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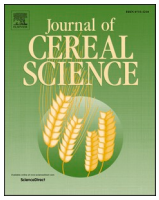

Influence of salts on the protein composition and functionality of gluten

Nina Hoeller [a], Katharina Anne Scherf [a,b,c,*]

[a] Department of Bioactive and Functional Food Chemistry, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20 a, 76131, Karlsruhe, Germany
[b] Leibniz Institute for Food Systems Biology at the Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany
[c] Technical University of Munich, TUM School of Life Sciences, Professorship of Food Biopolymer Systems, Lise-Meitner-Str. 34, 85354, Freising, Germany

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ABSTRACT

Different salts were added to gluten to investigate the effects of different cations on a fully developed gluten network. The chloride salts KCl, NaCl, $MgCl_2$, $CaCl_2$ were mixed with pre-isolated wet gluten in a low and high dose, and the gluten was dried at 40 °C and 80 °C to yield vital gluten. The impact of the individual cations on the gluten protein composition and functionality was determined by analysis of chemical and rheological properties of vital and wet gluten. All cations influenced the gluten protein composition and the rheological behaviour. While the observed changes could not be attributed to the order of the Hofmeister series, monovalent, kosmotropic cations K^+ and Na^+ and divalent, chaotropic cations Mg^{2+} and Ca^{2+} showed similar behaviour in terms of protein composition and functional properties. Salts therefore have an influence on the gluten protein composition of a fully developed gluten network and gluten protein composition and functionality can be altered by an after-treatment process in a targeted way.

1. Introduction

The viscoelastic properties of gluten play a key role in shaping the texture, structure and overall quality of various wheat-based products. In the baking industry, adding vital gluten to flour can compensate for the variable quality of wheat by stabilizing the existing gluten network. Vital gluten is obtained as a by-product in the starch extraction process after drying. The process parameters are adapted to the quality depending on natural variations in climate or soil conditions of the raw material wheat in order to guarantee consistent starch quality. In most cases though, no particular attention is paid to any changes in the gluten protein composition and functionality of the by-product gluten. Therefore, commercially available gluten exhibits a range of manufacturer-dependent quality differences (Schopf and Scherf, 2020; Schopf et al., 2021).

Besides vital gluten, NaCl is traditionally used in doughs, because it is known to strengthen the dough structure, to delay the dough development time, to increase the gas retention time and to yield a higher loaf volume (Carcea et al., 2020; McCann and Day, 2013; Wehrle et al., 1997). Therefore, numerous studies have investigated the influence of adding NaCl to wheat dough or the washing water during gluten isolation. One main focus was to elucidate the interaction of NaCl with gluten proteins on a molecular level (Chen et al., 2018; Fu et al., 1996; Melnyk et al., 2011; Tuhumury et al., 2014; Ukai et al., 2008). NaCl initiates changes of the molecular conformation of gluten proteins leading to more non-covalent interactions between gliadins and glutenins (Fu et al., 1996; Tuhumury et al., 2014). The addition of NaCl to gluten changes the dough matrix as water molecules interact with sodium and chloride ions. Thus hydrogen bonds between polypeptide chains of gluten proteins are enhanced resulting in a more dense and compact gluten network (Fu et al., 1996).

Other studies on salt-protein-interactions were based on the Hofmeister series to elucidate a systematic impact of ions on gluten structure and functionality (Kinsella and Hale, 1984; Melnyk et al., 2011; Preston, 1981; Tuhumury et al., 2016a, 2016b; Wellner et al., 2003). The Hofmeister series classifies anions and cations according to their ability to precipitate proteins in solution in the order $K^+ < Na^+ < Mg^{2+} < Ca^{2+}$ with kosmotropic cations on the left side of the scale and chaotropic cations on the right side of the scale (Kunz, 2010; Kunz et al., 2004). Kosmotropic ions promote protein stability and cause the precipitation of proteins. Chaotropic ions decrease protein stability and lead to improved protein solubility. They promote protein unfolding and thus protein denaturation (Kunz, 2010; Zhang and Cremer, 2006).

In classical theories, kosmotropes are designated by the term “water

* Corresponding author. Leibniz Institute for Food Systems Biology at the Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany.
E-mail address: katharina.scherf@tum.de (K.A. Scherf).

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structure makers" and chaotropes are referred to as "water structure breakers" (Zhang and Cremer, 2006). Kosmotropes and chaotropes influence the structure and dynamics of water, for example by interacting with the hydration layer of proteins, but this phenomenon probably only occurs locally (Lo Nostro and Ninham, 2012; Xie and Gao, 2013). In fact, ions can interact directly with proteins via their charged side chains and amino acid backbone (Lo Nostro and Ninham, 2012; Okur et al., 2017; Xie and Gao, 2013). However, model predictions about specific ion effects are challenging as ions act differently depending on their charge, size, geometry and the macromolecule with which they interact (Kunz, 2010; Okur et al., 2017; Preston, 1981; Xie and Gao, 2013). Furthermore, the effects of anions and cations are not additive, but the composition of the ion pair plays a crucial role in affecting the macromolecule (Xie and Gao, 2013). Salts enhance the aggregation of gliadin-rich proteins in general, but the intensity of this effect is dependent on the specific salt. Therefore, it is presumed that gliadin aggregation is affected by the interaction of a specific ion with a certain amino acid residue on the one hand and by the concentration of salt on the other hand (Kinsella and Hale, 1984; Preston, 1981; Ukai et al., 2008).

Even though many studies investigated the interactions of salts and gluten proteins in dough or during washing of gluten from dough, to the best of our knowledge, no study has ever investigated the effect of salts on isolated gluten. Therefore, it is unknown if salts can cause effects in a fully developed gluten network similar to the observations previously reported. In our study, we aimed to systematically investigate the synergistic effect of the important process parameter drying temperature and different salts in a low and high concentration on gluten protein composition and functionality.

2. Materials and methods

2.1. Materials

A mixture of different flours of type 550 (according to the German flour classification system, i.e., ash content 0.51%–0.63% based on dry matter) as commonly used in the baking industry, without the addition of ascorbic acid was kindly provided by Bavaria Mühle Dorfner Aktienmühle (Aichach, Germany). The flour was stored airtight at 22 °C. The protein content of the flour was 10.8% and the moisture content was 13.3%. All chemicals used were of analytical grade and the salts (NaCl, KCl, $MgCl_2$, and $CaCl_2$) were purchased from VWR International GmbH (Darmstadt, Germany), Carl Roth GmbH (Karlsruhe, Germany) and Acros Organics as part of Thermo Fisher GmbH (Schwerte, Germany).

2.2. Preparation of wet and vital gluten

The water-binding capacity of the flour was determined with a farinograph with a 50 g mixing bowl (Brabender GmbH & Co. KG, Duisburg, Germany) according to ICC Standard 115/1 to reach a standardised dough consistency at the beginning of the sample preparation procedure of wet and vital gluten samples. The settings of the farinograph were adjusted to achieve 500 Brabender units. The volume of water was scaled down to 20 ± 0.1 g flour (equals 11.98 mL of water) and the flour and desalted water were kneaded manually in a beaker with a spatula for 2.5 min. The kneading time corresponded to the dough development time previously determined in the farinograph. After kneading, the dough was divided into two portions of equal weight. The initial mixing step in the Glutomatic device (PerkinElmer Inc., Waltham, MA, USA) without water was skipped to avoid clogging the sieves. Then, each dough ball was rolled into a strand and placed along the side of each washing chamber. The dough was washed according to ICC Standard 137/1 using polyamide sieves (0.88 μm) with 540 mL of desalted water per washing chamber within 10 min.

The isolated wet gluten was placed in the sieves (600 μm) of the Glutomatic centrifuge and centrifuged at 6000 rpm for 1 min to remove excess water from the previous washing step. To incorporate the salts, the wet gluten was mixed with either a low dose of salt corresponding to 0.64% KCl, 0.50% NaCl, 0.41% $MgCl_2$, and 0.48% $CaCl_2$ or a high dose of salt, corresponding to 3.83% KCl, 3.00% NaCl, 2.44% $MgCl_2$, and 2.85% $CaCl_2$. The salts were added in solid form in an electric coffee grinder (Rommelsbacher ElektroHausgeräte GmbH, Dinkelsbühl, Germany) and were mixed with the wet gluten for 3 × 5 s. Between the mixing steps, the wet gluten was collected from the lid and sides of the cup of the coffee grinder to ensure the best possible incorporation of the salt into the gluten.

The molar concentration of salts was calculated as the amount of 0.50% or 3.00% NaCl (w/w), based on wet gluten (0.086 mmol/g and 0.513 mmol/g) for the salts KCl and NaCl. The required amount of the divalent salts $MgCl_2$ and $CaCl_2$ was halved accordingly (0.043 mmol/g and 0.257 mmol/g). The exact molar concentration per gram of wet gluten can be found in Table S1. The reproducibility of the sample preparation procedure was tested by analysing three technical replicates, which were analysed by Osborne fractionation. The content of individual gluten protein types was not statistically different in all three replicates.

To obtain vital gluten, the wet gluten was divided into balls of roughly 1 cm diameter and pressed between two Teflon molds with an even surface for 4 min. In this way, all samples had a thickness of no more than 1 mm to ensure even drying. The pressed wet gluten was dried on a rack in a drying oven for 4 h at 40 °C or 80 °C. The dry gluten was ground to vital gluten in an ultracentrifugal mill (Pulverisette 14, classic line, Fritsch GmbH, Idar-Oberstein, Germany) with a sieve insert with trapezoidal perforations of 200 μm at 13000 rpm. To avoid excessive temperature development in the mill, the grinding process was carried out at intervals of <10 s.

In total, 18 samples were produced. The salts KCl, NaCl, $MgCl_2$ and $CaCl_2$ were added to gluten in a low concentration of 0.64%, 0.50%, 0.41% and 0.48% or in a high concentration of 3.83%, 3.00%, 2.44% and 2.85%, respectively. Each of these samples was dried at 40 °C and 80 °C, respectively. Additionally, a control without salt was dried at 40 °C and 80 °C. For better readability, the concentrations of all salts are simply referred to as low or high salt concentration in the following.

2.3. Microscale extension tests

For the microscale extension test, wet gluten was placed in a two-piece Teflon mold with one smooth and one notched part (Scherf et al., 2016). The Teflon mold was centrifuged in cylindrical inserts for 10 min at 22 °C and 3060×*g* to remove excess water (Scherf and Koehler, 2018). The preformed gluten strands were transferred to another two-piece Teflon mold with a smooth and a notched part (Kieffer et al., 1981) which was slightly lubricated with paraffin oil and pressed for 30 min. The strands were then transferred to the SMS/Kieffer Dough and Gluten Extensibility Rig of a TA.XT plus Texture Analyzer (Stable Micro Systems, Godalming, U.K.). The device was equipped with a 5 kg load cell and the measurement parameters were set to test mode: extension, pre-test speed: 2.0 mm/s, test speed: 3.3 mm/s, post-test speed: 20.0 mm/s, rupture distance: 4.0 mm, distance: 150 mm, force: 0.049 N, time: 5 s, trigger type: auto, trigger, force: 0.049 N, break detect: rate and break sensitivity: 0.020 N. All measurements were carried out in quadruplicate from three separate sample preparations. The software version Exponent 6.2.4 was used.

2.4. Rheology

For rheological measurements, oscillatory tests were performed on wet gluten with a Physica rheometer MCR301 (Anton Paar Group GmbH, Graz, Austria) equipped with a circulating Viscotherm VT2 cooling water bath (Anton Paar) and a temperature-controlled hood (H-PTD200, Anton Paar). For the amplitude and frequency sweep, 1.3 g of wet gluten was weighed and pressed for 5 s between two smooth Teflon

plates using light pressure. The wet gluten sample was then placed on a profiled parallel plate measuring geometry with a diameter of 2.5 cm of the rheometer and the sample was allowed to relax for 20 min at the measuring gap of 2 mm before the respective measurement was started. The edges of the gluten sample that were not covered by the measuring plates, were coated with paraffin oil to prevent dehydration. Additionally, the temperature hood was lowered as a cover. The edges of the sample were not trimmed in order to avoid excessive stress on the gluten structure. The amplitude sweep was performed at 25 °C at a shear deformation of 0.01–100% and a frequency of 1 Hz. The frequency sweep was performed at 25 °C at a shear deformation of 0.1% and a frequency of 0.1–20 Hz. All measurements were carried out in triplicate with a new sample preparation for each measurement.

For the temperature sweep, 2.0 g of wet gluten was weighed and relaxed for 20 min between two smooth Teflon plates. The Teflon plates were fitted with a spacer so that the gluten samples were pressed to a height of 3 mm. For measurement, the gluten samples were tilted from the Teflon plate directly onto the profiled parallel plate measuring geometry with a diameter of 2.5 cm of the rheometer without readjusting the gluten sample on the measuring plate to avoid additional impact on the gluten structure. The edges of the gluten sample, which were not covered by the measuring plates, were coated with paraffin oil. Before starting the measurement, the temperature hood was placed on the measuring geometry. The temperature sweep was performed at a shear deformation of 0.1%, a frequency of 1 Hz and a temperature ramp of 25–95 °C at a heating rate of 1 °C/min. All measurements were carried out in triplicate with a new sample preparation for each measurement.

For comparison of functional changes of gluten proteins during the temperature sweep, inflection points of the loss factor tan(δ) (G'/G″) as a function of the temperature were identified. Therefore, the zero point of the second derivative after second order polynomial 20-point smoothing was calculated.

2.5. Crude protein content

The crude protein content of all vital gluten samples was determined by the Dumas combustion method according to ICC 167 with a Dumatherm N Pro (C. Gerhardt GmbH & Co. KG, Königswinter, Germany). To convert the nitrogen content, a protein conversion factor of N × 5.71 was used. The measurement was performed in triplicate.

2.6. Gluten protein composition

Protein fractionation according to Wieser et al. (1998) was performed with modifications. Gliadins were extracted in triplicates from 20 mg of vital gluten with 1.5 mL of 60% aqueous ethanol (v/v) and shaken for 2 min. The tubes were then stirred for 10 min and centrifuged for 25 min at 22 °C and 3550 rcf (Z446K, Heraeus, Hanau, Germany). The resulting supernatant was transferred to a 5 mL volumetric flask. The extraction was repeated once more and the supernatants were combined. The volumetric flasks were filled with 60% ethanol and filtered through a 0.45 μm membrane filter prior to HPLC analysis. To the remaining residue, 1.5 mL of 50% (v/v) propan-1-ol, 0.05 mol/L Tris–HCl (pH 7.5), 2 mol/L (w/v) urea and 1% (w/v) dithiothreitol (DTT) were added, treated with argon and mixed for 2 min. After stirring for 30 min in a water bath at 60 °C, the samples were centrifuged for 25 min at 22 °C and 3550 rcf. The supernatant was transferred to a 5 mL volumetric flask. The extraction was repeated once more, the supernatants were combined and the flask was filled with glutenin extraction solution. The solution was filtered through a 0.45 μm membrane filter prior to HPLC analysis (Schopf and Scherf, 2021).

For HPLC analysis, a Prominence UFLC-DAD HPLC system (Shimadzu Corp., Kyōto, Japan) was used with a YMC Triart C_{18} (3 μm, 2.1 × 150 mm) column at 60 °C, a flow rate of 0.5 mL/min and UV absorption at 210 nm. The eluents were A: 0.1% trifluoroacetic acid (TFA) in water and B: 0.1% TFA in acetonitrile at a gradient of 0 min: 5% B, 0.4 min: 5% B, 0.5 min: 30% B, 16 min: 60% B, 16.1 min: 100% B, 22.1 min: 100% B, 22.2 min: 5% B, 35 min: 5% B. For gliadins 20 μL of the extract and for glutenins 15 μL of the extract were injected. Prolamin Working Group (PWG)-gliadin with a protein content of 93.1% and a concentration of 2.5 mg/mL was used for quantification (van Eckert et al., 2006). For calibration, 5 μL, 10 μL, 15 μL and 20 μL of 2.5 mg/mL (60% EtOH, v/v) of PWG-gliadin was injected. Integration was performed with the software Lab Solutions 5.93 according to Schalk et al. (2017).

2.7. Content of SDSS proteins and GMP

The content of SDS-soluble (SDSS) proteins and glutenin macropolymer (GMP) was determined according to the method of Thanhaeuser et al. (2014). PWG-gliadin with a concentration of 1 mg/mL was used for quantification (van Eckert et al., 2006). A mix of albumin (molecular weight = 66 kDa), carbonic anhydrase (molecular weight = 29 kDa) and cytochrome C (molecular weight = 12.4 kDa) in water was used as a molecular weight marker.

For the determination of SDSS, 20 mg of vital gluten was extracted twice with 1.0 mL of 1% (w/v) SDS and 0.05 mol/L NaH_2PO_4 (pH 6.9) in triplicate. The tubes were mixed on the multi-tube vortexer for 2 min. The samples were then mixed for 25 min at 1500 rpm in a thermoshaker at 22 °C and centrifuged for 30 min at 22 °C and 21,300 rcf (Z216MK, Heraeus, Hanau, Germany). The supernatant was collected in 5 mL volumetric flasks, made up with SDS extraction solution and filtered through a 0.45 μm membrane filter prior to HPLC analysis. The residue of the SDS extraction was extracted twice with GMP extraction solution consisting of 50% (v/v) propan-1-ol, 0.05 mol/L Na_2HPO_4/KH_2PO_4 (pH 7.5) and 1% (w/v) DTT. The samples were treated with argon and mixed for 2 min. After stirring for 30 min at 60 °C and 1500 rpm in a thermoshaker, the samples were centrifuged for 30 min at 22 °C and 21,300 rcf. The supernatant was transferred to 5 mL volumetric flasks, filled with GMP extraction solution and filtered through a 0.45 μm membrane filter prior to HPLC analysis (Schopf et al., 2021).

For HPLC analysis, a Nexera LC-40 UHPLC system (Shimadzu Corp., Kyōto, Japan) was used with a BioResolve SEC mAb (20 nm, 2.5 μm, 4.6 × 150 mm, molecular weight range 10–450 kDa) at 30 °C, a flow rate of 0.3 mL/min and UV absorption at 210 nm. The eluents used were A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile (1 + 1) with isocratic elution. For SDSS extracts 5 μL were injected and for GMP extracts 15 μL. For calibration, 5 μL, 10 μL, 15 μL and 20 μL of 1 mg/mL (60% EtOH, v/v) PWG-gliadin was injected. Integration was performed with the software Lab Solutions 5.106.

2.8. Content of free and bound thiols

The determination of free and bound thiol groups was done according to Schopf and Scherf (2021). Vital gluten (10 mg) was mixed with 900 μL of buffer consisting of 0.05 mol/L Na_2HPO_4/NaH_2PO_4 (pH 6.5), 2% (w/v) SDS, 3 mol/L urea and 0.001 mol/L EDTA and incubated for 60 min in a thermal shaker at 22 °C and 500 rpm. Subsequently, 0.1% (w/v) 5,5′-dithiobis-2-nitrobenzoic acid solution (DTNB) was added to the samples and shaken for 45 min at 22 °C and 500 rpm. After centrifugation at 22 °C and 11000 rcf for 5 min, 200 μL of the supernatant was pipetted into a 96-well plate and the absorbance was measured at 412 nm at 22 °C using a photometer (Infinite M Nano + Multiplate Reader, Tecan AG, Männedorf, Switzerland). For calibration, a dilution series of glutathione standard (GSH) with concentrations between 10 μmol/L and 209 μmol/L was prepared. The standards were treated according to the sample preparation. All measurements were performed in triplicate.

To determine the bound thiols, 200 μL of 2.5% $NaBH_4$ solution was added to 2 mg of vital gluten for reduction. The samples were incubated for 60 min at 50 °C in a thermal shaker and then mixed with 100 μL of 1 mol/L HCl. The further steps were carried out according to the sample preparation for the determination of free thiols. Oxidized glutathione (GSSG) in the range of 3.8 μmol/L to 152.09 μmol/L was used for

calibration. The rest of the work-up was carried out according to the sample work-up. The content of bound thiols was determined by subtracting the content of free thiols from that of total thiols.

2.9. Statistics

IBM SPSS Statistics 27 (International Business Machines Corporation, Armonk, NY, USA) was used for statistics. Significant differences between control samples and gluten samples with additive were determined by analysis of variance (ANOVA) with two-sided Dunnett's *t*-test ($p \leq 0.05$). Significant differences to identify the influence of drying temperature or concentration of a certain salt were determined by ANOVA with Tukey's test ($p \leq 0.05$). Identification of inflection points of the loss factor tan(δ) of the temperature sweep was done with Origin 2023 (OriginLab Corporation, Northampton, MA).

3. Results & discussion

3.1. Effect of salts on uniaxial extension rheology of wet gluten

The resistance to extension ranged from 0.91 N to 1.47 N considering all 9 wet gluten samples (Fig. 1). The addition of high concentrations of NaCl and KCl to wet gluten resulted in a non-significant increase of the resistance to extension. Divalent salts and the low dose of KCl significantly decreased the resistance to extension. Monovalent and divalent salts each showed similar values for resistance to extension. The extensibility varied from 84.3 mm to 123.3 mm and was lowest for the control with no salt added and highest for samples with a high concentration of NaCl and KCl (Fig. 1). All salts increased the extensibility significantly compared to the control. Overall, the addition of a high dose of salt led to greater deviations from the control than the addition of low salt doses. Higher salt levels are therefore likely to have a greater effect on protein-protein interactions in wet gluten.

Similar to our findings, a study by Tuhumury et al. with salt-washed gluten showed that all salts resulted in a decrease of the resistance to extension compared to the sample without salt. The extensibility in the study of Tuhumury et al. was also decreased by the addition of different salts. This finding is not consistent with our results where salts led to an increase of extensibility. Tuhumury et al. attributed the different effects on the resistance to extension and extensibility caused by the salts to subtle microstructural differences within the gluten network. Using confocal laser scanning microscopy (CLSM) images, they showed that monovalent salts led to a fibrous structure of the gluten network. It was hypothesized that this structure is created by hydrogen bonds between glutenin molecules formed during hydration and mixing of dough. The presence of hydrogen bonds and the associated fibrous structure may determine the resistance to extension during large deformation measurements (Tuhumury et al., 2016a).

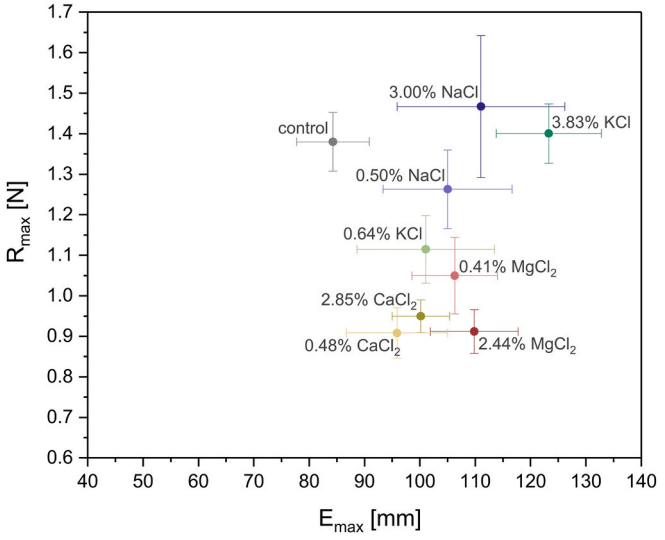

Fig. 1. Resistance to extension (R_{max}) and extensibility (E_{max}) determined with the microscale extension test in salt-treated wet gluten samples and the control sample without salt. All values are given as means ± standard deviation (n = 12).

This is consistent with Belton's train and loop model (Belton, 1999). The model explains the functionality of gluten proteins as interactions of unbound mobile regions (loops) and bonded regions (trains) on a molecular level. Loops are formed by hydrogen bonds between protein and solvent whereas trains consist of protein-protein interactions. Train regions, namely hydrogen bonds between high-molecular-weight glutenin subunits (HMW-GS), are responsible for elasticity. The resistance to extension depends on the ratio of loops and trains, since the deformation of loop regions requires less energy than the breaking of trains (Belton, 1999). As is typical for a kosmotropic salt, NaCl in gluten strengthens inter-protein hydrogen bonds and hydrophobic interactions, leading to more trains (Tuhumury et al., 2014). Due to the higher deformation energy required, more trains imply a higher resistance to extension (Belton, 1999). In contrast, chaotropic salts form more loop regions through distinct solvent-protein interactions and show less resistance to extension. In CLSM, chaotropic divalent salts therefore show a honeycomb structure with less fibrous protein structures (Tuhumury et al., 2016a). The results of our study are in line with the literature as the kosmotropic salts KCl and NaCl showed a higher resistance to extension compared to the chaotropic salts $MgCl_2$ and $CaCl_2$. This indicates more train regions and inter-protein hydrogen bonds in gluten treated with KCl and NaCl and more loop regions in gluten with $MgCl_2$ and $CaCl_2$.

Apparently, salts not only influence the formation of hydrogen bonds during hydration and mixing of the initial gluten network. Our study showed that the incorporation of salt into a fully developed gluten network combined with mechanical energy input still significantly alters the functionality of wet gluten. This may originate from the formation of new hydrogen bonds between glutenin molecules during the after-treatment process of wet gluten.

3.2. Effects of salts on dynamic rheological properties of wet gluten

The linear viscoelastic region (LVR) describes the range of the shear strain in which the test can be performed without destroying the structure and it provides information about the functionality of the gluten network. The LVR of wet gluten varied from 7.06% to 11.88% (Table 1). It was lower in gluten with high concentrations of KCl and NaCl compared to the control without salt, which indicates a weakening of the gluten network with large deformations due to the addition of these salts. The LVR remained unchanged with the addition of low doses of KCl, NaCl and $CaCl_2$. Compared to the control, the LVR was increased for gluten samples with a low and high concentration of $MgCl_2$ and a high dose of $CaCl_2$.

The storage modulus G′ corresponds to the elastic deformation of the sample whereas the loss modulus G″ corresponds to the flow behaviour. For all salts, regardless of the type of cation or salt concentration, as well as for the control, G′ was always higher than G'' (Table 1), which is characteristic for cross-linked polymer networks with solid-like, viscoelastic behaviour (Ahmed et al., 2013). G′ and G″ of all samples with salt and the control were frequency-dependent, as G′ and G″ increased with increasing frequency. This shows that there is a relatively high degree of protein chain mobility within the gluten network (Singh and Singh, 2013).

For better comparability and statistical evaluation, G′ and G″ of each point measured at different frequencies were added up (Table 1). The sum of G′ ranged from 39,250 Pa to 76,980 Pa. The sum of G″ ranged from 21,995 Pa to 40,222 Pa. KCl and NaCl in the high salt dose showed similar G′ and G″, which were higher than the corresponding moduli of the control. The addition of high concentrations of $CaCl_2$ and $MgCl_2$

Table 1
Rheological characterisation with oscillatory amplitude and frequency sweep. Linear viscoelastic range (LVR), sum of storage modulus, loss modulus and tan δ of salt-treated wet gluten samples and the control sample without salt.

Sample	LVR	Standard deviation	Storage modulus G′	Standard deviation	Loss modulus G″	Standard deviation	Tan δ	Standard deviation
	[%]		[Pa]		[Pa]		[1]	
Control	8.85	0.80	71853	7256	33421	2670	9.88	0.19
0.64% KCl	9.10 [b]	0.77	62196 [a,b]	215	29965 [a,b]	452	10.10 [b]	0.08
3.83% KCl	7.45 [a,b]	0.17	74983 [b]	237	38653 [a,b]	849	10.73 [a,b]	0.15
0.50% NaCl	9.19 [b]	0.45	65423 [b]	3477	31650 [b]	1964	10.15 [b]	0.17
3.00% NaCl	7.06 [a,b]	0.70	76980 [b]	3090	40222 [a,b]	1923	10.85 [a,b]	0.10
0.41% $MgCl_2$	10.64 [a]	0.57	49956 [a]	2212	25922 [a]	856	10.70 [a,b]	0.12
2.44% $MgCl_2$	10.92 [a]	0.12	43244 [a]	1378	24690 [a]	970	11.74 [a,b]	0.14
0.48% $CaCl_2$	9.77 [b]	0.19	47703 [a]	907	23879 [a]	504	10.33 [a,b]	0.18
2.85% $CaCl_2$	11.88 [a,b]	1.05	39250 [a]	1888	21995 [a]	865	11.50 [a,b]	0.38

All values are given as means ± standard deviation (n = 3).
[a] significant difference to the respective control (ANOVA with Dunnett's *t*-test, $p \leq 0.05$, n = 3).
[b] significant difference between different concentrations of the same salt (ANOVA with Tukey's test, $p \leq 0.05$, n = 3).

showed similar G′ and G″ as well, which were lower than the G′ and G″ of the control sample. G′ of the gluten samples with low doses of salt added were all lower than the control and showed very similar curves and sums of G′ and G″ for the monovalent and divalent salts. Like in the present study, G′ and G″ were previously found to be higher for kosmotropic cations than for chaotropic cations in rheological measurements with gluten of flour that was treated with different salt solutions. This finding was attributed to different water contents of the gluten samples due to different influences of the cations on the interaction between protein and water (Tuhumury et al., 2016a). The monovalent salts may be more strongly hydrated by the available water molecules. This is assumed to lead to intensified interactions between glutenins. Therefore, the higher elasticity for monovalent salts, which was also observed in our study, may be due to an increase in hydrogen bonds between HMW-GS. For this reason, gluten with monovalent salts shows a more aligned structure (Tuhumury et al., 2016a). Another reason for the differences between the individual salts could be specific protein-ion interactions with charged head groups of gliadin and glutenin molecules. However, it cannot be concluded from our experiments whether ion-water or ion-protein interactions are more pronounced. In general, the specific ion effects are concentration-dependent and should be more dominant at the higher salt concentration (Kunz, 2010).

Under large deformations, the interchain hydrogen bonds can break, allowing protein strands to slide over each other (Belton, 1999). To a certain extent, this may also be the reason for the increased flow behaviour in samples with NaCl and KCl compared to the control in small deformation oscillatory tests. The aggregated, honeycomb structure caused by divalent salts leads to higher friction within the gluten network. This means that the protein network is less resistant to flow, resulting in less viscous behaviour and a lower G'' (Tuhumury et al., 2016a).

All samples had in common that G″ increased with increasing frequency, indicating an increasingly viscous behaviour of the samples at higher frequencies. G′ was always higher than G″, but as G″ rose more strongly than G′, tan δ increased progressively. However, since tan δ remained <1 over the entire frequency range, the gluten samples still exhibited elastic-like behaviour even at high frequencies. Materials only begin to flow at a loss factor >1. The gluten samples thus showed a solid-like, viscoelastic behaviour which is typical for gluten networks (Tuhumury et al., 2014). The increase of G′ and G″ with increasing frequency was accompanied by a proportionally increasing loss factor tan δ for all salts and the control. Since tan δ represents the ratio of the two moduli, tan δ differed only slightly between the different samples.

In order to mimic the rheological behaviour and thus the functionality of gluten samples during a heating process like baking or extrusion, temperature sweeps were performed (Stathopoulos et al., 2008). Temperature sweeps operate at consistent deformation and frequency while the temperature is continuously increased from 25 to 95 °C. All changes in the gluten network can thus be attributed to the influence of temperature and salt. Temperature sweeps were only carried out with wet gluten with the high dose of salt added, as a stronger deviation from the control and therefore a bigger influence on the gluten network was observed in the frequency sweep.

G' of all samples with salt and the control was significantly higher than G″, which indicates an elastic behaviour of the samples (Fig. 2) (Ahmed et al., 2013). All samples with salt showed a higher G′ than the control. G′ increased in all samples from approximately 60 °C, G″ increased strongly from approximately 55 °C. For KCl and NaCl, G′ and G″ were very similar over the course of the temperature sweep. For $MgCl_2$ and $CaCl_2$, G′ was similar, but G″ was higher for $MgCl_2$ and showed especially higher values from 60 °C. Tan δ decreased rapidly for $MgCl_2$ and $CaCl_2$ from the beginning. In contrast, tan δ decreased only slowly for NaCl and KCl and even exhibited an upward trend for the control up to 55 °C, before decreasing similarly to the temperature sweep curves of the divalent salts. Tan δ remained <1 for all samples over the entire temperature ramp and decreased with increasing temperature. Tan δ was almost always higher for the salt samples than for the control, thus samples with salt exhibited a more viscous behaviour. For KCl and NaCl, tan δ was almost identical, for $MgCl_2$ and $CaCl_2$, tan δ had a similar shape, but was lower for $CaCl_2$.

The temperature sweep curves followed the characteristic course of the curve of heated gluten, whereby the shape of the curve changed due to the interaction of salts with gluten proteins. When gluten is heated, ionic interactions and hydrogen bonds are initially destroyed (Wang et al., 2017). Therefore, G′ decreases at the beginning of the temperature sweep (Singh and Singh, 2013). The influence of temperature leads to conformational changes within the protein network: hydrophobic amino acids and free thiols, which are usually buried inside the gluten structure, are exposed and surface hydrophobicity increases (Wang et al., 2017; Weegels et al., 1994). With rising temperature, proteins tend to unfold and a compact gluten structure is created by the formation of gluten aggregates and cross-links between proteins. The reorganization of proteins and the growing gluten network are responsible for the increase in G′ and G″ occurring at 60–90 °C (Schofield et al., 1983; Wang et al., 2017).

Inflection points of the temperature sweep curve indicate a change in viscosity or elasticity and can thus provide information about structural changes in the polymer network such as protein denaturation (Wehrli et al., 2021). Four to six inflection points were determined for all gluten samples over the entire temperature sweep. Gluten samples with all salts except for the high dose of $CaCl_2$ showed a first inflection point at 29.3–29.6 °C, which was lower than the inflection point of the control at 30.2 °C. Other common inflection points for all samples were 61.8–68.3 °C, 70.6–75.1 °C and 82.0–86.2 °C. The monovalent salts exhibited very similar, but slightly reduced inflection points compared to the control. $MgCl_2$ and $CaCl_2$ showed similar inflection points as well.

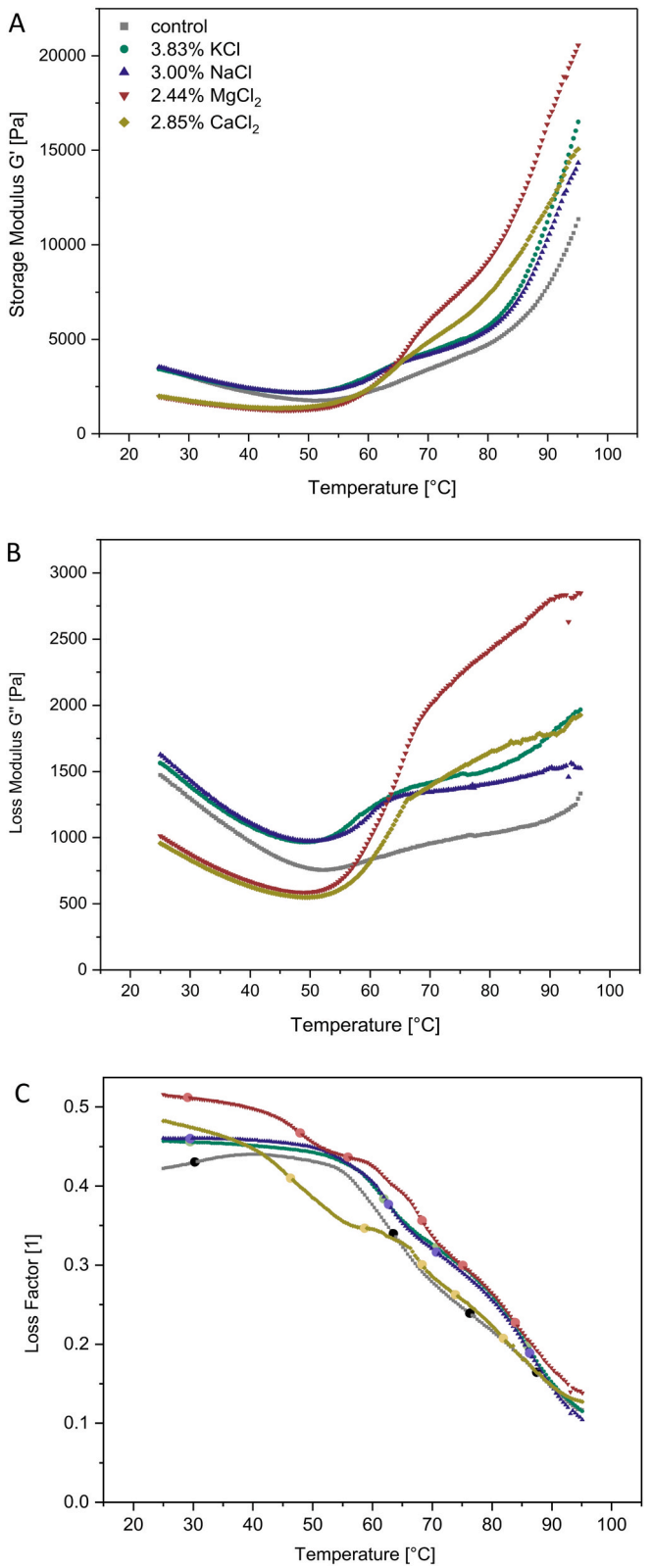

Fig. 2. Rheological characterisation with oscillatory temperature sweep. Curves of elastic modulus G' (A), loss modulus G'' (B) and loss factor tan δ (C) with inflection points of salt-treated wet gluten samples and the control sample without salt. All values are given as means (n = 3).

In gluten with the high $CaCl_2$ concentration, no inflection point occurred around 30 °C. The inflection points at 68.3 °C and 68.2 °C in gluten with high doses of $CaCl_2$ and $MgCl_2$, were higher than the comparable inflection point of the control. All other inflection points of gluten with high concentrations of $CaCl_2$ and $MgCl_2$ were lower than the respective inflection point of the control. Both divalent salts showed two additional inflection points at 47.9 °C and 46.4 °C and at 55.9 °C and 58.6 °C, respectively, which were not detected in the other samples.

The different curve characteristics could be attributed to the interaction of salt and protein and the reorganization and assembly of the protein network. The similar temperature sweep curves and inflection points for each monovalent and divalent salts indicate that the salts interact with gluten proteins in a similar way. This could be due to the kosmotropic and chaotropic nature of the salts, as these properties affect the availability of free water in hydrated gluten (Tuhumury et al., 2016a). It has been found that an increase in water content leads to a decrease in the denaturation temperature for glutenins (León et al., 2003).

3.3. Effect of salts on gluten protein composition

The crude protein content determined by Dumas varied between 71.4% and 82.5% for all samples (Table S2). To obtain information about the gluten protein composition of vital gluten samples, gliadins including the gluten protein types ω5-, ωb-, ω1,2-, α- and γ-gliadins, as well as LMW-GS and HMW-GS were determined by Osborne fractionation and RP-HPLC. The content of gliadins ranged from 419.4 mg/g to 514.6 mg/g. The content of glutenins ranged from 257.6 mg/g to 396.8 mg/g and was higher for samples, which were dried at 80 °C (Fig. 3, Table S3). All salt-treated samples contained more ω5-, ωb-, ω1,2-, α- and γ-gliadins than the respective control. Especially divalent salts led to an increase of gliadin protein types regardless of the salt concentration. The increase of gliadins for salt-treated samples compared to the control is in line with the increase in extensibility. However, divalent salts did not show the highest extensibility, but monovalent salts NaCl and KCl. At the same time, all salt-treated samples contained less LMW-GS and HMW-GS than the respective control. The decrease in glutenin content of all salt samples compared to the control is consistent with the decrease in resistance to extension since glutenins are associated with gluten strength. In samples dried at 80 °C, less gliadins, but more glutenins were extracted than in samples dried at 40 °C. This indicates that gliadins had been incorporated into the gluten network. Cross-linking between gliadins and glutenins only occurs at higher temperatures due to conformational changes within the gluten network (Lagrain et al., 2008; Schofield et al., 1983; Singh and Singh, 2013). Cross-linking between gluten proteins was observed during the temperature sweep in the present study. With rising temperature, the loss factor decreased, indicating a more viscoelastic network. In the literature, inflection points in the temperature sweep curves were used to demonstrate the influence of gliadins on the viscoelastic behaviour of the gluten network. It was shown that gluten with less gliadin has inflection points at lower temperatures since gliadin is more temperature-stable than glutenin (Schofield et al., 1983; Wehrli et al., 2021). In the present study, however, the order of inflection points did not match the amount of gliadin detected in the samples by Osborne fractionation.

In the literature, there are no consistent results on gluten protein composition after salt treatment. Fu et al. also found more gliadin in NaCl-treated dough and the resulting gluten, but less insoluble protein (Fu et al., 1996). In contrast, Chen et al. found less extractable gliadin but more glutenin when NaCl was incorporated into dough before washing the gluten (Chen et al., 2018). These opposite findings might be related to the protein composition of the different flours used. Moreover, the existing literature focuses on the incorporation of salt into dough or salt-washed gluten. In this experimental setup it has to be considered that starch has a major influence on the interaction of protein and salt (Chen et al., 2018). In our study, however, salt was added to pre-washed

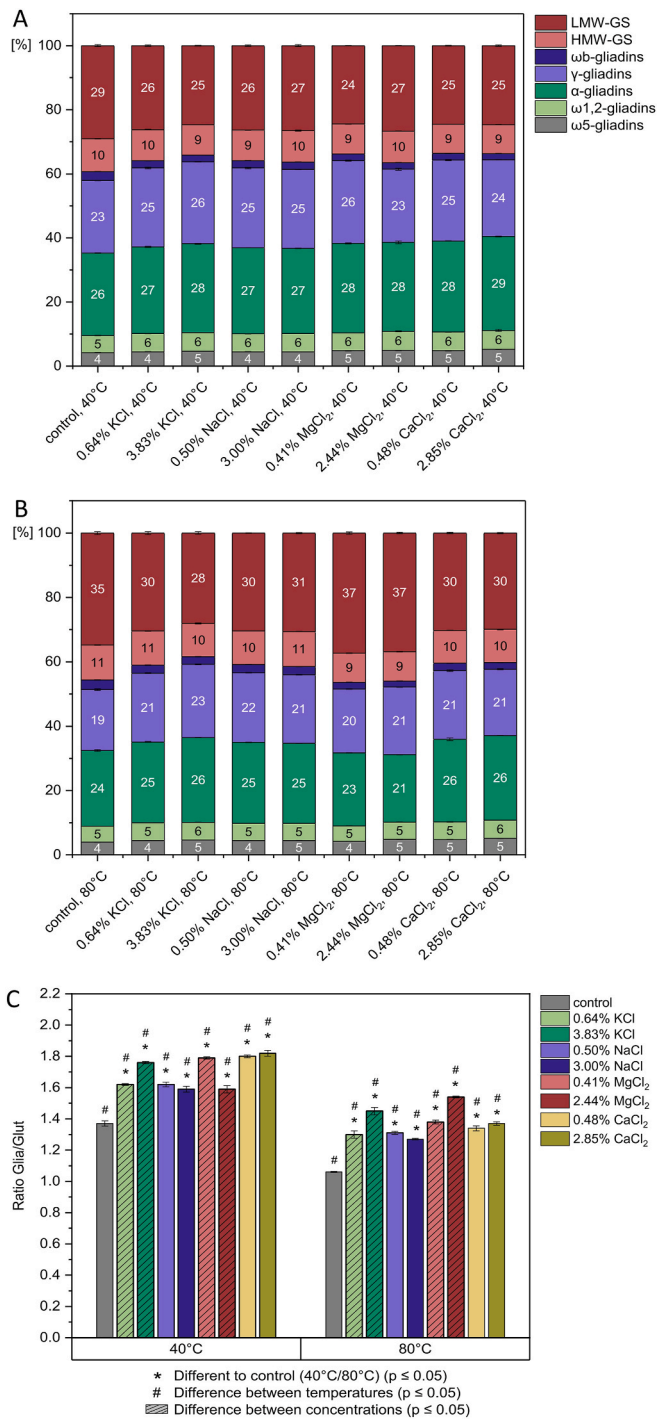

Fig. 3. Proportions of low-molecular-weight glutenin subunits (LMW-GS), high-molecular-weight glutenin subunits (HMW-GS), ω5-gliadins, ωb-gliadins, ω1,2-gliadins, α-gliadins and γ-gliadins (A,B) and gliadin-to-glutenin (glia/glut) ratio (C), in salt-treated samples and control samples without salt at different drying temperatures 40 °C (A) and 80 °C (B). All values are given as means ± standard deviation (n = 3). In part (C), asterisks show a significant difference to the respective control (ANOVA with Dunnett's *t*-test, $p \leq 0.05$, n = 3). Hashtags show a significant difference between the drying temperatures 40 °C and 80 °C for the same sample type (ANOVA with Tukey's test, $p \leq 0.05$, n = 3). Hatched bars show a significant difference between different concentrations of the same salt within each drying temperature, respectively (ANOVA with Tukey's test, $p \leq 0.05$, n = 3).

gluten, which is why starch interactions play a minor role.

The difference between the two sample sets with different drying temperature becomes more evident when looking at the gliadin/glutenin (glia/glut) ratio which ranged from 1.37 to 1.82 for gluten samples dried at 40 °C and from 1.06 to 1.54 for gluten samples dried at 80 °C (Fig. 3, Table S3). It was therefore lower for the samples that were dried at higher temperature. All samples treated with salt had a significantly higher glia/glut ratio than the control sample. The glia/glut ratio was also higher for all samples dried at 40 °C than the comparable sample dried at 80 °C. All salts increased the glia/glut ratio to a different extent, regardless of the concentration and the drying temperature. When comparing the relative increase or decrease of extractable gliadin or glutenin protein types compared to the respective control, it becomes clear that this was due to an increase of extractable gliadin and a decrease of glutenin in salt-treated samples. At the low salt dose, a trend regarding the valence of the added salts was observed, regardless of the drying temperature. Vital gluten with the addition of the monovalent salts KCl and NaCl had the same or almost the same glia/glut ratio at 40 °C and at 80 °C. The samples with the divalent salts $MgCl_2$ and $CaCl_2$ also showed almost identical glia/glut ratios at 40 °C and 80 °C. Besides the valence of the salts, this effect could also be attributed to the kosmotropic or chaotropic properties of the salts. This trend was no longer observed with the high salt concentration, as the glia/glut ratios varied significantly between the salts, depending on the drying temperature. The sample with $CaCl_2$ showed the highest glia/glut ratio of 1.82 at the high concentration and 40 °C drying temperature, while the ratio for $MgCl_2$ was only 1.67. This observation could be due to a synergistic effect between cation, concentration and drying temperature. KCl and $MgCl_2$ showed a significant difference of the glia/glut ratio between samples with low and high salt dose at both drying temperatures.

It is therefore likely that the kosmotropic or chaotropic properties of the salts only take effect at the higher salt concentration. Salts in low concentration ranges of <0.3 mol/L, represented by the low salt dose, have a very similar effect on protein aggregation, regardless of the type of salt. In this case, salts shield the electrostatic forces of the proteins, as ions neutralize charges of amino acids, and thus enable increased protein interactions (Fu et al., 1996; Kinsella and Hale, 1984; Preston, 1981; Ukai et al., 2008). The characteristic kosmotropic or chaotropic effect of the ions on the protein only emerges at concentrations above 0.5 mol/L, resulting in delayed or more rapid protein unfolding. It is assumed that electrostatic forces are negligible at higher salt concentrations and that hydrophobic interactions and hydrogen bonds play a role in protein aggregation instead (Kinsella and Hale, 1984; Preston, 1981).

3.4. *Effect of salts on SDSS and GMP*

Differences between the samples were seen in the SDS-soluble proteins (SDSS) and were more pronounced for the glutenin macropolymer (GMP). The absolute SDSS content ranged from 519.6 mg/g to 645.9 mg/g and that of GMP from 105.9 mg/g to 320.4 mg/g (Fig. 4, Table S4). The extractability of SDSS was mainly reduced by the addition of the high salt concentration and the drying temperature of 80 °C. The GMP content was higher in samples dried at 80 °C than at 40 °C. It was highest in the 80 °C control sample. For all samples with salt dried at 80 °C, significantly less GMP could be extracted than for the control. For some salt-treated samples dried at 40 °C, there was an increase in GMP compared to the control, but it was only significant for HMW-GMP in the sample with the high NaCl dose. For the high concentration of $CaCl_2$ significantly less LMW-GMP and HMW-GMP could be extracted than for the control at 40 °C. Rombouts et al. found that the amount of SDSS in hydrated vital gluten did not decrease when heated at 50 °C for 120 min. At 90 °C, the extractability declined by 50% and after the first 30 min of heating, the extractability stabilized at a level. The temperature treatment of gluten up to 110 °C did not affect protein extractability under reducing conditions. This indicates that gluten protein polymerisation in this temperature range is mainly due to disulphide bonding which is

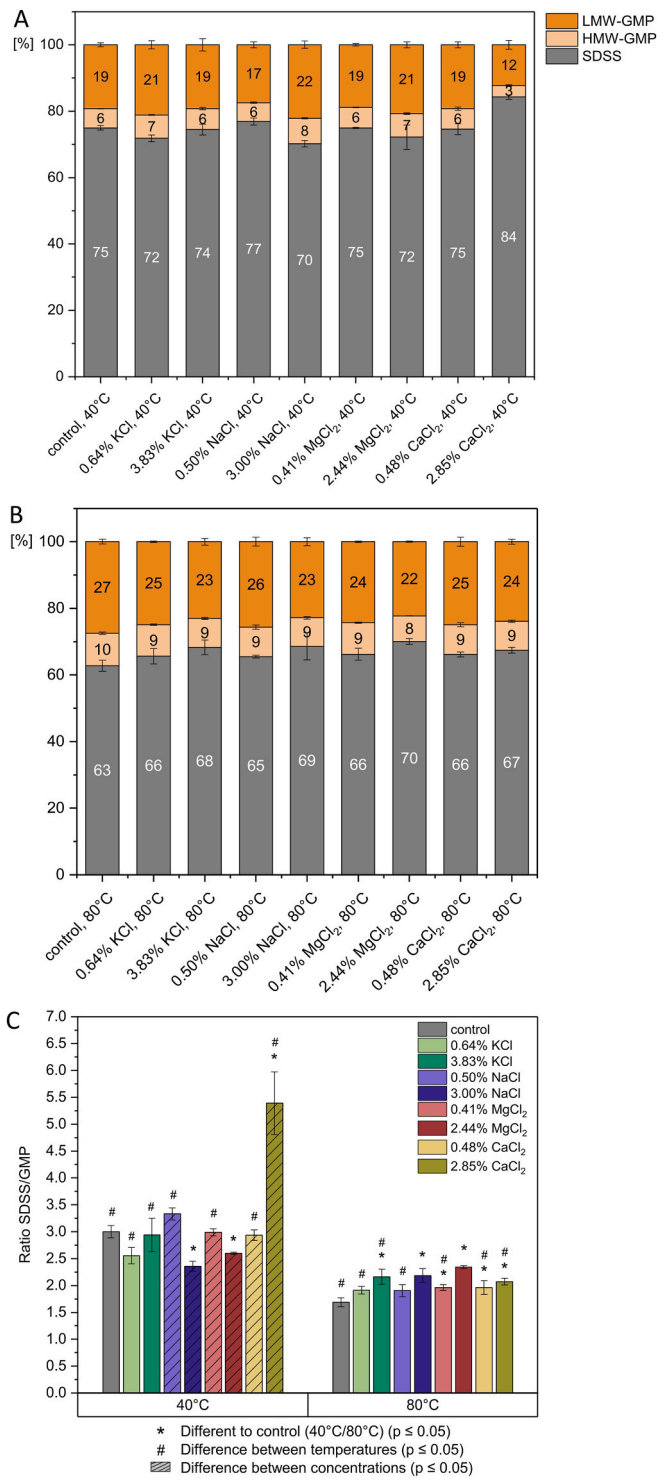

Fig. 4. Proportions of low-molecular-weight glutenin macropolymer (LMW-GMP), high-molecular-weight glutenin macropolymer (HMW-GMP) (A,B) and ratio of SDS-soluble proteins and glutenin macropolymer (SDSS/GMP) (C) in salt-treated samples and control samples without salt at different drying temperatures 40 °C (A) and 80 °C (B). All values are given as means ± standard deviation (n = 3). In part (C), asterisks show a significant difference to the respective control (ANOVA with Dunnett's *t*-test, $p \leq 0.05$, n = 3). Hashtags show a significant difference between the drying temperatures 40 °C and 80 °C for the same sample type (ANOVA with Tukey's test, $p \leq 0.05$, n = 3). Hatched bars show a significant difference between different concentrations of the same salt within each drying temperature, respectively (ANOVA with Tukey's test, $p \leq 0.05$, n = 3).

reversible by reducing agents. The extractability only decreased from 130 °C on when covalent non-SS cross-links get incorporated into the gluten network (Rombouts et al., 2012). This suggests that in our study, first, most of the molecular changes within the gluten network took place within the first hour of the 4-h long heating process. Second, if SDSS extractability is not affected by heating temperatures under 50 °C, any differences in extractability of SDSS of samples dried at 40 °C are due to the addition and individual effects of salts and salt may lead to the formation of covalent non-SDS-extractable bonding in gluten. Third, if the protein extractability under reducing conditions is fully reversible at process temperatures under 130 °C, all differences in GMP content are based on the addition and individual effects of salts and salt may lead to the formation of non-reducible, non-SS cross-links. This hypothesis is supported by the observation that SDSS was reduced mostly by the high dose of salt at 40 °C and 80 °C drying temperature, but at the same time GMP extractability was not increased most in these samples (Table S4).

The individual effect of salt treatment and drying temperature becomes clearer and easily comparable when looking at the ratio of SDSS and GMP. The SDSS/GMP ratio ranged from 1.69 to 5.39 (Fig. 4, Table S4). At 40 °C, only the samples with the high dose of NaCl, $MgCl_2$ and $CaCl_2$ were significantly different to the control, but all other samples were significantly different to the comparable sample at 80 °C. The vital gluten sample with the high $CaCl_2$ concentration showed the highest SDSS/GMP ratio of 5.39 among all samples. Additionally, the salts NaCl, $MgCl_2$ and $CaCl_2$ showed a significant difference between the concentrations at 40 °C. At 80 °C, all samples with the addition of a high dose of salt showed a significantly higher SDSS/GMP ratio than the control and a higher SDSS/GMP ratio than the corresponding sample with a low amount of salt added. Schopf et al. showed that commercial vital gluten samples with a rather low SDSS/GMP ratio of 4.8 as well as vital gluten with a high SDSS/GMP ratio of 21.6 exist on the market (Schopf et al., 2021). No tendency according to the Hofmeister series could be identified regarding the influence of the salts on the SDSS/GMP ratio at 40 °C and 80 °C. However, if the Hofmeister series is used to classify ions regarding their effect in gluten proteins, it must be noted that the Hofmeister series was originally established for the effect of salts on a mixture of egg white and water (Kunz et al., 2004). Since gluten is a non-water-soluble protein, it is questionable whether the Hofmeister series can be used to assess the systematic effect of ions in the gluten matrix. Furthermore, the original Hofmeister series does not take into account the individual effect of ions, but only the synergistic effect of salts. Depending on the biological system in which the effect of salts is observed, the order of the ions according to their influence on the proteins can therefore deviate from the original Hofmeister series (Kunz, 2010). In addition, it is not yet clear to what extent individual cations influence proteins through direct interaction with the protein backbone, with amides and charged side groups (Okur et al., 2013).

3.5. *Effect of salts on free and bound thiol groups*

The different treatments of wet gluten had individual effects on the SH content in vital gluten, which ranged from 0.57 μmol SH/g protein to 1.63 μmol SH/g protein (Fig. 5, Table S5). Increased drying temperature reduced the absolute content of SH. All salts led to a reduction of the SH content compared to the control, regardless of the salt concentration. The divalent salts $MgCl_2$ and $CaCl_2$ decreased the SH content the most at both drying temperatures. The SS content ranged from 17.88 μmol SS/g protein to 30.63 μmol SS/g protein and was not significantly increased by the addition of salts compared to the control (Fig. 5, Table S5).

Chen et al. also found a decrease in SH, but no increase in SS by adding NaCl to dough before gluten isolation (Chen et al., 2018). Tuhumury et al. found less free thiols in gluten from NaCl-treated dough than in untreated gluten from two different flour varieties and little or no increase in SS content (Tuhumury et al., 2014).

The SS content was higher for most samples dried at 80 °C compared to the respective sample dried at 40 °C. This is probably due to cysteine

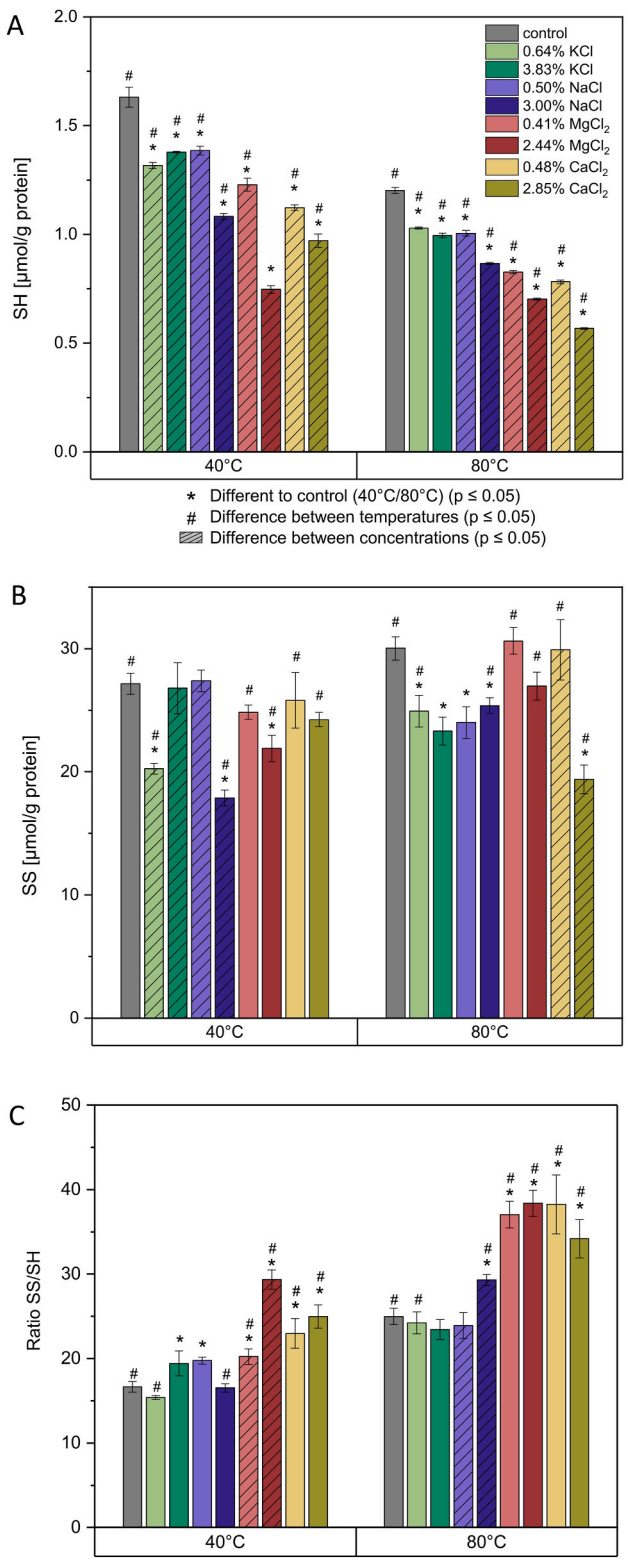

Fig. 5. Content of free (SH) (A) and bound (SS) (B) thiol groups and ratio of bound and free thiols (SS/SH) (C) in salt-treated samples and control samples without salt at different drying temperatures 40 °C (A) and 80 °C (B). All values are given as means ± standard deviation (n = 3). Asterisks show a significant difference to the respective control (ANOVA with Dunnett's *t*-test, $p \leq 0.05$, n = 3). Hashtags show a significant difference between the drying temperatures 40 °C and 80 °C for the same sample type (ANOVA with Tukey's test, $p \leq 0.05$, n = 3). Hatched bars show a significant difference between different concentrations of the same salt within each drying temperature, respectively (ANOVA with Tukey's test, $p \leq 0.05$, n = 3).

residues in LMW-GS and HMW-GS, which are particularly susceptible to embedding in aggregates through intra- and intermolecular disulphide bridges (Lagrain et al., 2008; Weegels et al., 1994). At temperatures above 75 °C, cysteine-containing α- and γ-gliadins, which are responsible for the viscous behaviour of gluten, polymerize or are incorporated into the polymeric structure of glutenins by inter-chain disulphide bonds (Lagrain et al., 2008; Schofield et al., 1983; Singh and Singh, 2013). This is the reason why tan δ decreases during the temperature sweep, indicating a more viscoelastic gluten network (Fig. 2). ω-gliadins do not contain free cysteine residues and are therefore not part of the aggregated network (Lagrain et al., 2008; Rombouts et al., 2012). As a result of the molecular processes described, the content of free thiols and hydrophobic regions on the gluten surface decreases (Weegels et al., 1994). In our study, all added salts led to a reduced SH content in the vital gluten samples, while no salt resulted in the formation of new SS bonds. This result shows that the decrease in SH content cannot be explained by SH/SS exchange reactions between cysteine containing proteins. This may also be the reason why no salt increased the resistance to extension in the microscale extension test. The analysis of SH and SS by Ellman assay can only provide information about the content of SH and SS in the samples, but not about the conformation of the thiols. The decrease in SH might be due to non–SS–crosslinking or hydrophobic interactions induced by salts, as suggested by Chen et al. (2018). Hydrophobic interactions and hydrogen bonding could also be the reason for increased tan δ values compared to the control in the frequency sweep in our study as suggested by Tuhumury et al. (2014). However, there was no trend between the content of free thiols or disulphides, salt type and drying temperature.

The ratio of SS and SH provides more detailed information about the protein composition of the vital gluten samples, as the absolute content of SS and SH does not reflect the proportional linkage of disulphides within the gluten network. The ratio of SS and SH varied between 15.4 and 38.4 for all samples (Fig. 5, Table S5). In particular, the divalent salts $MgCl_2$ and $CaCl_2$ showed a higher SS/SH ratio than the control for both drying temperatures and concentrations because the decrease in SH was especially high for these samples. In general, the SS/SH ratio was higher for samples with salts dried at 80 °C. Only NaCl and $MgCl_2$ showed a temperature dependent effect of salt concentration on the SS/SH ratio. A higher SS/SH ratio indicates that these samples contain relatively more SS than SH, suggesting a more cross-linked gluten network. However, since no new disulphide bonds were formed, other interactions than covalent SS-crosslinks must be responsible for the differences observed in rheological measurements and gluten protein composition.

4. Conclusion

The addition of salts into a fully developed gluten network influenced the gluten protein composition and the rheological behaviour. Although the salts mostly did not follow the order of the Hofmeister series, trends regarding the valence or the kosmotropic and chaotropic properties were observed. Both monovalent, kosmotropic cations K^+ and Na^+, as well as the divalent, chaotropic cations Mg^{2+} and Ca^{2+} showed similar behaviour in terms of gluten protein composition and functional properties. The salts showed distinct effects on SDSS and GMP, as well as the amount of free and bound thiol groups, suggesting a salt-induced modification of hydrophobic interactions, hydrogen bonds and non-covalent crosslinks.

The findings of our study indicate that the gluten protein composition and functionality of a fully developed gluten network can be influenced by the addition of salts. Therefore, a downstream after-treatment step of isolated gluten with common additives like salt would be easily feasible in order to alter the properties of vital gluten without having to intervene in the established, self-contained starch extraction process. The targeted production of vital gluten with defined properties opens up the possibility of expanding the diverse area of

application and of exploiting the maximum potential for gluten in the food and non-food industry.

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5. Compliance with ethics requirements

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

6. Informed consent

Not applicable.

CRediT authorship contribution statement

Nina Hoeller: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Katharina Anne Scherf:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Abbreviations

ANOVA	analysis of variance
G’	storage modulus
G″	loss modulus
GMP	glutenin macropolymer
HMW-GS	high-molecular-weight glutenin subunits
HMW-GMP	high-molecular-weight glutenin macropolymer
LMW-GS	low-molecular-weight glutenin subunits
LMW-GMP	low-molecular-weight glutenin macropolymer
LVR	linear viscoelastic region
PWG	Prolamin Working Group
SDSS	SDS-soluble protein
SH	free thiol groups
SS	bound thiol groups
tan δ	loss factor

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2024.103978.

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