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Short communication

Water electrolyte promoted oxidation of functional thiol groups

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ABSTRACT

The formation of disulfide bonds is of the utmost importance for a wide range of food products with gluten or globular proteins as functional agents. Here, the impact of mineral electrolyte composition of aqueous solutions on thiol oxidation kinetics was studied, using glutathione (GSH) and cysteine (CYS) as model systems. Interestingly, the oxidation rate of both compounds into their corresponding disulfides was significantly higher in common tap water than in ultrapure water. The systematic study of different electrolyte components showed that especially $CaCl_2$ improved the oxidation rate of GSH. However, this effect was not observed for CYS, which indicated a strong impact of the local chemical environment on thiol oxidation kinetics.

1. Introduction

Organosulfur compounds carrying functional thiol groups (RSH) or their oxidized disulfide bridge linked dimers (RSSR) play a crucial role in biochemistry and pharmaceutical, chemical and food industries (Chatterjee & Ranu, 2013). Thiol oxidation and thiol/disulfide interchange reactions in and between gluten proteins during wheat dough fermentation and bread baking impact dough processability and final bread quality (Lagrain, Thewissen, Brijs, & Delcour, 2008). Even small variations in thiol/disulfide ratios have a large impact on dough rheology and corresponding bread loaf volume (Jones, Phillips, & Hird, 1974).

Gel texture from globular proteins also depends on oxidation of functional thiols to inter- and intramolecular disulfides (Visschers & De Jongh, 2005). For instance, the functionality of whey protein gels was linked to the number of thiol groups, and larger disulfide cross-linked protein structures were correlated to increased gel hardness (Alting, Hamer, De Kruif, Paques, & Visschers, 2003). The importance of disulfide bonds has been demonstrated for gels from a wide range of food albumins and globulins including hen egg ovalbumin (Kitabatake, Hatta, & Doi, 1987). Furthermore, the conversion of wheat gluten proteins into bioplastics also relies on disulfide cross-linking. These sustainable low-cost alternatives to petroleum derived polymers offer good mechanical and rheological properties, superior biodegradability and low toxicity (Jansens et al., 2014; Lagrain, Goderis, Brijs, & Delcour, 2010). Thiol redox kinetics are also important for disulfide-cleavage-triggered drug delivery systems (Lee et al., 2013), and regeneration of certain self-healing polymer films through thiol/disulfide redox exchange reactions (Yoon et al., 2012).

Several studies have reported the influence of mineral salts on wheat flour dough rheology. While it is assumed that salts shield the charges of proteins, thereby reducing electrostatic repulsion and allowing proteins to associate and produce a stronger dough, this theory does not explain all results (Tuhumury, Small, & Day, 2014). For instance, effects depend on concentration as well as the type of salts. Remarkably, few if any studies investigated the possible impact of electrolytes on thiol oxidation kinetics. Increased insight into water electrolyte promoted thiol oxidation is of high societal relevance and can be expected to significantly influence industrial production streams.

This work studied the impact of the electrolyte composition on the oxidation activity of functional thiol groups for two organosulfur model compounds, glutathione (GSH) and cysteine (CYS). GSH is a tripeptide (γ-glutamylcysteinylglycine) which contains the amino acid CYS. Both compounds can be oxidized into their corresponding disulfides, glutathione disulfide and cystine, respectively. The oxidation of GSH and CYS was studied in the presence of an additional oxidant $KBrO_3$ under mild conditions (30 °C, pH 6.5) in ultrapure water, in tap water and in different electrolyte solution series to investigate the effect of the mineral electrolyte

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composition in tap water. $KBrO_3$ was a popular additive in the bread making industry. It oxidizes free thiol groups of low molecular weight peptides to disulfide compounds thereby enhancing gluten functionality (Joye, Lagrain, & Delcour, 2009).

2. Materials and methods

2.1. Materials

$Ca(NO_3)_2$ (99%), $Mg(NO_3)_2$ (99%), $NaNO_3$ (99%), $CaCl_2$ (99%), $CaSO_4$ (99%), $MgCl_2$ (99%), L-glutathione reduced, L-cysteine, DTNB, $KBrO_3$ and MOPS were purchased from Sigma–Aldrich (Steinheim, Germany); NaCl (99%) from VWR International (Leuven, Belgium) and $CaCO_3$ (99%) from Acros Organics (Geel, Belgium). Ultrapure water (MilliQ, minimum resistivity 18.2 MΩ) was used to prepare the mineral electrolyte solution series.

2.2. GSH and CYS model systems

First, MOPS buffers (25 mmol/L; pH 6.5) were prepared from ultrapure water, tap water and different mineral electrolyte solution series. MOPS buffer has negligible metal ion binding affinity, is typically used in biochemical and biological research and has an effective pH range of 6.5–8.0. Based on the mineral composition of tap water in Leuven, Belgium (Table 1), following electrolyte solution series were selected: a nitrate solution series [$Ca(NO_3)_2$; $Mg(NO_3)_2$; $NaNO_3$], a calcium solution series [$CaCl_2$; $CaSO_4$; $Ca(NO_3)_2$; $CaCO_3$] and a chloride solution series [$CaCl_2$; $MgCl_2$; NaCl]. Ions in tap water with concentrations lower than 0.1 mmol/L were not considered. Carbonate (CO_3^{2-}) was included in the experiments because its concentration in tap water can be expected to be in equilibrium with CO_2 in the air. Then, MOPS buffers containing 0.125 mmol/L GSH or CYS, and 0 or 0.055 mmol/L $KBrO_3$, were shaken and heated for 24 h at 30 °C. All experiments were performed in triplicate. The concentrations of monovalent and divalent cation salts were 8 mmol/L and 4 mmol/L in the reaction medium, respectively.

Table 1
Mineral ion composition in tap water in Leuven, Belgium (De Watergroep).

Ion	Average concentration (mmol/L)
Calcium (Ca^{2+})	3.08
Magnesium (Mg^{2+})	0.49
Sodium (Na^{+})	0.87
Chloride (Cl^{-})	1.44
Sulfate (SO_4^{2-})	0.51
Nitrate (NO_3^{-})	0.83

2.3. Free SH determination

The concentration of GSH or CYS in solution was determined by colorimetric analysis of the free thiol content after reaction with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB). 750 μL of a DTNB solution [0.26 mmol/L DTNB and 2.0 mmol/L EDTA in MOPS buffer (250 mmol/L; pH 8.0)] was added to 750 μL of the sample solution. The mixtures were shaken and extinction at 412 nm was determined exactly 8 min after adding the DTNB solution. Absorbance values were converted to the concentration of free thiol groups using a calibration curve with GSH. Control solutions containing either no DTNB or no sample were used to correct for background extinction of DTNB and sample (Lagrain, Brijs, Veraverbeke, & Delcour, 2005).

3. Results and discussion

3.1. Impact of tap water on GSH and CYS oxidation

Compared to the reference system prepared in ultrapure water, the use of tap water accelerated thiol oxidation kinetics of both GSH and CYS. In the reference system after 24 h, 32% and 75% of the initial GSH concentration remained unoxidized in presence and absence of $KBrO_3$, respectively. In tap water, GSH was fully oxidized after 24 h, either with or without $KBrO_3$ (Fig. 1A). In the presence of $KBrO_3$, CYS was already completely oxidized after 2 h in tap water, while this was only the case after 24 h in ultrapure water (Fig. 1B).

CYS was found more reactive towards oxidation than GSH. In ultrapure water with $KBrO_3$, CYS was fully oxidized after 24 h while 32% of GSH remained unoxidized. In tap water with $KBrO_3$, CYS and GSH oxidation were complete after 2 h and 24 h, respectively (Table S1).

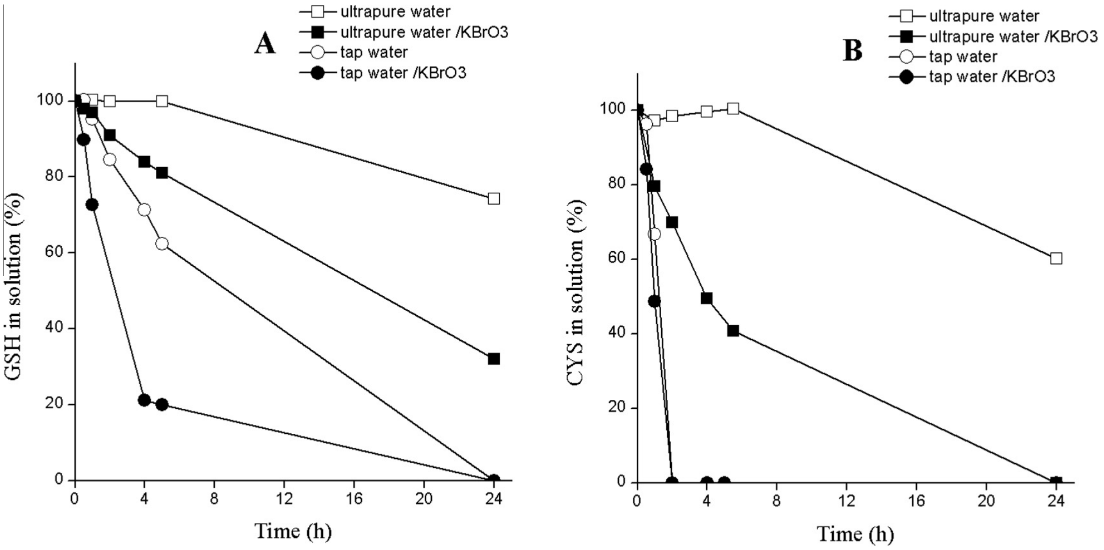

Fig. 1. The oxidation of GSH (A) and CYS (B) in ultrapure water, with and without $KBrO_3$.