How to impact gluten protein network formation during wheat flour dough making

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ABSTRACT

Gluten proteins strongly affect the structure and texture of various wheat flour-based baked goods. During dough making, gluten proteins are the main determinants of dough properties. Be it for research purposes or as a way of controlling dough properties in an industrial environment, different approaches have been taken to alter gluten network structure and, thus, functionality. In this brief review, we summarize these strategies, considering both processing-based interventions to gluten network formation and some additives commonly used to steer gluten protein functionality at the dough level.

KEYWORDS

Gluten protein network, glutenin, gliadin, redox agents, enzymes, processing

1. INTRODUCTION

Wheat is grown on more land area than any other food crop and is the second most-produced cereal after maize (FAOSTAT, 2016). The visco-elastic dough making capability of its flour resides primarily in gluten, wheat's main storage protein [1]. Since the discovery of wheat gluten was reported by Beccari in 1728 [2], research on its structure, functionality and how to control these aspects has been ongoing.

2. THE GLUTEN NETWORK

Gluten makes up 80-85% of the total wheat flour protein [3] and consists of monomeric gliadins and polymeric glutenin proteins [4]. Gluten characterization is difficult, as the term 'gluten' groups a complex mixture of homologous proteins that vary widely in molecular mass and charge [2]. Nevertheless, many researchers have taken on this challenging task, thereby applying an extensive range of analytical techniques [5-8]. The results of their efforts have been thoroughly summarized and reviewed elsewhere [1, 2, 4, 9-11].

Gluten proteins determine the visco-elastic properties of wheat flour dough [4] as they are able to form a network upon hydrating and mixing wheat flour [12]. Gluten proteins consist of polymeric glutenin proteins and monomeric gliadins. Differences in the structures of glutenin and gliadin proteins provide them with different functionalities during dough formation. It is generally accepted that glutenin proteins build up the polymeric protein network that provides cohesiveness and elasticity to dough [13], whereas gliadins act as plasticizers of the glutenin network and contribute to dough viscosity and extensibility [1].

Gliadin proteins are a heterogeneous mixture of proteins with molecular masses of 30 to 75 kDa [3, 10]. They are subdivided into α-, γ-and ω-gliadins. The α-and γ-gliadins contain cysteine residues, which are all involved in intramolecular disulfide (SS) bonds at ambient conditions [1]. ω-Gliadin lacks cysteine residues [3]. Gliadin's primary structure consists of a short N-terminal domain, a central repetitive domain containing mainly glutamine, proline and hydrophobic amino acids and a non-repetitive C-terminal domain, which contains most of the cysteine residues, if present [1]. The secondary structure of gliadins consists of predominantly β-turns in the N-terminal domain and α-helix and β-sheet structures in the C-terminal domain [4]. All gliadins are

assumed to in their native state be globular monomers [2]. **Glutenins** are the largest polymers found in nature. They have molecular masses ranging up to several tens of millions kDa. They consist of glutenin subunits (GS) linked together by intermolecular SS bonds [10]. In addition, GS also contain intramolecular SS bonds [1,3,4]. Typically, a distinction is made between high molecular mass (HMM-GS) and low molecular mass (LMM-GS) GS. As is the case for gliadin, the primary structure of GS consists of an N-terminal and C-terminal non-repetitive domain enclosing a repetitive central domain [13]. The hydrophilic central domain is rich in glutamine [10], whereas the hydrophobic N-and C-terminal domains contain most of the cysteine residues [1]. While the secondary structure of HMM-GS shows a predominance of aperiodic and α-helix conformations in both the N- and C-terminal domains [124], the central area shows a β-spiral structure [2]. Although relatively little is known about the structures of LMM-GS, the N-terminal domain is thought to contain mainly β-turns, whereas a-helices are predominant in the C-terminal domain [125].

Different types of reactions and interactions are crucial for the formation of a gluten network upon dough mixing. Intermolecular **disulfide (SS) bonds** between glutenin polymers are key in this respect. The oxidation of free thiol (SH) groups to SS bonds, which increases the molecular weight of the glutenin aggregates, as well as SH-SS exchange reactions are of utmost importance for building a three dimensional gluten network during mixing [4, 14]. SH-SS exchange reactions are initiated either by low molecular weight SH compounds or by free SH groups in glutenin proteins [15]. Other covalent bonds have also been suggested to be important for gluten network formation. Morel *et al.* (2002) found indications for the formation of **isopeptide bonds** during mixing. Nevertheless, they reasoned that their contribution to gluten network formation would be negligible [16]. Tiley *et al.* (2001) claim that **dityrosine linkages** are also formed during dough making. According to Hanft and Koehler (2005), it is unlikely for dityrosine to play an important role in in wheat gluten structure, since only very a low amount of dityrosine is present in dough. Peña *et al.* (2006) confirm that the amount of crosslinking between tyrosine residues appears to be small during bread making and of little importance compared to disulfide bond formation. The importance of non-covalent interactions has been established. The high levels of glutamine in gluten proteins allow for intermolecular as well as intramolecular **hydrogen bonds** [10]. Although

they are much weaker than covalent bonds, their large number and their ability to interchange under stress renders them main determinants of the gluten network's properties [122]. Indeed, the dough weakening effect of hydrogen bond breaking agents such as urea and the dough strengthening effect of heavy water when compared with that of ordinary water illustrates the importance of hydrogen bonds in the structure of the gluten network [4]. According to Sapirstein and Fu (2000) [123], as cited in [122], gliadin and glutenin from different flour samples differ in the number of interacting hydrogen bonds. Therefore, the specific surface area of glutenin determines the rate of interactions and, thus, the mixing time required for full dough development [123]. **Hydrophobic bonds** also contribute to gluten network structure [19]. They result from interactions of non-polar groups in the presence of water. Their functionality in the gluten network is likely similar to that of hydrogen bonds, although their overall contribution is thought to be smaller, as evidenced by the rheological effects of adding organic solvents to dough systems [122]. Finally, although gluten proteins have a low charge density due to the relatively low level of basic amino acids and the presence of the amide form of acidic amino acids [2], the importance of **ionic interactions** has nevertheless been established [20].

Over the years, several models have been proposed to describe the gluten network's structure, often focusing on explaining dough visco-elastic properties [3-9]. For an extensive overview of these models, the interested reader is referred to the review papers by Bock and Seetharaman (2012) [2]• and Ortolan and Steel (2017) [124]•. One of the models commonly accepted is the so-called 'loop-train model' proposed by Belton (1999) [47]. In this model, the HMM-GS are represented by long chains that are comprised of zones dominated by polymer-polymer interactions (i.e. 'trains') and zones dominated by polymer-solvent interactions (i.e. 'loops'), both of which are mainly hydrogen bonds. Hydration of the gluten network results in the formation of more hydrated loop regions. Belton associates the train regions with the formation of β-sheets and the formation of the loops with hydrated β-turn structures. Stretching of the gluten network would result (i) in deformation of the loop regions and (ii) in the trains being pulled apart. The elongation of the chains results in a loss of entropy. When the extension is removed and the polymers relax, the structure returns to the equilibrium of loops and trains.

3. IMPACTING GLUTEN NETWORK FORMATION

Multiple approaches have been taken to alter gluten network structure and, thus, functionality during wheat flour dough making. Gluten protein functionality strongly depends on the specific dough recipe, as water and other typical dough components such as salt significantly impact gluten network formation. The different mechanical unit operations during dough making such as mixing and sheeting determine the degree of gluten development. Furthermore, a range of improving agents are commonly used in the baking industry. They allow better control of production processes, improve product quality and/or increase shelf-life. Redox agents and enzymes are much used for altering gluten network formation.

In what follows, we briefly outline all strategies above. Where appropriate, the reader is referred to more in-depth review papers on specific approaches.

2.3.1 Influence of processing steps

Flour treatments

Oxidizing agents can be added to flour in order to accelerate its natural maturation, i.e. '**flour bleaching'**. One of the most commonly used oxidizing agents for bleaching is benzoyl peroxide [21]. This component is assumed to exhibit its decolorizing action without influencing flour baking properties [22]. Other peroxides, such as acetone peroxide, are also used [23]. Where allowed, chlorine gas can also be used for bleaching. The hypochlorite ion that is formed when chloride gas reacts with water in the flour is a strong oxidizing agent. Chlorinated flour has exceptional cake-making properties, as its use in cake systems tends to prohibit collapse after baking [24] and results in cakes with high volumes and uniform grain and good sensory properties [25]. Bosmans *et al.* (2019)•• recently showed that gluten proteins lose part of their network forming capabilities as a result of chlorination. Furthermore, chlorination also influences starch and lipid functionality [24, 25]. Azodicarbonamide is another oxidizing agent that is commonly added to flour as a bleaching agent, although, as is also the case for chlorine gas, its use is prohibited in the European Union. This oxidant rapidly oxidizes free SH groups of flour proteins. It is therefore used as a dough improving agent during dough mixing (*cfr. infra*) [25].

Flour may also be subjected to **heat treatment**. Like chlorinated flour application, heat treated flour use prevents collapse during baking of cake systems [26]. Although it is assumed that mainly starch properties are affected during heat treatment [27]•, it does affect gluten extensibility [28]. Van Steertegem *et al.* (2013) reported crosslinking of protein in flour particles as a result of flour heat treatment. They related this upfront polymerization to poor hydration and network formation during mixing. Nevertheless, the precise mechanisms by which heat treatment alters flour properties are still subject of debate.

Dough formulation

During dough mixing, flour particles are hydrated and sheared to such extent that they no longer exist as separate entities. As a result, gluten proteins form a continuous network. Dough mechanical behavior of course strongly depends on its **water** content [30].

Nowadays, the baking industry faces with the increasingly important consumer demand for **salt** reduction. Based on the National Diet and Food Survey in the UK, cereals and cereal products have been estimated to contribute 35% of total sodium consumption [112]. However, salt reduction may result in weaker gluten networks and impair dough handling characteristics [31]. In this context, Lynch *et al*. [113] reported that omission of salt leads to a significant reduction in dough and bread quality. However, reducing salt level from 1.2% to 0.3% did not significantly affect the rheological properties and bread-making performances of wheat dough. Salt likely shields charges on the gluten proteins, thus limiting electrostatic repulsion between gluten polymers and allowing them to aggregate [12, 114]. Wellner *et al.* [114] reported an increase in the level of intermolecular β-sheets in gluten proteins isolated from flour-water doughs containing small amounts of table salt, compared to those of control flour-water dough. The presence of these structures alone may increase molecular rigidity and, as a consequence, dough strength [115]. Increasing the level of table salt from 0.2 to 1.0 M did not induce any further changes in secondary protein structure [114]. According to McCann and Day [32], the presence of salt delays the formation of the gluten network. This has been attributed to a reduction of the rate of gluten hydration. Finally, salt is thought to, to some degree, inhibit proteolytic enzymes

[12]. Although table salt is typically used in wheat bread making, it is of note that different salts may induce different gluten protein conformations, at least at higher concentrations. Indeed, at low salt concentration (0.1 - 0.3 M), all ions have a similar effect on protein aggregation in dough systems, most likely by limiting electrostatic repulsion between polymers, as described above [116]. At higher salt concentrations (> 0.3 M) however, salt type and the different effect of chaotropic and kosmotropic anions on water structure dictate protein aggregation [117]. Indeed, at high salt concentrations, gluten protein extractability and aggregation depends on the anion type and follow the lyotropic anion (i.e. 'Hofmeister') series [116]. In this context, Wellner *et al.* [114] reported different effects of increasing concentrations of sodium bromide and sodium iodide on the equilibrium between β-turn and β-sheet structures than those observed for table salt. They ascribed these differences to the different chaotropic properties of the anions released.

Yeast and, in particular, its **metabolites** produced during fermentation also impact the gluten network. The effects on dough rheology of ethanol [33], succinic acid [34] and glycerol [35]•• which are produced during fermentation, and of glutathione, which is released by yeast after cell death [36]• (*cfr. infra*), have all been shown to be significant.

Although wheat gluten is the main (or only) network forming component in most traditional wheat bread recipes, other wheat flour based dough systems may be more complex. For example, pasta dough, cookie dough, donut dough and (pre)dough for multiple types of pastry products typically include **proteins from other sources** such as eggs and milk (powder). Some basic work on co-protein network formation between gluten proteins and some common (globular) food proteins was carried out by Lambrecht *et al.* (2017). They reported synergistic co-protein effects (*i.e.* increased heat-induced polymerization of proteins mixtures in comparison with the isolated proteins) in dispersions of isolated wheat gluten with S-ovalbumin, egg white, whole egg, defatted egg yolk, bovine serum albumin, wheat albumin and wheat globulin. Soy glycinin did not partake in co-protein network formation with wheat gluten, whereas hen egg lysozyme even resulted in antagonistic co-protein effects. They concluded that the level of (accessible) free SH-groups and the surface hydrophobicity of unfolded globular proteins are the main determinants of co-protein network formation with isolated gluten during heating in water.

In the context of complex food systems, co-protein network formation has been studied to some extent in e.g. pound cake and noodles [for a recent review on the subject, the reader is referred to Lambrecht *et al.* (2018)••]. Nevertheless, research on mixed protein network development during bread dough mixing is limited. Egg yolk and egg white respectively increase and decrease wheat dough development time, strength and stability [37]. Inclusion of sodium caseinate or hydrolyzed casein in a wheat flour dough recipe results in low proof times, high bread volume and crumb softness [38]. Whey protein concentrates on the other hand have been reported to increase dough development time [39] and to increase proof time and decrease loaf volume [38]. Soy products interfere with gluten formation, weaken dough strength and decrease its gas retention capacity [40]. According to Pérez *et al.* (2008), soy and wheat proteins interact through non-covalent interactions as well as through SS bond formation during dough mixing and resting. The dough weakening effect can thus be ascribed to SH-SS exchange reactions and the loss of some gluten protein from the gluten network. Bonet *et al.* (2006) also reported that inclusion of soy flour in wheat flour dough recipes significantly modifies the mixing characteristics, but concluded from capillary electrophoresis studies that interactions occurred mainly within proteins from the same source. Nevertheless, the exact mechanisms by which mixed networks may be formed at the bread dough level are not well documented.

Other typical bakery ingredients also impact gluten networks. The presence of **sugar** for example has been associated with a reduction in bread dough consistency, increased stickiness and improved extensibility. This has traditionally been linked to sugar's high affinity for water, leaving less water available to hydrate gluten and starch [44]. Furthermore, sucrose containing solvents are less potent gluten plasticizers than pure water [118]. According to Uedaira and Uedaira (1980) [119], sucrose solutions are less favorable solvents for aliphatic and aromatic amino acids than pure water. It would thus require more energy for nonpolar side chains to be exposed in a sucrose solution [119]. This implies that gluten protein conformation may be different in the presence of sucrose/water than in water. In bakery products with high sucrose contents, such as cookies [118] and cakes, the presence of sucrose is indeed known to increase the temperature necessary for protein cross-linking during baking. **Shortening** and other fat sources have been

suggested to lubricate the gluten proteins and limit their water uptake during pastry making [45, 46].

Energy-input

Belton (2005) [47] subdivided dough formation in two, simultaneously occurring stages: a hydration stage and an energy input stage through deformation during **dough kneading**. In the second stage, depolymerization and (re)polymerization reactions take place [47, 48]. Mixing intensity and energy are critical parameters that have a large impact on final product properties. Both must be above a minimum critical level to develop the dough properly, the level varying with flour and mixer type [49]. Multiple researchers have examined the impact of mixing time and intensity on gluten protein network development [37, 49, 50].

A typical unit operation during industrial wheat flour dough making is passing dough (sheets) through a pair of cylindrical rolls, i.e. 'dough sheeting'. The pressure exerted by the sheeting rolls transfers energy to dough, resulting in dough strengthening [51]. In the case of laminated pastry products, which undergo multiple sheeting and folding steps, this additional energy input is anticipated for by working with under-mixed dough. Dough sheeting aligns the gluten network along the final direction of sheeting [51], which results in dough 'snapback' or 'elastic recoil. Some researchers have attempted to model this dough contraction behavior [52-54].

The input of mechanical energy should not exceed an optimal level. Indeed, when wheat flour dough mixing is continued beyond the optimum, dough breakdown takes place, during which the proportion and the average molecular weight of large non-extractable polymeric protein significantly decreases [55]. According to Danno and Hoseney [120], overmixing leads to breakdown of disulfide bonds, which would explain these observations. Skerritt *et al.* [121] confirmed these findings, but state that the cleavage of SS bonds during overmixing is not a random process. Based on sodium dodecyl sulfate polyacrylamide gel electrophoresis and reversed phase high performance gel electrophoresis experiments, they concluded that specific HMM-GS are lost from the gluten network during dough breakdown, as are B-type LMM-GS. On the other hand, the inclusion of C-type LMM-GS, which have a higher hydrophobicity, in the

gluten network increased. According to Bock and Seetharaman (2012), the Belton-model (*cfr. supra*) implicitly assumes that during prolonged dough mixing β-sheet structures (*i.e*. trains) develop at the expense of β-turns (*i.e*. loops) or, presumably, all other secondary protein conformations, and that β-sheets confer less elasticity than β-turns. Eventually, at a threshold ratio of β-sheet to β-turns, the dough would become resistant to further deformation and prolonged mixing would result in breakdown of the gluten network. Whatever be the case, dough breakdown and the mechanisms behind this phenomena during prolonged mixing are not fully understood.

2.3.2 Use of redox agents

Redox agents are frequently used in the bread-making industry to optimize gluten performance in different applications or for research purposes to selectively alter the gluten network and/or study the resulting product properties. In general, addition of oxidizing agents (in appropriate dosages) increases dough strength since they promote SS-bond formation within glutenin polymers [56]. Reducing agents on the other hand, weaken wheat flour dough by reducing the molecular weight of glutenin polymers through SH-SS interactions [25]. As different components are characterized by different reaction rates, the impact of seemingly similar agents on dough properties is not always comparable. For example, potassium bromate ($KBrO_3$) is a slow acting oxidant mostly active during fermentation [in the absence of molecular oxygen (O_2)] and baking [57], whereas potassium iodate (KIO_3) is fast acting and mostly active during mixing [58]. Furthermore, the working mechanism of certain redox agents such as ascorbic acid is more complex and despite excellent research on the topic [59-61] still not completely understood. The use of oxidants is more permitted in the United States but restricted in the European Union, which permits only the use of ascorbic acid. In general, due to the great number and the complexity of oxido-reduction reactions occurring during bread making, the effects of oxidative reagents are insufficiently understood.

In **Table 1**, an overview of some of the most commonly used redox agents in wheat flour dough making is given, along with some key references in which their mode of action is thoroughly examined, as these are outside of the scope of this article.

2.3.2 Use of enzymes

Since enzymes are (in most cases) fully denatured during baking and do not need to be labelled [25], they provide a 'clean-label' alternative to chemical agents. Typically, redox enzymes (oxidoreductases) in dough making are used to directly or indirectly crosslink gluten proteins through various covalent bonds, i.e. for strengthening the dough system [25]. Nevertheless, appropriate use of (endo)peptidases, which hydrolyze gluten too some extent during dough mixing and fermentation, may also improve bread crumb textural properties [62, 63]. The effects of peptidases strongly depend on the dough-making methods used, on flour quality and on the presence of other functional ingredients [64]. They also serve as a useful tool to study gluten protein functionality. In this context, Verbauwhede *et al.* (2018)•• recently examined the use of aqualysin 1 from *Thermus aquaticus*, the hydrolyzing action of which is inhibited by wheat endogenous serin peptidase inhibitors during mixing and fermentation, but no longer during baking. Multiple studies have focused on combinations of enzymes [65, 66]. It is indeed worth noting that commercial enzyme-based dough improvers are hardly ever single enzyme preparations. Besides the main enzyme activity, a range of other enzymes can be present, either as natural side activities coming from the microorganism producing the main activity, or deliberately added [64].

In **Table 2**, an overview of some of the most commonly used gluten-impacting enzymes in wheat flour dough making is given, along with some key references. For a more extensive review on enzyme use in wheat flour dough making, the reader is referred to Joye *et al.* (2009b) and Van Oort (2010).

2.3.2 Other additives

Some emulsifiers strengthen or stabilize dough systems, presumably at least in part through interaction with the gluten network [67]. Diacetyl tartaric esters of monodiglycerides and ethoxylated monoglycerides both exhibit excellent dough stabilizing properties. Although their exact working mechanism is unclear, they have been suggested to be able to form liquid lamellar films between gluten and starch, thereby improving the film forming properties of the gluten [68]. Sodium stearoyl lactylate can also strengthen dough [46] and presumably preferably interacts with or binds to gliadin proteins [37].

For a thorough review on the role of emulsifiers and other lipids in wheat flour dough making, the reader is referred to Pareyt *et al.* (2011).

4. CONCLUSIONS AND PERSPECTIVES

A lot of focus has been on the importance of SS bond formation during dough making. Redox agents and oxidoreductase enzymes are the most commonly used tools for altering the gluten network. Although SS bonds are of major importance, the contribution of non-covalent hydrogen bond and hydrophobic interactions is less well documented. Also, in spite of the large number of excellent studies dedicated to the topic, the exact working mechanism of certain bread improving agents, such as ascorbic acid, still remains unclear and deserves more attention.

All the above shows that small differences in the ingredient bill or dough making procedures may result in significantly altered gluten protein networks. In specific bakery products and industrial applications, learnings from (simplified) models, such as water-flour dough systems may thus not always (fully) apply. For research purposes, it is therefore of utmost importance to clearly specify dough formulations and mixing procedures, also when comparing results of different authors.

Finally, as this review points out, the amount of available literature on gluten proteins is somewhat overwhelming. Therefore, there is a need for integrating knowledge coming from different approaches (e.g. structural features of gluten proteins as investigated through proteomics-based approaches vs. empirical measurements of dough rheology in realistic applications) and for a regular review of the scientific literature on gluten, highlighting recent developments in the field.

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343 ***Table 1:*** *An overview of chemical redox agents used in dough systems either as a tool for studying gluten*
344 *network formation or as an improving agent. Underlined references are review articles.*

REDOX AGENT	APPLICATION	KEY REFERENCES
Molecular oxygen *The most important oxidant in bread making. It is incorporated during dough mixing.*	Flour-water dough Bread dough	Xu, 2001 [69] Hawthorn *et al.*, 1955 [70] Marston, 1986 [71] Campbell, 2003 [72] Decamps *et al.*, 2016 [73]••
Potassium bromate *Slow acting oxidant, mainly active during fermentation and baking*	Hydrated gluten Bread dough	Lagrain *et al.*, 2006 Dong and Hoseney, 1995 [15] Mair *et al.*, 1979 [74] Lagrain *et al.*, 2007 [75] Joye *et al.*, 2009b [25]
Potassium iodate *Iodates are strong oxidants and have a fast effect during dough mixing. Iodate oxidizes free thiol groups and is itself reduced to iodide.*	Hydrated gluten Bread dough Cookie dough Laminated dough	Veraverbeke et al, 1999 [58] Lagrain *et al.*, 2006 Joye *et al.*, 2009b [25] Gaines, 1990 [76] Pareyt *et al.*, 2010 [77] Ooms *et al.*, 2017 [51]
Asorbic acid/dehydroascorbic acid *Ascorbic acid is essentially a reducing agent, but in dough is converted to dehydroascorbic acid in the presence of molecular oxygen by endogenously present ascorbic acid oxidase. Dehydroascorbic acid can reduce glutathione, hence, causing dough strengthening. The exact mechanism is unclear.*	Bread dough	Elkassabany and Hoseney, 1980 [78] Stear, 1990 [79] Nakamura *et al.*, 1997 [80] Every *et al.*, 1999 [59] Wieser, 2003 [23] Grosch and Wieser, 1999 [60] Joye *et al.*, 2009b [25]
Azodicarbonamide *A fast acting oxidant that rapidly oxidizes free thiol groups and is itself reduced to biurea.*	Flour-water dough Bread dough	Miller and Hoseney, 1999 [81] Yamada and Preston, 1992 [82] La *et al.,* 2004 [83] Yasui *et al.*, 2016 [84]
Calcium peroxide/acetone peroxide *In presence of water peroxides releases hydrogen peroxide, which is presumably the active compound and strenghtens the gluten network through radical crosslinking reactions.*	Bread dough	Tieckelmann and Steele, 1991 [85] Wieser, 2003 [23] Takasaki *et al.*, 2005 [86] Joye *et al.*, 2009b [25]
Glutathione *A reducing agent containing a thiol group, which can easily be oxidised to protein bound glutathione. Often added in the form of inactive dry yeast.*	Hydrated gluten Cookie dough Bread dough	Lagrain *et al.*, 2006 Pareyt *et al.*, 2010 [77] Lagrain *et al.*, 2007 Joye *et al.*, 2009a [87]
L-Cysteine *A reducing agent. Its supplementation results in dough weakening, with decreases in the elastic and viscous properties, mixing time and tolerance to mixing.*	Bread dough Laminated dough	Angioloni and Dalla Rosa, 2007 [88] Joye *et al.*, 2009a [87] Ooms *et al.*, 2017, 2018 [51, 89]
Sodium metabisulfite *This reducing agent is hydrolysed by water to bisulfite, which reacts with protein disulfide groups by interchange, leaving a thiolsulfate ester on one protein. This ester is hydrolysed by water, yielding a free thiol group on the protein and a sulfate ion.*	Cookie dough	Oliver *et al.*, 1995 [90] Wieser, 2003 [23] Pendersen *et al.*, 2005 [91]

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347 ***Table 2:*** *An overview of chemical redox agents used in dough systems either as tool for studying gluten*
348 *network formation or as improving agent. Underlined references are review articles.*

ENZYME	APPLICATION	KEY REFERENCES
Glucose oxidase [EC 1.1.3.4] *Specifically catalyzes the oxidation of C1 of β-D-glucose, producing hydrogen peroxide H_2O_2 and D-glucono-δ-lactone. Hydrogen peroxide may indirectly oxidise the gluten thiol groups crosslinking the gluten proteins*	Bread dough Laminated dough	Bonet *et al.*, 2006 [92] Hanft *et al.*, 2006 [93] Bankar *et al.*, 2009 [94] Steffolani *et al.*, 2010 [95] Decamps et al, 2012a [96] Rasiah *et al.*, 2005 [97]
Hexose oxidase [EC 1.1.3.5] *Catalyzes the oxidation of several mono- and oligosaccharides to lactones and hydrogen peroxide, which is thought to be the active compound inducing the formation of disulfide bonds.*	Bread dough	Poulsen and Hostrup, 1998 [98] Hanft and Koehler, 2005 [99]
Pyranose oxidase [EC 1.1.3.10] *Catalyzes the oxidation of C2 or C3 of mono- and disaccharides by molecular oxygen to the corresponding dicarbonyl derivatives and hydrogen peroxide*	Bread dough	Decamps et al, 2012b [100] Decamps *et al.*, 2013 [101]
Sulfhydryl oxidase [1.8.3.2] *Catalyzes the formation of disulfide bonds from a variety of thiol groups. However, its affinity for thiol groups in protein chains seems limited.*	Bread dough	Kaufman *et al.*, 1987 [102] Van Oort, 2010 [64] Faccio *et al.*, 2012 [103]
Transglutaminase [EC 2.3.2.13] *Introduces covalent isopeptide bonds through catalyzation of acyl-transfer reactions.*	Bread dough Laminated dough	Gerrard *et al.*, 1998 [104] Bauer *et al.*, 2003 [105] Caballero, 2007 [65] Steffolani *et al.*, 2010 [95] Kieliszek and Misiewicz, 2014 [111]• Gerrard *et al.*, 2000 [106] Hozova *et al.*, 2002 [107] Ooms *et al.*, 2016 [108]
Peptidases *Hydrolyze peptide bonds and, if active during mixing, lower dough strength. Enzymes with affinities for different amino-acid sequences and with different optimal working conditions have been tested.*	Bread dough	Martínez-Anaya, 1996 [109] Harada *et al.*, 2000 [110] Van Oort, 2010 [64]

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