 0.0$	$60.0^* \pm 0.2$	$73^{**} \pm 2.9$	$76.0^* \pm 2.9$	119.0 ± 1.7	$1015^{**} \pm 41$	$16.3^{**} \pm 8.4$	$999.0^{**} \pm 48$
30	$53.0^{**} \pm 2.1$	$120.0^{**} \pm 0.0$	$53.0^{**} \pm 2.1$	$85^{**} \pm 5.5$	$63.0^{**} \pm 5.5$	120.0 ± 0.0	$800^{**} \pm 104$	$6.7^{**} \pm 3.5$	$793.0^{**} \pm 103$
35	$47.0^{**} \pm 0.2$	$118.0^{**} \pm 1.5$	$46.0^{**} \pm 1.5$	$98^{**} \pm 3.9$	$57.0^{**} \pm 2.9$	115.5 ± 0.0	$696^{**} \pm 54$	$4.3^{**} \pm 1.2$	$691.0^{**} \pm 52$
40	$40.0^{**} \pm 2.2$	$120.0^{**} \pm 0.0$	$40.0^{**} \pm 2.2$	$118^{**} \pm 3.6$	$47.0^{**} \pm 1.8$	119.5 ± 0.9	$492^{**} \pm 31$	$5.7^{**} \pm 5.5$	$486.0^{**} \pm 27$

Results are shown as mean $\overline{X}$95% confidence interval, $n = 3$. From 0 g NaCl $100\,g^{-1}$ flour significant different values are marked (* $P < 0.05$; ** $P < 0.01$).
[a] For $T_{2\,g\,NaCl}$: from 20 g NaCl kg^{-1} flour significant different values are marked (* $P < 0.05$; ** $P < 0.01$).

Table 4. Correlation matrixes of bread quality results

	Bread volume	Dough development			Gas release		DSC	
		H_m	h	H'_m	V_T	V_R	T_{max}	ΔH
Bread volume		0.939**	0.923**	0.915**	0.896**	0.889**	-0.889**	0.707*
Crumb firmness								
1 day	-0.745**	-0.853**	-0.856**	-0.911**	-0.866**	-0.872**	0.888**	-0.625
4 day	-0.806**	-0.829**	-0.819**	-0.824**	-0.793**	-0.793**	0.875**	-0.692*
7 day	-0.819**	-0.893**	-0.897**	-0.809**	-0.781**	-0.771**	0.827**	-0.654*
Dough development								
H_m			0.978**	0.913**	0.921**	0.909**	-0.831**	0.786**
Gas release								
H'_m					0.985**	0.991**	-0.848**	0.773**
V_R				0.991**	0.998**		-0.811**	0.829**

To explore relationships between variables a simple linear regression analysis was calculated using Statgraphics Centurion. The correlation coefficient r was used for illustrating the degree of correlation (* $P < 0.05$; ** $P < 0.01$).

increase in the size of gas cells, and a well-developed gluten network is required for holding this gas and preventing CO_2 from dissolving into the dough system. Thus the amount of lost CO_2 and therefore the retentions coefficient (RC) is higher with a less developed gluten network, due to low amounts of NaCl.

Correlation of bread quality results

The most important correlations within bread quality values are summarized in Table 4. No correlation higher than 0.500 was found with crumb springiness and other parameters. In contrast, a highly significant ($P \leq 0.01$) relationship ($r \geq -0.745$) between bread volume and crumb firmness over all days of storage was observed, since decreasing crumb firmness occurs with rising loaf volume. High volumes are probably based on elevated dough heights during and at the end of proofing due to good leaving ability in yeast cells. Thus bread volume

significantly ($P \leq 0.01$) correlated ($r \geq 0.889$) with parameters of the dough development curve (H_m) and the gas release curve (H'_m, V_T, V_R), measured by Rheofermentometer. Thus a highly significant ($P \leq 0.01$) correlation with bread volume showed the maximum height of gas release curve H'_m ($r = 0.939$). The results suggest that predicting loaf volume and crumb firmness by measuring the gas release curve, in particular H'_m, during proofing is feasible.

A significant negative correlation ($r \geq -0.625$) between crumb firmness and melting enthalpy (ΔH) was found. This inverse correlation means that the rate of firming is not directly based on starch retrogradation. This result seems to be a further important extension of the theory of bread staling. Excessive NaCl addition indirectly causes (by gluten-strengthening effects[7] and less gas production) an increase in crumb firmness that correlates negatively with bread volume.

304

70

CONCLUSIONS

Dough ingredients show an important effect on bread quality. Thus in this work the impact of NaCl on bread and dough characteristics was shown. The three most important results are summarized here:

1. Reducing NaCl illustrated higher yeast leavening ability. At low concentrations, NaCl provides a stimulatory effect on yeast leavening ability or a possible optimum between gluten strength and gas-holding capacity of wheat dough. Thus dough volume increases somewhat up to 5 g NaCl kg^{-1} flour. At higher concentrations, NaCl shows an inhibitory effect on fermentation, as indicated by reduced loaf volume.

2. Starch retrogradation increased almost linearly with NaCl reduction. This is probably based on the theory that by NaCl addition the larger Na$^+$ ions are entrapped instead of smaller H$^+$ ions inside the starch molecules, leading to less starch recrystallization. However, there is less knowledge about starch retrogradation and its kinetics as affected by various salts. The investigation of starch and bread crumb alteration using different ingredients seems to be an important aim for the future.

3. NaCl reduction showed no changes in crumb firming between 0 and 20 g NaCl kg^{-1} flour. However, at higher concentrations, NaCl illustrated a rapid decrease in crumb firming (between 20 and 40 g NaCl kg^{-1} flour). These results correlate inversely with bread volume. However, starch retrogradation seems not to be the main cause for the increase in the rate of crumb firming.

Overall, the results may provide a good basis for reducing sodium in bread. However, the reduction of sodium alone will produce less flavorsome breads with altered quality (e.g. larger crumb pores, harder-to-handle dough). This work illustrates a basis for explaining alterations in the end-product due to reduced sodium content and the subsequent development of sodium substitutes.

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305

71

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2.5 Starch re-crystallization kinetics as a function of various cations

Research Article

Starch re-crystallization kinetics as a function of various cations

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Starch retrogradation is the main cause of quality deterioration in starch-containing foods during storage. The current work investigates the effect of different cations on the retrogradation of corn starch and the potential of reducing starch retrogradation with the aim of preparing products with an extended shelf life. To gelatinize the starch, starch–water suspensions containing various chloride salts ($LiCl$, $NaCl$, KCl, $MgCl_2$, $CaCl_2$, and NH_4Cl) were heated in a DSC, stored up to 504 h at 8°C, and reheated again. Analysis of gelatinization behavior for each salt type indicates a relationship to the a_W-value of the starch–water system. The degree of re-crystallization was calculated using the Avrami equation, and indicates that the starch re-crystallization rate (k) is significantly ($p < 0.01$) reduced with the addition of a cation, unlike the reference (starch–water systems without salt). Further, bivalent cations such as Ca^{2+}, Mg^{2+} decreased the starch re-crystallization rate (k) more than univalent cations (Li^+, NH_4^+, Na^+, and K^+). This result may be based on the theory that high cations with higher charge densities show greater hydration, and, therefore, lower a_W-values, than cations with lower charge densities. The results illustrate important results for predicting starch quality change when using sodium replacements.

1 Introduction

The correlation of starch re-crystallization (retrogradation) with staling or bread firming has been discussed in several publications [1, 2]. Further, Axford et al. [2] found a strong negative correlation between consumer acceptance and compressibility, or rather, firmness. Thus, understanding the characteristics of retrogradation following the addition

of other ingredients, e.g., salt or salt replacers is of great interest to food scientists since it affects the quality, acceptability, and shelf-life of starch-containing foods.

Starch, is regarded to be a partially crystalline and partially amorphous polymeric system [3]. In native starch, amylose (AM) and amylopectin (AP) molecules are built up in granules as alternating semi-crystalline and amorphous layers (growth rings). The semi-crystalline layer is composed of ordered regions of double helices formed by short AP branches and show birefringence. The amorphous regions in the starch granules consists of AM and non-ordered AP branches [3]. Starch normally gelatinizes due to the irreversible swelling, or even disruption, of the granules while cooking. The alteration of starch from an initially amorphous state after gelatinization to a more ordered or crystalline state during storage has been described as starch retrogradation [4, 5]. Further, starch retrogradation is regarded as a crystallization process which starts with spontaneously formed nuclei, from which crystallites

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Abbreviations: AM, amylose; **AP,** amylopectin; a_W, water activity; **DSC,** differential scanning calorimetry; ΔH, gelatinization enthalpy (J/g) based on dry matter; ΔH_R, gelatinization enthalpy after storage (J/g) based on dry matter; k, starch re-crystallization rate; n, Avrami exponent; **NaCl,** sodium chloride (g/100 g flour); T_0, onset temperature (°C); T_E, end temperature (°C); T_p, peak temperature (°C)

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grow [6]. Some authors argue that it is important to differentiate between the short-term development of the starch gel structure including retrogradation due to AM re-association (re-crystallization) and the long-term development of AP [7, 8]. Starches containing AM as well as AP form a network of swollen AP enriched granules that fill an interpenetrating AM gel [7, 8]. During retrogradation AP molecules re-crystallize and increase the firmness of gelatinized starch. Contrastingly, AM forms double-helical associations of 40–70 glucose units during retrogradation [8, 9], similar to the crystalline structure of native granular starch [10].

The influence of starch size distribution on bread characteristics has been the subject of various investigations [11]. A significant effect of granule size distribution on dough has been shown for both rheological and pasting properties [12–17]. Starch from sources such as wheat, rye, and triticale are non-uniform in size since their particle size distribution is bimodal (A-and B-type), and, therefore, affect the pasting characteristic. Starch granules with a distribution as small as 2–10 μm in diameter (B-type) exist, while large starch granules can have a diameter of up to 63 μm (A-type) [18]. Corn is comprised of homogenously distributed starch granules (A-type) with a granule size distribution of 5–25 μm [19] and similar AM content to wheat starch (25%). Thus, corn offers a unique model system to explore differences in pasting and the retrogradation of electrolytes [20].

Several methods have been employed to measure the degree of retrogradation. An extremely valuable tool for quantifying crystalline amounts in starch is differential scanning calorimetry (DSC). In a typical DSC measurement, the endothermic changes (ΔH) monitor structural changes that occur during heating. The energy absorption measured by DSC reflects the energy input into both the substance and the reference material as a function of temperature and/or time. Thus, the energy absorbed during the DSC test shows the energy transformation that occurs during the melting of re-crystallized AP, and consequently can be used to measure the amount of retrograded starch.

Previous studies have shown that retrogradation depends on water content, ingredients, and the ratio of AM/AP [5]. Different food constituents, e.g., monoacyl lipids have long been known to retard the re-crystallization process. However, the effects of sugar and sodium chloride seem to be more complex and less well understood [21]. Further, based on the current discussion on reducing the sodium chloride content of starch-based food [22], it is important to analyze the effect of several sodium replacers (as chloride salts) on starch. Additionally, less information about different chloride salts and their impact on retrogradation have been published. Thus, this work investigates the effect of various cations on the mode and kinetics of the retrogradation of model corn starch gels.

2 Materials and methods

2.1 Materials

Corn starch with a ratio of AM:AP of 25:75 and a moisture level of 12.7% was used. The starch is commercially available and was kindly supplied by National Starch, Hamburg, Germany. The NaCl, MgCl$_2$, and NH$_4$Cl were delivered by Merck, Darmstadt, Germany. Meanwhile, the CaCl$_2$, KCl, and LiCl was obtained from Alfa Aesar, Karlsruhe, Germany.

2.2 Gelatinization properties

Gelatinization parameters were measured using a differential scanning calorimeter DSC Diamond (DSC, Perkin Elmer GmbH, Überlingen, Germany) and following the procedure of Paredes-López et al. [23]. The starch (5 mg based with 12.7% moisture) was weighed directly into an aluminum pan part no. BO 14-3004 and cover 0.15 mm (Perkin Elmer GmbH) of 40 μL capacity. Deionized water was added to achieve a starch–water ratio of 1:2 to ensure the equilibration of the starch–water samples as well as complete hydration [24]. Equivalent amounts of cations were used to reach the same amount of sodium, that is, 20 g NaCl/kg starch. The pans were hermetically sealed and allowed to stand at room temperature for 1 h to ensure the even distribution before DSC measurements were taken [24]. An empty aluminum pan was used as a reference. Sample pans were heated at a rate of 10°C/min from 25 to 120°C then held for 5 min at 120°C (achieving of a complete gelatinization). The heated pans were then immediately cooled to 8°C and kept at 8°C inside a refrigerator for varying durations. Onset temperature (T_0; °C), peak temperature (T_p; °C), end temperature (T_E; °C), and gelatinization enthalpy of the dry sample (ΔH; J/g) were calculated automatically from the gelatinization curve. All results are the average of three experiments.

2.3 Retrogradation properties

Subsequent to the gelatinization test, the samples were stored for 96, 168, 240, 336, and 504 h at 8°C before rescanning. The temperature range and heating rate were 25–120°C and 10°C/min, respectively. An empty pan was used as reference for all measurements. The received enthalpy peaks were observed between 30 and 80°C, featuring the re-crystallized AP fraction [25]. The peak size or rather the melting enthalpy is regarded as a measure of the amount of re-crystallized starch [25]. Thus, melting enthalpy (ΔH_R) was calculated from the area under the achieved curve and was expressed in J/g (based on corn starch with a moisture content of 12.7%), representing the

amount of retrograded starch. All results are the average of three experiments.

2.4 Avrami model

The crystallization process consists of nucleation and crystal growth. Specifically, nucleation is the stage where the solute molecules start to assemble into very small agglomerates [26]. These clusters become stable due to defined operating conditions (temperature, supersaturation, etc.) and generate the nuclei [27]. However, if the clusters do not become stable they redissolve. To avoid dissolution, the clusters need to reach a critical size in order to and become stable nuclei. During nucleation the crystal structure of the relative arrangement of the atoms will be defined, however, the macroscopic characteristics of the crystal (size and shape) will not, even if those results of the internal crystal structure. The crystal growth is the subsequent growth of the nuclei that occurs after reaching critical cluster size and basically depends on supersaturation. Depending on the process, either nucleation or growth will prevail. As a result, crystals with different sizes and shapes are achieved [27]. Further, the crystallization process follows.

The common crystallization kinetics of starch are usually analyzed using the Avrami equation [28]. When applied in conjunction with DSC, it is assumed that the differential area (gelatinization enthalpy; ΔH) under the curve with time is in good agreement with the dynamic changes in the alteration of amount of re-crystallized starch [29]. Therefore, the received (ΔHR) for each sample were fitted to the Avrami equation [30–32] using a non-linear procedure equation for analyzing the form and gradient of crystallization. Re-crystallized starch (expressed as gelatinization enthalpy after storage) subjected to storage time were fitted to the Avrami equation:

$$\Theta = \frac{A_\infty - A(t)}{A_\infty - A_0} = e^{(-kt^n)} \tag{1}$$

where Θ is the non-crystallized fraction developed at time (t) and constant temperature. A_o, A_t, and A_∞ are experimental values of the property at time zero (A_o), at time t ($t = 96$, 168, 240, 336, or 504 h), and infinity (or limiting value after $A_\infty = 504$ h), k is a rate constant, and n is the Avrami exponent, which is characteristically related to the way that the nucleation of crystallites occurs. Once the nucleation stage is complete, nuclei may grow into larger crystals and thus, this stage is known as crystal growth. Therefore, the Avrami exponent is defined as the number of dimensions in which growth takes place [33]. Depending on the objectives and applications of growth rate measurements, the growth rate may be expressed as (1) a rod-like growth from instantaneous nuclei (nuclei appear at once;

Avrami exponent = 1), (2) as a rod-like growth from sporadic nuclei (nuclei growth randomly; Avrami exponent = 2), (3) as a disc-like growth from instantaneous or sporadic nuclei (Avrami exponent = 3), and (4) as a spherulitic growth from instantaneous or sporadic nuclei (Avrami exponent = 4).

2.5 A_W-values measurement

The a_W-values of each unheated corn starch–water solution subject a different salt were measured with an a_W Sprint, Swissmade, Novasina TH-500, MT-Technologie Messtechnik GmbH, Gummersbach, Germany.

2.6 pH-value measurement

The corn starch–water solutions were heated (100°C for 8 min) with various salts, and after having cooled down the pH was measured with a precisions-pH-mV-Meter, no.: 100298, Science—Technical Factory GmbH, Weilheim, Germany.

2.7 Statistical analysis

Statistical examinations were realized by one-way analysis of variance (ANOVA) to establish which samples provides differences amongst the control (blank) and the cations. The data were analyzed with the software Statgraphics Centurion (version 15.1.02 Statpoint Technologies Inc. Warrenton, Virginia).

3 Results and discussion

3.1 Gelatinization of corn starch subjected to different cations

If starch molecules are heated in a water solution a phase transition from an ordered to a disordered state occurs (gelatinization). DSC was used to study whether and to what degree corn starch gelatinization process is affected by the presence of various chloride salts at a concentration of 20 g/kg starch. As seen in Fig. 1, the gelatinization enthalpy (ΔH) of corn starch depends on the characteristics of the cations, whereby, all cations showed significantly different values ($p < 0.01$) to the reference. The order of ΔH comes along with:

Reference (without cation)

$$< NH_4^+ < K^+ < Na^+ < Li^+ < Mg^{2+} < Ca^{2+}$$
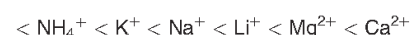
Starch gelatinization when subjected to various salts seems to be an interplay of (a) the effect of the ions on
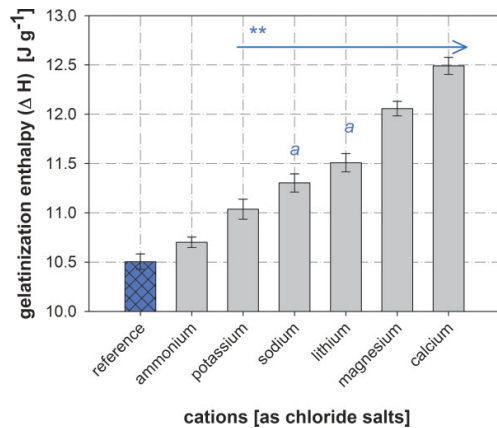
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Figure 1. Gelatinization enthalpy (ΔH) of corn starch–water suspensions (ratio: 1:2) subjected to various cations; $n = 3$. $^{**}\longrightarrow$ = significant differences ($p < 0.01$) to the reference (blank). a = same letters illustrate no significant ($p < 0.05$) difference among the respective samples.

water and (b) the effects of the ions on polymers (starch). Therefore, the order may be explained by the hydration effect of cations in water [34–37]. The hydration energy of cations depends on the diameter and the charge of the cation. The smaller the ion and the higher its charge, the stronger the solvation of the cation [38]. Smaller cations produce thicker hydration shells with equal electric charges. Cations with smaller diameters exhibit an enlarged electrostatic field due to higher charge density, and thus increase hydration. An increase of the diameter of univalent cations can be detected: $NH_4^+ > K^+ > Na^+ > Li^+$. Lithium features the smallest diameter of the monovalent cations used and, therefore, the highest hydration in comparison to Na^+, K^+, and NH_4^+, thus rendering less water available for starch gelatinization [39]. Consequently, more energy is required for starch gelatinization [34, 35], and Li^+ has the highest gelatinization enthalpy (ΔH) of the monovalent cations. The bivalent cations used follow the hydration order: $Mg^{2+} < Ca^{2+}$; this is in accordance with the theory explained above. Bivalent cations with higher charges cause greater hydration than those with lower charges. Further, small bivalent cations (Mg^{2+}) feature a higher solvation than larger cations (Ca^{2+}).

Differences in hydration shell formation by various solutes is suggested to be one of the most important factors of starch gelatinization [40]. Therefore, the theory is that the more the cation reduces the a_W-value, the more energy is required for starch gelatinization and the higher the ΔH-value. Native starch is a partially crystalline polymer where water operates as a plasticizer which decreases the T_g

(reversible transition of amorphous materials from a relatively hard state into a rubber-like state) of the amorphous areas [41–43]. Water enhances the mobility of the starch chains and thus alleviates the melting of starch granules [41, 42, 44]. Changes in the ΔH of the starch–water samples may be supported by the a_W-values of salts in the respective matrix. The different cations compete with the starch for water, thereby, reducing the amount of water available for gelatinization [45]. The reduction of a_W-values illustrated significant differences ($p < 0.05$) to the reference due to the addition of cations (with the exception of ammonium; Fig. 2).

Figure 3 illustrates the onset temperature (T_0), peak temperature (T_p; maximum peak value of DSC curve), and end temperature (T_E) of gelatinization using varying cations. An increase in T_0, T_E, and T_p was achieved with cations, compared with a starch–water suspension without ions. However, the changes do not correlate with the change in ΔH. Thus the effect of gelatinization is complex and comprised of various factors [42].

A basis for explaining changes in starch gelatinization temperatures due to electrolytes may be found in the theory of Oosten [37]. Oosten illustrates, that starch may act as a weak acid ion exchanger, capable of exchanging some starch alcoholic group protons for cations (Na^+) from NaCl. At equilibrium, starch molecules feature a negative charge, while the water has a positive charge [20]. The resulting potential between the water and the starch, defined as a negative Donnan-potential, stabilize the starch granule's structure, preventing the penetration of the anions into the starch, as schematically illustrated in
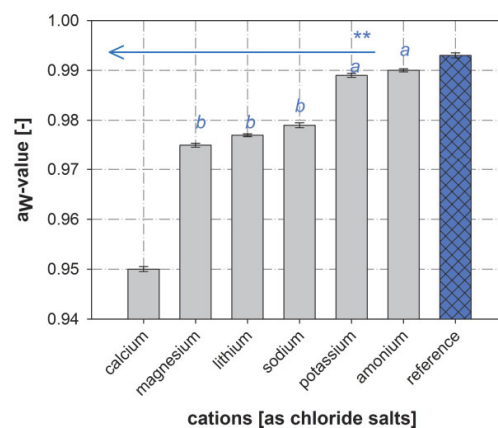

Figure 2. a_W values of corn starch–water suspensions (ratio: 1:2) subjected to various salts; $n = 2$. $^{**}\longrightarrow$ = significant differences ($p < 0.05$) to the reference (blank). a and b = same letters illustrate no significant ($p < 0.05$) difference among the respective samples.
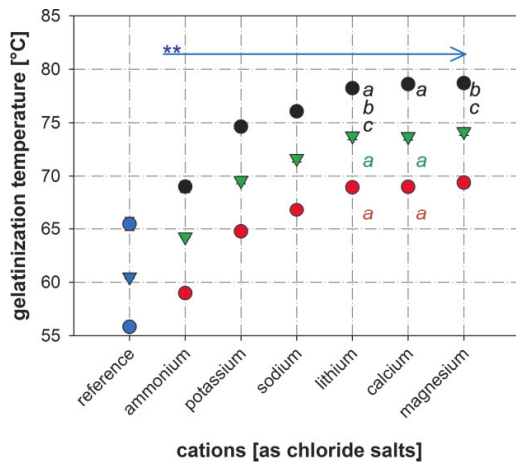

Figure 3. Onset temperature ($\bullet = T_0$), peak temperature ($\triangle = T_p$), and end temperature ($\bullet = T_E$) of corn starch–water suspensions (ratio: 1:2) subjected to various cations; $n = 3$. $^{**}\!\!\longrightarrow$ = significant differences ($p < 0.01$) to the reference (blank). a and b = same letters illustrate no significant ($p < 0.05$) difference among the respective samples.

Fig. 4. Further, the concentration gradient tends to push the cations (e.g., Na^+) into the starch particle, replacing hydrogen ions [20, 37]. As a result the hydrogen ions move to the water phase, lowering the pH of the solution (as seen in Fig. 5). Additionally, the chloride ions may support gelatinization by breaking hydrogen bonds within starch chains [35].
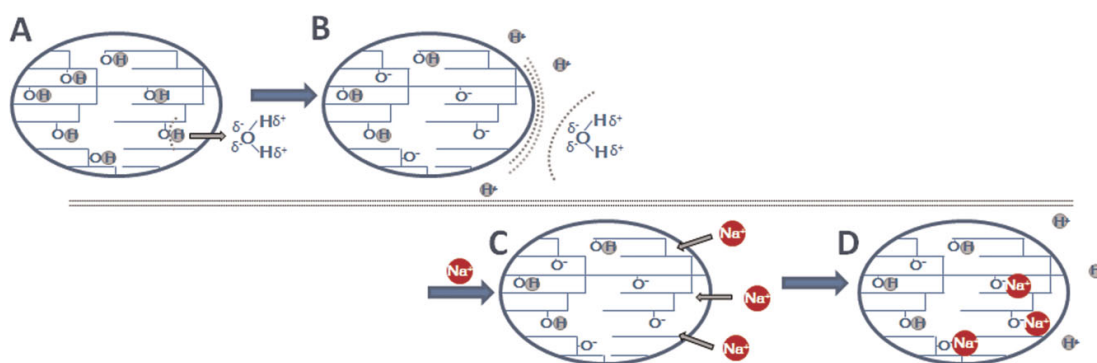
3.2 Retrogradation of corn starch subjected to different cations, and an estimate of the retrogradation rate using Avrami equations

Gelatinized starch re-crystallizes when starchy foods such as bread are stored with sufficient moisture content. The change from a post gelatinization disordered state to a re-crystallized state during storage is called retrogradation. Retrogradation can be measured by melting the re-crystallized starch molecules in the DSC: The more starch is re-crystallized, the higher the melting enthalpy (ΔH_R). Starch retrogradation in the corn starch analyzed was observed to have melting temperatures (ΔH_R) between 30 and 80°C. This temperature range is taken from the melting point of the AP fraction after storing the gelatinized starch gel [46]. Retrogradation takes place in two stages; where the first and faster stage depends on the formation of crystalline regions from altered amlyose, the second stage affect the formation of an ordered structure within AP [47]. Since the storage of the samples was up to 504 h, the measured re-crystallization illustrates AP retrogradation.

In previous studies it has been reported that the Avrami equation is regarded to be a useful tool for studying the kinetics of starch retrogradation during staling. Further, Armero and Collar [6] argue that the equation will provide a good basis for comparing different bread staling processes if the Avrami parameters are used correctly. Thus, it is possible to model the crystallization of starch gels, starch retrogradation, and bread staling using the Avrami equation. In some studies the crystallization rate constant (k) has been determined. Other authors have used Avrami plots to analyze the Avrami index (n) to gain insight into the mechanism of crystallization [47].

Figure 4. (A) The hydrogen ion (gray) concentration inside the starch granules (blue) is higher than in the water phase [37, 61]. Therefore, hydrogen ions will migrate from the starch to the water phase due to the concentration gradient. (B) The starch charge will become negative and the water phase positive. The resulting potential between starch and water is called Donnan potential [37]. Thus, the migration of hydrogen ions from the starch molecule to the water phase will be stopped due to a ultimately dynamic equilibrium [37]. (C) Due to the potential, the starch tends to push cations with a higher charge density (Na^+; red) into the starch. (D) Sodium ions have a greater ion radius and therefore probably disturb retrogradation.
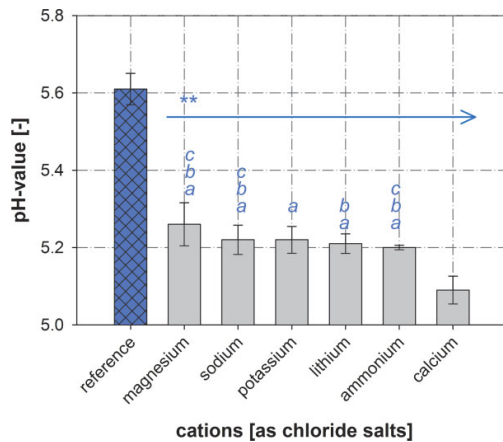
Figure 5. pH values of corn heated (100°C for 8 min) starch–water suspensions (ratio: 1:2) subjected to various salts; $n = 3$. ˙→ = significant differences ($p < 0.05$) to the reference (blank). a–c = same letters illustrate no significant ($p < 0.05$) difference among the respective samples.
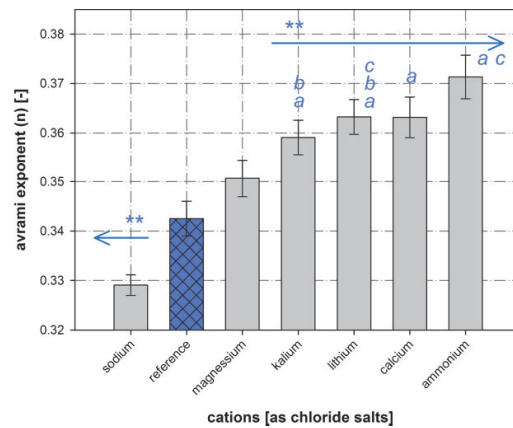

Figure 6. Avrami exponent (n) of corn starch–water suspensions (ratio: 1:2) subjected to various salts; $n = 2$. Avrami equation has been used to examine stored samples. Storage time was up to 504 h. ˙→ = significant differences ($p < 0.05$) to the reference (blank). a–c = same letters illustrate no significant ($p < 0.05$) difference among the respective samples.

Therefore, this work uses the Avrami equation to estimate the rate of retrogradation of starch gels as a function of storage time (up to 504 h at 8°C) as time variable, and the Avrami exponent (n) and re-crystallization rate (k) were obtained. The Avrami exponent (n) reflects the crystal morphology of the nucleation process. Whereby, the Avrami exponent (n) significantly changes due to the addition of all cations (with the expectation of magnesium; $p < 0.05$) in comparison to the reference (Fig. 6). The values achieved for the Avrami exponent (n) indicate that the mode of all nucleation in starch re-crystallization follows an instantaneous mechanism (nuclei appear at once) and that the growth of crystallites is 1D [47].

Starch re-crystallization rate (k) is affected by AM and AP concentration [48] and their molecular size [48], temperature, hydration [49], pH [50], starch granule size distribution [51, 52], and non-starch components [48]. The amount of re-crystallized starch increases with low storage temperatures [53] since less symmetrical crystals are generated at a lower temperature, increasing growth [54]. Thus, a storage temperature of 8°C was chosen for the current work. Furthermore, water content seems to be an important factor since retrogradation increases with increasing water content [49]. With a decreasing water content, both particle movement (entropy) and re-crystallization decrease [55, 56]. This assumption can be supported by the results of the a_W-values (Fig. 2) since the values of k and a_W-value increase in the same order:

Figure 7. Starch re-crystallization rate (k) of maize starch–water suspensions (ratio: 1:2) subjected to various salts; $n = 2$. Avrami equation has been used to examine the stored samples. Storage time was up to 504 h. ˙˙→ = significant differences ($p < 0.01$) to the reference (blank). a–c = same letters illustrate no significant ($p < 0.05$) difference among the respective samples.
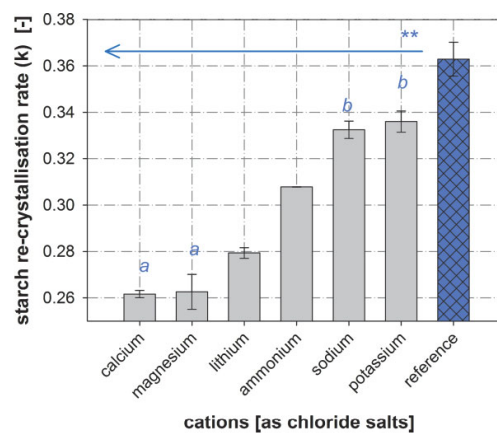
$Ca^{2+} < Mg^{2+} < Li^+ < Na^+ < K^+$ (Fig. 7). Further, cation addition significantly decreases the a_W-values ($p < 0.05$) compared to the blank sample (without cations). These observations may be explained by the inverse function

between ion size and hydration energy; whereby, hydration energy decreases in the order [57] as discussed above.

However, it appears that an additional and far more complex phenomena for the retrogradation process should be considered, based upon the gelatinization mechanism of Oosten [37]. As chloride salts may adsorb starch molecules and limit the formation of hydrogen bonds between glucose molecules, retrogradation is reduced. Thereby, starch acts as a weak ion exchanger: cations penetrate the starch molecule, and H^+ ions of starch hydroxyl group are pushed out. The entrapment of larger cations instead smaller H^+ ions leads to less starch molecule re-crystallization and thus to decreased melting enthalpy (ΔH). The enrichment of H^+ ions in the starch–water suspensions leads to a decrease in pH values in the suspension. Thus, all of the cations illustrate lower pH values than the reference without salt (Fig. 5). Furthermore, a trend to a similar order of pH-values was achieved compared with the starch re-crystallization rate (k): $Ca^{2+} < Li^+ < Na^+ < K^+$.

Another explanation is that high charges alter the solubility of the starch in water [58, 59]. Changes in starch solubility may induce changes in gelatinization enthalpy. Khairy et al. [58] illustrate solubility indices for starch with different salts and analyze low cation solubility (Li^+, Mg^{2+}, and Ca^{2+}) that largely reduces starch retrogradation. In comparison, the cations which do not decrease retrogradation to same extent (K^+ and Na^+) showed higher starch solubility. Thus, it seems that low starch solubility decreases starch retrogradation.

4   Conclusions

Analyzing maize starch gelatinization and re-crystallization with different cations by DSC shows that the measured enthalpy (ΔH_R) depends on the cation type. Although various mechanisms of starch gelatinization were proposed, the effect of different chloride salts on the retrogradation of starch is still complex: Two explanations were selected to explain the significant changes in retrogradation due to the different cations:

(A) Cations presumably prevent the reorganization of starch chains, resulting in a slowing of the retrogradation process. Whereby, cation ions replace hydrogen ions inside the starch molecules and the hydrogen ions move to the water phase, lowering the pH of the solution. The entrapment of large cations instead smaller H^+ ion leads to less re-crystallization of the starch molecules and thus to decreased melting enthalpy (ΔH_R). Further, starch solubility may change due to the ions' action.

(B) Low a_W-values resulting from cation hydration shell formation lead to a rising retrogradation rate: The higher the hydration energy of the cations, the lower the a_W-value due to water elimination. Thus the rate of retrogradation might have increased as a result. Therefore, further efforts have to be made to prove whether the elimination of water is the principal point. An approach toward this goal may be a modification of the water amount of the starch–water cation systems so that all samples feature the same a_W-value.

Finally, in this work, the effects of different cations and the mechanisms of starch–water suspension gelatinization and retrogradation in starch-based foods were effectively elucidated.

5   References

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799

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3.   DISCUSSION

The political pressure to reduce NaCl in cereal-based food products is evident and still increasing. However, NaCl influences yeast leavening ability, rheological, technological as well as starch re-crystallization properties and processing, and this must be taken into account when reducing NaCl in cereal-based products as it will affect product quality. The effects of NaCl reduction on cereal-based foods are examined step by step and analyzed in detail in this work.

NaCl causes changes in wheat dough structure which directly affect the end-product structure. The most important alterations in wheat dough and end-product structure due to NaCl addition are based on the following four points.

a)   Modification of the yeast-leavening ability

b)   Protein structure alteration

c)   Change in end-product quality and volume

d)   Alteration of starch retrogradation during storage

The addition of NaCl to wheat dough modifies its yeast-leavening ability during proofing. The effect of NaCl on the activity of *Saccharomyces cerevisiae* (baker's yeast) has been determined by measuring dough development behavior and gas release properties with a Rheofermentometer. At low concentrations, 5 g NaCl kg^{-1} flour, the dough reached its maximum volume since this level of sodium chloride both stimulates the gas production of yeast and provides the optimum gas retention capacity in wheat dough. At higher concentrations NaCl illustrated an inhibitory effect on yeast since the total volume of gas produced by yeast cells significantly decreased with increasing NaCl levels. This result can be attributed to different reasons, first, the rise in osmotic pressure in the yeast cells, as schematically illustrated in Figure 9.

A quantitative estimation of the osmotic pressure (p_{osm}) in wheat dough due to NaCl addition may be achieved through a simplification in calculating p_{osm} in a model water-NaCl solution with NaCl addition:

$$p_{osm} = z \cdot [NaCl] \cdot R \cdot T, \tag{23}$$

where z is the amount of substance, R is the gas constant, and T is the temperature (here: 30 °C). An equivalent amount of 15 g NaCl kg^{-1} flour in the model water-NaCl solution exhibited an osmotic pressure of 2.2 MPa and showed a significantly lower total CO_2 volume compared to the reference without NaCl. Thus, this solution and all higher NaCl amounts are hypertonic and therefore negative for yeast-leavening ability. In contrast, one third of the NaCl (equivalent with 5 g NaCl kg^{-1} flour) has an osmotic pressure of 0.75 MPa and is isotonic, or rather optimal, for yeast solutions [118], suggesting that 5 g NaCl kg^{-1} flour provides a good medium for yeast. Therefore, low amounts of NaCl provide a stimulatory effect for yeast, and thus, the total volume of produced CO_2 increased with 5 g NaCl kg^{-1} flour. A second reason for the inhibitory effect of NaCl on yeast is that the yeast cell membrane changes in electro-chemical potential due to contact with Na^+ and Cl^- ions [119]. And third, NaCl may also indirectly affect yeast leaving rates by lowering the oxygen solubility [9]. Future work should systematically analyze how other substances, when used as sodium replacers, affect both yeast leavening rates and gas retention in the dough system. The comparison of these results with NaCl may provide essential information on which substance is technologically similar to NaCl since, next to sensory attributes, high product volume based on CO_2 retention in the dough is essential for consumer acceptance.
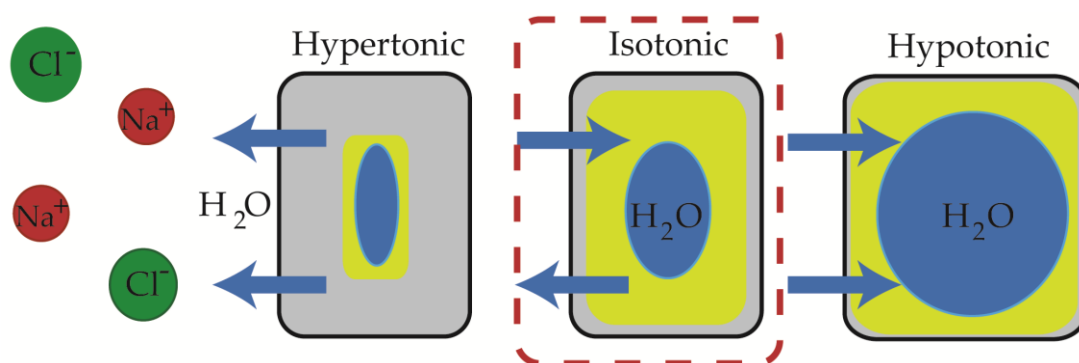
Figure 9: Schematic illustration of the plasmolysis of yeast cells by NaCl. Cell liquid (blue) will dissolve out of the cells with the osmotic effect of Na^+ and Cl^- ions. Further, effects on yeast-leavening ability, which are based on effects of ions on the cell membrane and on reducing water activity in the system, lead to decreasing yeast activity.

Although yeast gas production decreased with high amounts of NaCl, gas retention (retention volume divided by the total gaseous release in %) increased resulting in a nearly equivalent end-product volume with up to 20 g NaCl kg^{-1} flour. Increased gas retention can

be attributed to changed dough structure, and was therefore investigated further. The usage of NaCl enhances the dough system due to protein strengthening effects, which was shown by fundamental rheological analysis (e.g. dynamic rheometry) as well as empirical rheological measurements (e.g. Farinograph analyses). All rheological measurements indicated an increase in elastic parameter, which may be based on protein cross-linking in particular. However, other factors may change dough elastic behavior, thus it is conceivable that a) other flour fractions (starch, pentosan, lipid) increase their elastic parameter due to NaCl addition and b) that water availability decreases with NaCl addition and elastic behavior increases because water acts as a plasticizer so elasticity increases if there is less water available (up to a certain amount of water) [59]. So, analysis of the free available water, or rather water activity in the dough and in the crumb with different NaCl amounts, may provide more information about this increase in elasticity. Furthermore, rheological analyses of NaCl in both pentosan- and starch-water systems can provide useful information about the effect of NaCl in the complex flour systems. However, here it is meaningful to measure these systems using a constant a_w-value, to reduce the effect of the water.

The rheological change that NaCl facilitates may be caused by the neutralization of the charge repulsion that emanates from the (presumably) positively charged amino acid moieties on the surface of the gluten proteins [120, 121]. This charge neutralization results from the sodium and chloride ions; thus, raised gluten cross-linking is generated, as schematically illustrated in Figure 10 [122]. Although the theory of the electrical double layer defines most particles or colloids as negatively charged [123, 124], some gluten charge measurements illustrate more positive charges at pH 6 [120, 121]. Therefore, it seems necessary to analyze the charge of gluten molecule by itself and with different NaCl amounts.
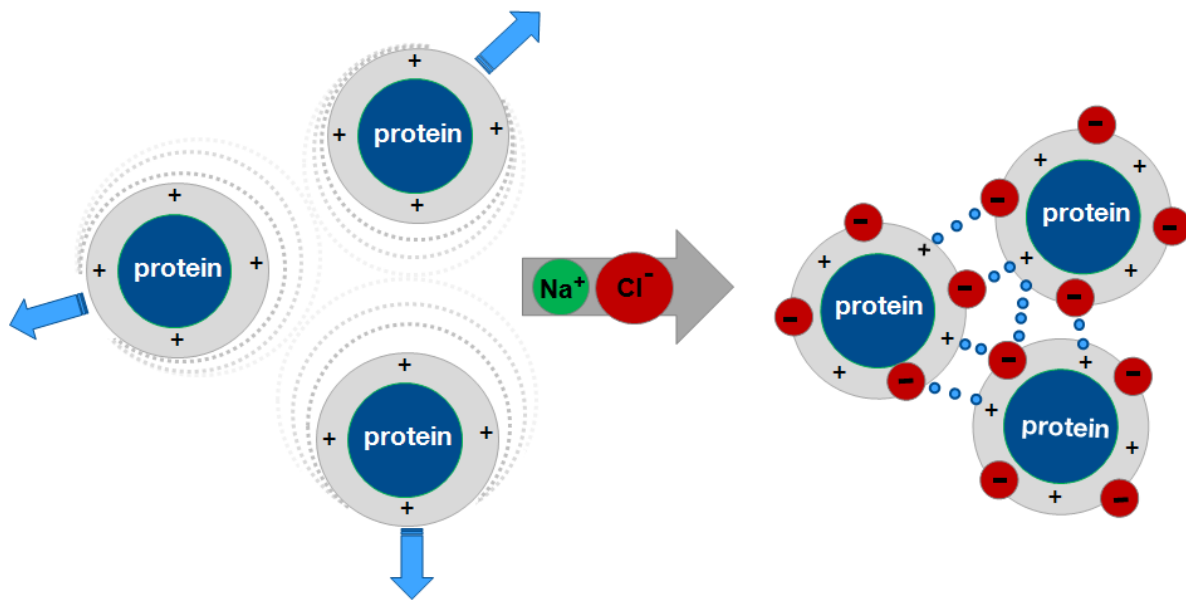
Figure 10: Schematic illustration of the influence of NaCl on the formation of gluten proteins in a flour-water solution. On the left: gluten protein features in a solution (pH 6). Here, there are more positive charges than negative [120, 121], and therefore, the proteins repulse each other. On the right: NaCl ions shield gluten charges, and subsequently improve protein network formation.

This change in protein has been visualized and proven using CLSM imaging (Figure 11). Increasing amounts of NaCl demonstrated visible changes in the protein structure since NaCl addition leads protein structures to exhibit more elongated and higher cross-linked proteins. Furthermore, it is important to analyze dough rheology as well as changes in microstructure (CLSM) resulting from different substances in order to identify suitable sodium replacers for bread recipes. It is also being of interest to investigate whether starch or pentosan demonstrate changes (visually or when measured with Rheometer) when NaCl is added. Therefore analyzing these structures by itself as well as in systematically ratios with gluten would enlarge scientific knowledge.
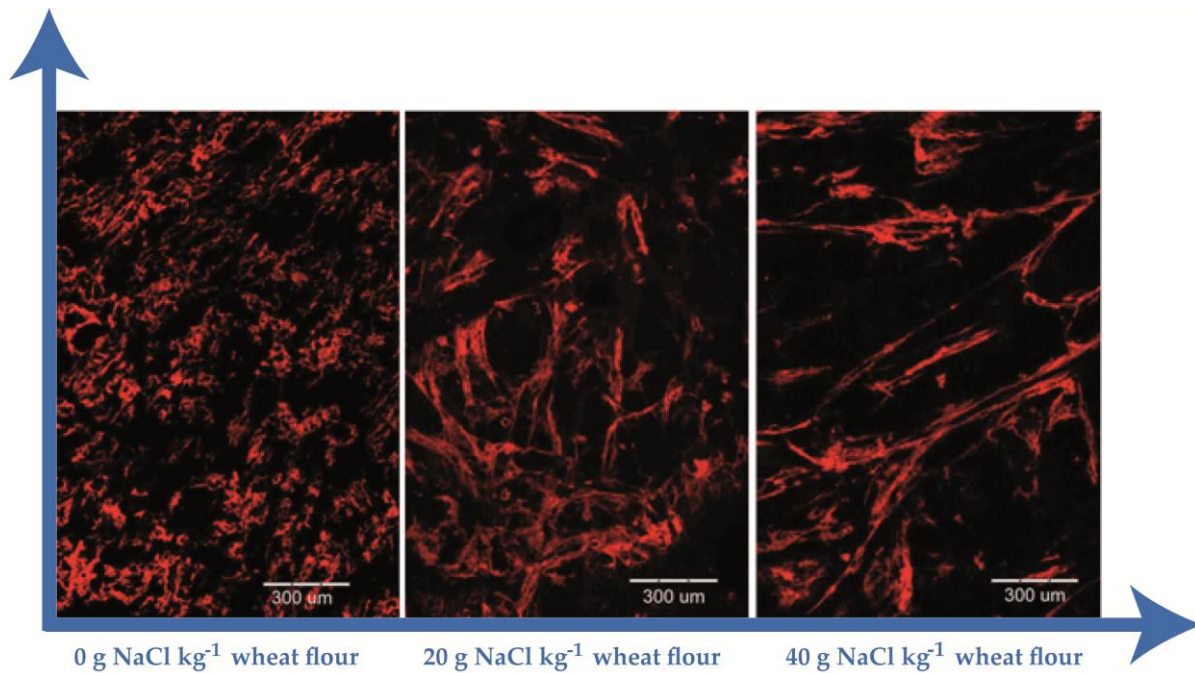
Figure 11: 2-D confocal laser scanning microscopy (CLSM) images of wheat dough. Left = 0 g NaCl kg⁻¹ wheat flour and on the right = 40 g NaCl kg⁻¹ wheat flour. The dough were stained with Rhodamine B, and analyzed after 60 min proofing at 30 °C. Each micrograph (1714 x 1542 μm) was obtained by the composition of twelve individual images to create an enlarged section of the protein structure. A detailed scale is evident.

Moreover, NaCl addition reduces the flour's capacity for water absorption. This decrease is based on a change in gluten polarity. As the protein polarity alters with NaCl addition, the proteins hydrate to a lesser extent. Consequently, less water is bound by the proteins (flour water absorption decreases), and the remaining, non-protein adsorbed water amount in the dough system is higher. Theoretically, this is thought to decrease the viscosity of the dough system and thus also dough stickiness. However, due to the increase of gluten network cross-linking, the viscosity (e.g. $|G^*|$) was actually raised with higher NaCl. Additionally an increase in dough stickiness was detected with the universal stickiness device (Chen and Hoseney Rig) [125, 126] that accompanies rising amounts of NaCl. Analytically, the dough is more liquid due to higher content of non-protein adsorbed water. Therefore, the contact area between the surface of the dough and the surface of the plunger is higher and the dough sticks to the plunger surface in the TPA assembling more than in lower NaCl dough, and thus, stickiness increases. This contradicts the norm accepted by bakery engineers that NaCl reduces dough stickiness [127]. This was established by subjective measurements in

an earlier work by Danno and Hoseney [127], which describes the experiences of countless bakery engineers and dough scientists, past and present. The finding of increasing stickiness, using the objective method of Chen and Hoseney [125, 126], is not equal to subjective stickiness, and although it commonly correlates well with the subjective perception, it does not in this particular circumstance. Non-standardized stickiness measurements undertaken by humans analyze the overall rheological characteristics, and predominantly changes in visco-elasticity. Further work should estimate the cause of the stickiness in detail and develop new, improved advice regarding stickiness. Interestingly, such an improved stickiness rig would also prove beneficial for other applications such as measuring the adhesion of dough to proofing baskets.

In the formed gluten network, gas bubbles are entrained and remain there during proofing, thus dough volume increases. However, high NaCl addition (40 g NaCl kg^{-1} flour) lead to increased dough resistance and a decrease in the total volume of gas produced by the yeast cells. Therefore, the gas amount was to less, and insufficient to extend the dough system (which illustrates higher viscous as well as elastic parameters compared to the reference). As a result, the end-product volume was less and featured a dense, fine pored crumb structure (Figure 12).

0 g NaCl kg^{-1} wheat flour   20 g NaCl kg^{-1} wheat flour   40 g NaCl kg^{-1} wheat flour
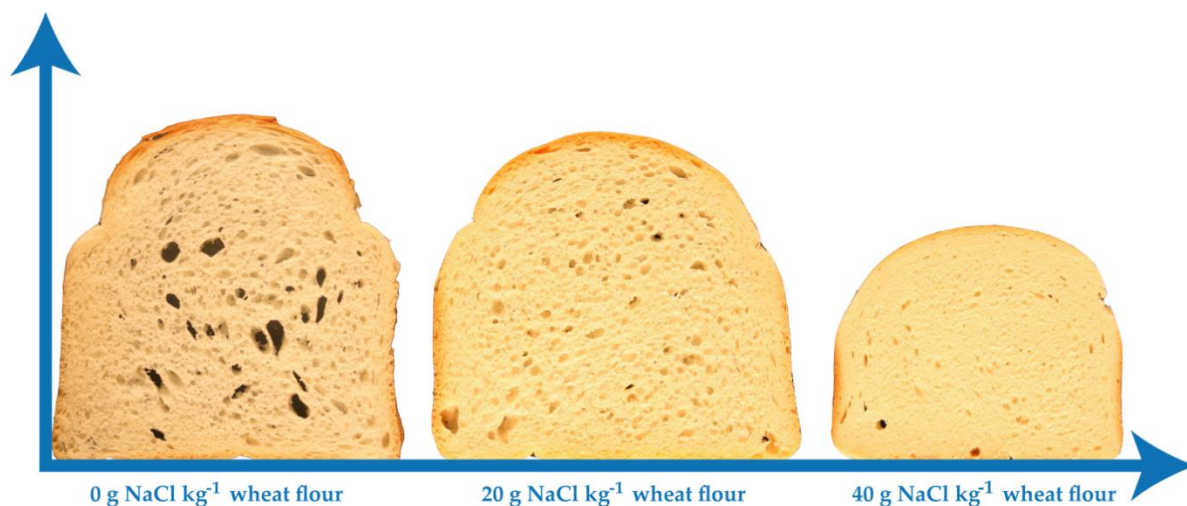
Figure 12: Crumb structure of wheat bread crumbs with different amounts of NaCl wheat flour. 0 g NaCl kg^{-1} flour illustrates large crumb pores and less cohesive crumb structure. Increasing NaCl amounts (20 g kg^{-1} flour) illustrates increased crumb coherence and less porosity. High NaCl addition (40 g kg^{-1} flour) shows a very dense crumb structure as well as less volume.

The reason for this crumb structure is that the dough was a) too highly viscous to retain gas and allow the bubbles to expand [128] and simultaneously b) insufficiently capable of retaining the gas, thus the bubbles may coalesce or disperse during proofing and oven rise. In detail, an intensive protein cross-linking, due to NaCl, strengthens the protein system and increases the dough viscosity so that the gas produced by the yeast is unable to extend the dough volume further. The pressure of a gas bubble in a dough system is the interaction of the Laplace pressure based on the surface tension and the visco-elasticity of the system [77], and can be defined by the Young-Laplace-equation (equation (19)). Therefore, if viscous dough behavior increases due to the increment of gluten cross-linking caused by NaCl, the pore cell pressure in the bubbles should also rise to achieve constant bread volume. If the cell pressure in the bubbles rises to an insufficient extent, maybe due to low gas production, the pore cell radius as well as the bread volume decreases and crumb structure becomes finer. This leads directly to an increase in crumb firmness. It would, therefore, be interesting to establish the pressure measurement inside the crumb pores. This could be measured using a very small and sensitive pressure sensor, which could be left in the bread during baking. This should be considered for future work.

The gas retention effect, or rather foam stability of the dough, could be explained using the Gibbs-Marangoni effect (equation (18)). The Gibbs-Marangoni effect defines the mass transfer along an interface between two fluids due to surface tension gradient. Foam stability elicited by Gibbs-Marangoni effect increases due to a) salt, which commonly increases the surface tension [129] or b) protein changes, which can alter the surface tension. To achieve optimal foam stability, the pH of the dough should be near the isoelectric point at which the proteins are uncharged [130]. Since the author's own measurement show that NaCl alters both proteins and pH of dough [131], it is possible that increased gas retention that results from a stronger protein network may depend on NaCl addition. Therefore, although no significant changes in end-product volume were determined with the addition of NaCl 0 up to 20 g kg^{-1} flour, gas production decreased while gas retention increased. It seems that with higher NaCl (>20 g kg^{-1} flour), the amount of produced gas is more important for the bread volume than the change in gas retention, because end-product volume decreased significantly with high amounts of NaCl. To predict foam stability through pH changes, it would be interesting for further works to

analyze which flour fraction and composition (starch, gluten, pentosan, lipid, and water) change dough pH in the presence of NaCl to the largest extent.

Crumb structure is an important quality factor of baked goods, since lack of crumb stability is undesired by consumers. In considering the crumb firmness, it was recognized that crumb firmness slightly increases due to NaCl addition. This is based on the crumb pore distribution, which became more equal and more compact, and the inverse proportional relation between crumb firmness and specific end-product volume. Less volume due to high amounts of NaCl are reflected in high crumb firmness with small, compact pores.

Additionally, higher end-product volume showed an inverse correlation with crumb firming. With high end-product volume, the crumb pores are larger and therefore, the crumb firmness is low. This occurs because the ratio of air to crumb is high, resulting in a lower resistance during the TPA measurement compression. High amounts of NaCl decrease bread volume and increase crumb firmness. Thus, to manufacture healthy, nutritionally high grade yeast-leavened products with high end-product volume and fluffy crumb structure, less NaCl (around 5 g NaCl kg^{-1} flour) is optimal. However, the higher the NaCl addition, the better the crumb cohesion and dough handling ability. Further, in our measurements the most sensory pleasing products were found to be those with relatively high amounts of NaCl, around 17.5 g NaCl kg^{-1} flour [132], the equivalent of most bread products. Further measurements should undertake the analysis of the effect of different sodium replacers on bread volume and crumb texture. Furthermore, since there is an inverse relation between bread volume and crumb firmness due to the relation between high bread volume and large air bubbles, crumb firmness decrease. Thus, it would be interesting to develop alternative methods to measure the firmness of a crumb, which are independent of the density of the crumb.

Upon analyzing the structure alteration of wheat crumb during storage, a high staling rate with NaCl addition of >20 g NaCl kg^{-1} wheat flour was observed. The available literature primarily presents an increase in bread staling as a function of starch re-crystallization (retrogradation) [46, 47]. Interestingly, crumb, or rather starch retrogradation showed an inverse correlation with crumb staling. The addition of NaCl to wheat bread in this study shows, therefore, novel information about starch retrogradation (measured as melting

enthalpy ΔH in the DSC) in the respective crumb. Increased NaCl concentration (up to 40 g NaCl kg^{-1} flour) showed a significant decrease in retrogradation after four days of storage. Several studies discuss the change in starch gelatinization due to NaCl addition; but however, do not thoroughly analyze changes in starch retrogradation.

Few hypotheses have postulated to explain the modification of starch gelatinization by NaCl. The most likely explanation is the theory, that starch acts as a weak ion exchanger, which is schematically illustrated in Figure 13. Sodium ions may be pushed into the starch due to a DONNAN gradient [133]. Since the diameter of sodium is larger than that of hydrogen ions, the re-association of the starch chains probably decreases, resulting in fewer retrograded starch molecules.
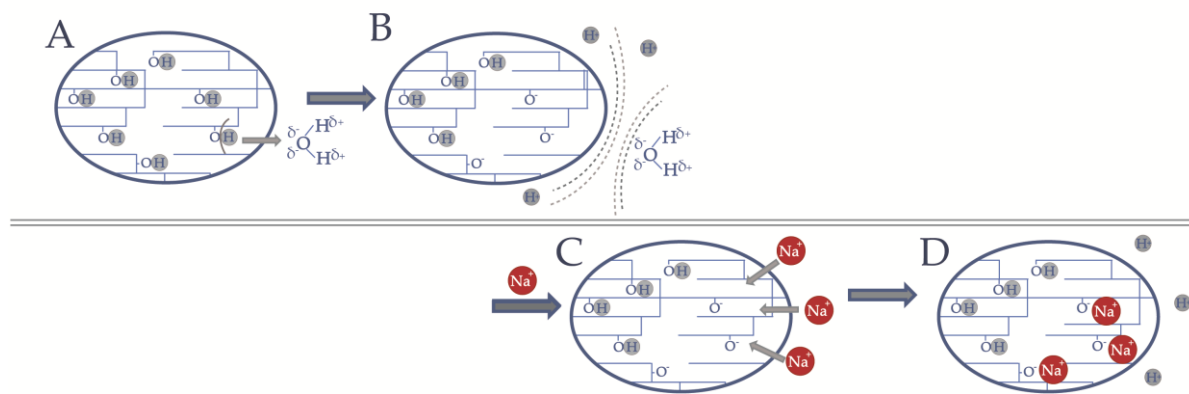

Figure 13: A) The hydrogen ion (grey) concentration inside the starch granules (blue) is higher than in the water phase [133, 137]. Therefore, hydrogen ions will migrate from the starch to the water phase due to the concentration gradient. B) Starch total charge will become negative and the water phase positive. The resulting potential between starch and water is called DONNAN potential [133]. Thus, the migration of hydrogen ions from the starch molecule into the water phase will be stopped by a dynamic equilibrium [133]. C) Due to the potential, the starch tends to push cations with higher charge density (Na$^+$; red) into the starch. D) Sodium ions have a greater ion radius and therefore probably disturb retrogradation.

As a conclusion, the received results about the alteration of starch retrogradation due to NaCl addition raised some questions: a) is the above mentioned theory about starch acting as a ion exchanger appropriate and b) how do other ions influence starch gelatinization and retrogradation. Therefore, different chloride salts (NH$_4^+$, K$^+$, Na$^+$, Li$^+$, Ca^{2+}, Mg^{2+}) were

analyzed regarding their ability to change the gelatinization and retrogradation of starch-water samples. All of the chloride salts affected starch gelatinization, and thereby, gelatinization onset (T_0) occurred later in samples with chloride salts than in the sample without salt. A possible explanation for this change is a reduced a_w-value. The water-controlled gelatinization reaction requires more energy, and thus, onset is delayed and gelatinization enthalpy is higher with the addition of salts [134]. In addition, complex reactions between NaCl, water and starch may also affect starch gelatinization [135, 136].

Changes in gelatinization temperatures and the gelatinization enthalpy of starch-water suspensions occurs in the order $NH_4^+ < K^+ < Na^+ < Li^+ < Ca^{2+} < Mg^{2+}$. This equates an inverted version of the Hofmeister series [138, 139]. In the Hofmeister series, the ions are classified in order of their ability to change the structure of water and dissolve proteins in water. Thus, our rheological measurements in DoughLab (Farinograph equivalent) as well as with the CLSM illustrated that gluten protein definitely changes in line with the Hofmeister Series due to different solubilization effects [131]. However, the effects of the Hofmeister series on starch is not entirely clear; therefore other reasons were investigated as to why starch gelatinization changed with different cations. The order of the gelatinization temperatures can also be clarified by the hydration energy of the chloride salt in water (Figure 14) [136, 140]. Thereby, hydration depends on the diameter and charge of the cations. Bivalent cations with higher charges (Ca^{2+} and Mg^{2+}) show higher hydration than monovalent cations with less charge (NH_4^+, K^+, Na^+, Li^+) [136].
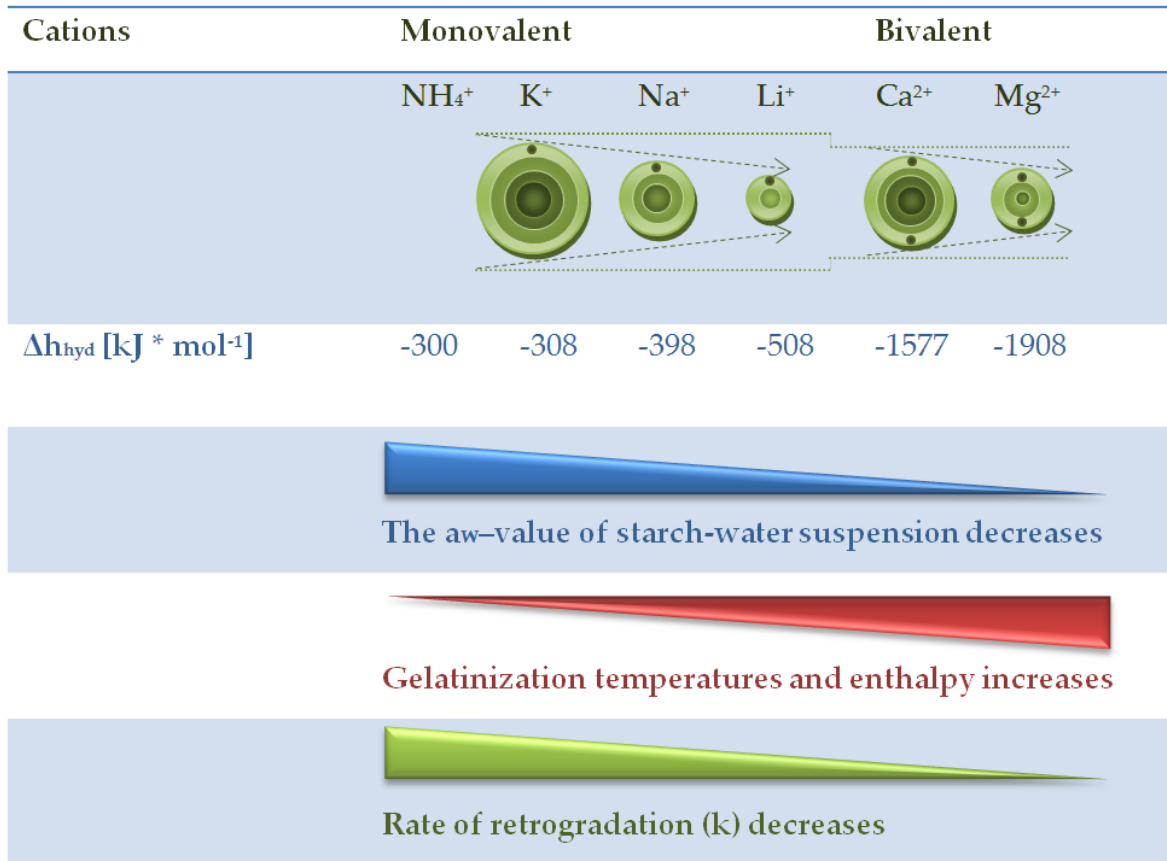
Cations	Monovalent				Bivalent	
	NH_4^+	K^+	Na^+	Li^+	Ca^{2+}	Mg^{2+}
Δh_{hyd} [kJ * mol^{-1}]	-300	-308	-398	-508	-1577	-1908

The aw–value of starch-water suspension decreases

Gelatinization temperatures and enthalpy increases

Rate of retrogradation (k) decreases

Figure 14: Hydration energy (Δh_{hyd}) [kJ mol^{-1}] of different cations [140]. The order is listed (trends are shown with arrows), in which the ions influence aw–value, starch gelatinization temperatures, starch gelatinization enthalpy and starch retrogradation.

Gelatinization behavior has been extensively analyzed in the literature, but up till now there is little detailed scientific knowledge about the alteration of starch retrogradation. Therefore, the rate of re-crystallization was analyzed over a storage period up to 504 h by means of the Avrami equation (equation (22)). All cations used decreased the rate of retrogradation (k) compared to the reference. Further, the order in which the cations decreased the rate of retrogradation (k) was the same as for the gelatinization behavior (Figure 14). Bivalent cations showed the lowest values of k; this phenomenon is probably based on the aw–value [52], which was lowest in bivalent cations. Low water content may hinder molecular movement and thus reduce the starch re-crystallization process [141].

On the other hand, the addition of cations might reduce the reversion of amylopectin into crystalline form, whereby retrogradation decreases. The proposed theory of

Oosten [133, 137], that starch acts as a weak ion exchanger (illustrated in Figure 12) may be applied to starch retrogradation, however the influence of the a_w-value may be stronger. The hypothesis of the ion exchanger was proven in this study by the decreasing pH-values in the starch-water samples since the pH-value of all samples with salt were significantly lower than reference (without salt). Additionally, the starch solubility, or swelling, may also change due to cation addition [142], and thereby effect retrogradation. However, to discover to which extent starch solubility changes with different ingredients, this effect should be systematically analyzed in further works. It is also important that the starch-water-salt samples show same a_w-values.

Salt addition to starch-water suspensions demonstrated a remarkable alteration of the rate of starch retrogradation (k). The a_w-value is probably one of the main important values which affect the gelatinization as well as retrogradation. Therefore, further work should include measurements with constant a_w-values and the same number and type of cations as analyzed in this study. Subsequently, confirmation of the results for both flour-water systems and the end-product should be undertaken to evaluate how the end-product will behave due to different cations. For further measurements it would improve scientific knowledge if DSC analysis would comprise other complex DSC analysis models which provide additional data about starch alteration. As an example the Williams-Landel-Ferry Modell is an empirical equation associated to time-temperature superposition and maybe helpful to understand baking behavior regarding temperature dependent starch alteration.

Currently, less favorable sodium-free salt alternatives are available on the market. This is in contrast to the widespread usage and development of sugar substitutes. Therefore, a promising approach would be to discover or develop compounds that either substitute the cation sodium or enhance the saltiness perception of NaCl. Sodium replacements by other cations such as potassium, ammonium, calcium, or magnesium are the most commonly used method of sodium reduction. However, our measurements show that with a replacement of sodium with a larger amount of potassium chloride (more than 30-50% substitution) wheat bread remained bitter and with a metallic, off taste although both the rheological and technological results of KCl were very similar to NaCl. Wheat bread with other chloride salts such as magnesium or calcium also tasted bitter or metallic with considerably lower levels of sodium substitution (Table 1) [131, 132].

Thus, the usage of sodium replacers such as chloride salts are primarily restricted by associated bitterness or metallic taste. The reduction of NaCl and the perception of bitterness in food has been investigated for many years, and several compounds have been proposed to prevent this bitterness [143-145]. However, an appropriate bitterness inhibitor for general food products has yet to be developed. In mixing two taste compounds there is a potential for one substance to interfere with the taste receptor of the other substance. It has been shown, that sodium salts and bitter compounds interact so that bitterness is inhibited and saltiness is unaffected [146, 147]. Several substances has been demonstrated to be effective in reducing bitterness: e.g. sweeteners (sucrose, lactose) [9, 148], adenosine 5'-monophosphate (AMP) [149] and trehalose [150]. Thus, with a bitter blocker, the usage of KCl may be possible. However, our preliminary sensory measurements with bitter blockers and KCl in wheat bread indicated that while AMP (up to 1600 ppm) did not reduce bitterness, 5% saccharose (based on flour) significantly reduced bitterness, but significantly increased the sweetness. Wheat breads with more than 7.5% lactose (based on flour) or more than 5% trehalose (based on flour) showed a slight decrease in bitterness (Table 1); however sweetness and off-flavor increased [131]. Although first trials with bitter blockers in wheat bread did not show the expected results, there are several other bitter blockers available (e.g. 4-2,2,3-trimethylcyclopentyl- butyric acid [151]) which may help in developing sodium replacers. Therefore, future work should systematically investigate which bitter blocker may be useful for wheat breads.

Another more innovative and not widely used way, to reduce sodium in food products is the use of a saltiness enhancer (also called salt booster). A saltiness enhancer can be defined as a substance which raises the saltiness perception of a salty substance without showing any significant saltiness. Saltiness enhancers suitable for use in yeast-leavened products are L-lysine and L-arginine [152], monosodium glutamate [153], lactates [154], trehalose [150], L-ornithine [155]. Preliminary trials with more than 0.25% L-arginine and more than 0.75% L-lysine presented increased saltiness in wheat breads, however, the off-flavour also increased. K-lactate (around 0.5%, based on flour) presented more aromatic bread; however, the wheat bread was not significantly saltier (Table 1). Rheological and technological parameters were not significantly influenced by the salt enhancers

used [131, 132]. Further trials, should systematically analyze other salt enhancers as well as combinations of different salt enhancers on sensory, rheological and technological effects.

Table 1: Strategies for improving saltiness perception while sodium reduction.

Substance		First own results with wheat bread
Mineral salt	KCl	More than 30-50% substitution: bitter or metallic off-flavour
	$MgCl_2$	More than 20-30% substitution: bitter or metallic off-flavour
	$CaCl_2$	More than 10-20% substitution: bitter or metallic off-flavour
Bitter blocker	AMP	did not significantly reduce bitterness
	Sucrose	More than 5% (based on flour) superimpose bitterness, however sweetness increased significantly
	Lactose	More than 7.5% (based on flour) slightly decrease bitterness, however sweetness and off-flavour increased
	Trehalose	More than 7.5% (based on flour) slightly decrease bitterness, however sweetness and off-flavour increased
Salt enhancer	Arginine	More than 0.25% (based on flour) increased saltiness, however off-flavor also increased
	Lysine	More than 0.75% (based on flour) increased saltiness, however off-flavor also increased

Decreasing the overall sodium intake is also possible through taste adaptation. Taste adaptation is defined as the customization of the personally preferred sodium level to a certain sodium concentration [156]. Several works discuss the positive effect of a general, low-sodium diet with the theory that after individuals adjust their salt perception to lower sodium concentration after having been on a low sodium diet, and subsequently find food with high NaCl levels too salty, less preferred, and less pleasant [157, 158]. This statement is based on the fact that adults with a very low-sodium diet are able to detect lower levels of NaCl in water compared to adults with a higher sodium intake [158].

Since the attention of consumers and governments will undoubtedly focus on a healthy diet, baked goods are a central part in the discussion of sodium reduction. In conclusion the usage of 5 g NaCl kg^{-1} flour presented the highest bread volume and lowest rate of staling,

and seems to be a good solution for high volume end-products. However, such a low concentration of NaCl features less dough extensibility as well as problems with handling due to stickiness. Further, the most sensory pleasing products were found to be those with relatively high or rather common amounts of NaCl, around $17.5\,g\,NaCl\,kg^{-1}$ flour. The reduction of sodium in yeast-leavened products, while achieving the same structural and sensory effects, is therefore only feasible in small doses, or rather, undesirable without substitution by other substances. Therefore, sodium reduction is a challenge for the food industry because, as seen in the current work, sodium chloride causes essential structural changes (rheological and technological) as well as sensory end-product changes in yeast-leavened products.

As a compromise between rheological, technological, preservatory, and sensory effects, the reduction of sodium chloride should be aimed at 1.25% (based on flour). The further reduction of sodium needs a) sodium replacers or b) a long term taste adaption to achieve high quality, tasteful yeast-leavened products. Overall, this work provides broad, detailed, fundamental and novel information about the effects of sodium chloride in yeast-leavened products as well as strategies for sodium reduction with replacers. Only with an extensive background in the fundamental effects of sodium chloride and some replacers will it be possible to develop sodium-free salt alternatives.

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5.   FURTHER PUBLICATIONS

Non-reviewed papers

1. Beck, M., Jekle, M., Becker, T.: Protein cross-linking - a method for improving the quality of rye baked goods. Baking+Biscuit 5 (2009), 78-81.

2. Beck, M., Jekle, M., Becker, T.: Proteinvernetzung - Ein Weg zur Verbesserung von Roggenbackwaren. Brot und Backwaren 6 (2009), 36-41.

3. Beck, M., Hofmann, S., Jekle, M., Becker, T.: Untersuchung unterschiedlicher Materialien auf die Eignung als Gärgutträger. Getreidetechnologie 63 (2009), 58-65.

4. Beck, M., Mitzscherling, M., Becker, T.: Darstellung rheologischer und technologischen Eigenschaften von Transglutaminase auf Roggenteige und -backwaren. Getreidetechnologie 62 (2008), 349-353.

Book contribution

1. El Fawakhry, H., Beck, M., Jekle, M., Hussein, M., Becker, T.: Dough density determination using ultrasound. In Review: bread, editor: f2m baking+biscuit international. Hamburg, Germany: f2m food multimedia GmbH (2010), 110-115.

Oral presentations

1. Beck, M., Jekle, M., Becker, T.: Impact of different sodium replacers on starch re-crystallization kinetics. 2011 AACC International Annual Meeting, Palm Springs, USA, 2011-10-28.

2. Beck, M., Jekle, M., Becker, T.: Impact of sodium chloride and sodium replacers on micro and macro texture of wheat baked goods. ICC Cereals & Europe Spring Meeting 2011, Freising, Germany, 2011-04-12.

3. Beck, M., Jekle, M., Becker, T.: Ermittlung der thermischen sowie lagerungsbedingten Stärkeveränderungen in Abhängigkeit verschiedener Elektrolyten bei der Herstellung stärkebasierter Lebensmittel. Jahrestreffen des ProcessNet-Fachausschusses Lebensmittelverfahrenstechnik, Vlaardingen, The Netherlands, 2011-03-03.

4. Beck, M., Jekle, M., Becker, T.: Natriumreduktion in Backwaren - Hintergründe, Alternativen und Möglichkeiten für die Zukunft. Tagung für Bäckerei-Technologie, Detmold, Germany, 2010-10-26.

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8. Beck, M., Mitzscherling, M., Becker, T.: Improvement of the technological properties of rye dough and rye bread by Transglutaminase. 13th ICC Cereal and Bread Congress, Madrid, Spain, 2008-06-18.

9. Beck, M., Mitzscherling, M., Becker, T.: Verbesserung der technologischen Eigenschaften von Roggenteigen und –backwaren durch Transglutaminase. 59. Tagung für Getreidechemie, Detmold, Germany, 2008-06-19.

10. Beck, M., Mitzscherling, M., Becker, T.: Untersuchung innovativer Materialien für Gärgutträger. 58. Tagung für Bäckerei-Technologie, Detmold, Germany, 2007-11-08.

11. Beck, M., Mitzscherling, M., Becker, T.: Untersuchung innovativer Materialien für Gärgutträger. GDL-Kongress Lebensmitteltechnologie, Hamburg, Germany, 2007-10-13.

Poster presentations

1. Beck, M., Jekle, M., Becker, T.: Impact of sodium chloride and different sodium replacers on the retrogradation kinetics of baked goods. iCEF11 International Congress on Engineering and Food, Athens, Greece, 2011-05-22.

2. Beck, M., Jekle, M., Becker, T.: Rheological alteration of wheat dough due to sodium chloride addition. Sitzung des Wissenschaftlichen Ausschusses der FEI, Neustadt a. d. Weinstraße, Germany, 2010-09-07.

3. Beck, M., Jekle, M., Becker, T.: Risks and impact of sodium chloride in baked goods. 15th World Congress of Food Science and Technology, Cape Town, South Africa, 2010-08-22.

4. Beck, M., Jekle, M., Becker, T.: Transglutaminase and its usage for producing rye products. Jahrestreffen des Fachausschusses Lebensmittelverfahrenstechnik, Lausanne, Switzerland, 2009-03-25.