

Molecular Oxygen and Reactive Oxygen Species in Breadmaking Processes: Scarce, but Nevertheless Important

Karolien Decamps, Iris J. Joye, Dirk E. De Vos, Christophe M. Courtin & Jan A. Delcour

To cite this article: Karolien Decamps, Iris J. Joye, Dirk E. De Vos, Christophe M. Courtin & Jan A. Delcour (2015): Molecular Oxygen and Reactive Oxygen Species in Breadmaking Processes: Scarce, but Nevertheless Important, *Critical Reviews in Food Science and Nutrition*, DOI: [10.1080/10408398.2013.795929](https://doi.org/10.1080/10408398.2013.795929)

To link to this article: <http://dx.doi.org/10.1080/10408398.2013.795929>



Accepted author version posted online: 09 Jun 2015.



Submit your article to this journal



Article views: 51



View related articles



View Crossmark data

Molecular oxygen and reactive oxygen species

in breadmaking processes:

Scarce, but nevertheless important

Karolien Decamps*, Iris J. Joye, Dirk E. De Vos,

Christophe M. Courtin and Jan A. Delcour

Department of Microbial and Molecular Systems & Leuven Food Science and Nutrition

Research Centre (LFoRCe), KU Leuven,

Kasteelpark Arenberg 20 - box 2463, B-3001 Heverlee, Belgium

*Corresponding author:

E-mail address: Karolien.Decamps@biw.kuleuven.be

Keywords: oxidants, enzymes, radicals, wheat, dough.

Abbreviations: arabinoxylan (AX), ascorbic acid (AH₂), catalase (CAT), dehydroascorbic acid (DHA), disulfide (SS), ferulic acid (FA), glucose oxidase (GO), glutathione (GSH), hexose oxidase (HO), laccase (LAC), lipoxygenase (LOX), peroxidase (POD), polyphenol oxidase (PPO), polyunsaturated fatty acids (PUFA), pyranose oxidase (P₂O), reactive oxygen species (ROS), sulfhydryl (SH), sulfhydryl oxidase (SO), superoxide dismutase (SOD), tyrosinase (TYR), tyrosine (Tyr).

Abstract

In breadmaking, O₂ is consumed by flour constituents, yeast, and, optionally, some additives optimizing dough processing and/or product quality. It plays a major role especially in the oxidation/reduction phenomena in dough, impacting gluten network structure. The O₂ level is about 7.2 mmol per kg dough, of which a significant part stems from wheat flour. We speculate that O₂ is quickly lost to the atmosphere during flour hydration. Later, when the gluten network structure develops, some O₂ is incorporated in dough through mixing-in of air. O₂ is consumed by yeast respiration and in a number of reactions catalyzed by a wide range of enzymes present or added. About 60% of the O₂ consumption in yeastless dough is ascribed to oxidation of fatty acids by wheat lipoxygenase activity. In yeasted dough, about 70% of the O₂ in dough is consumed by yeast and wheat lipoxygenase. This would leave only about 30% for other reactions. The severe competition between endogenous (and added) O₂ consuming systems impacts the gluten network. Moreover, the scarce literature data available suggest that exogenous oxidative enzymes but not those in flour may promote crosslinking of arabinoxylan in yeastless dough. In any case, dough turns anaerobic during the first minutes of fermentation.

Introduction

Gluten proteins are most important for wheat flour functionality in breadmaking as they impart unique viscoelastic properties to dough (Shewry et al., 2001). During doughmaking, monomeric gliadin and polymeric glutenin form a three-dimensional gluten network in which glutenin inter- and intramolecular disulfide (SS) bonds play a crucial role (Wrigley et al., 2006). Upon mixing, shear forces reorganize inter- and intramolecular SS bonds between low and high molecular weight glutenin subunits, resulting in a polymeric glutenin structure (Veraverbeke and Delcour, 2002). Results of flow relaxation tests have suggested that, next to the existing primary gluten network, also a secondary arabinoxylan (AX) network is formed when adding peroxidase (POD, E.C. 1.11.1.7) to yeastless dough recipe (Dunnewind et al., 2002).

Whatever the effect of O₂ mediated reactions discussed in this review, one must keep in mind that O₂ is consumed early on in the breadmaking process, rendering the dough system anaerobic very quickly (Joye et al., 2012). Indeed, both yeast (Eyoum et al., 2003) and several enzymes endogenous to wheat, such as lipoxygenase (LOX, E.C. 1.13.11.12), polyphenol oxidase (PPO) or ascorbic acid (AH₂) oxidase (E.C. 1.10.3.3.) compete for O₂ in dough. It is evident that their effect heavily depends on the availability of O₂ as substrate.

Furthermore, oxidizing agents can also be added to the dough system. At optimal dosage, their effect increases dough stability and improves bread quality such as in the case of glucose oxidase (GO, E.C. 1.1.3.4) and pyranose oxidase (P₂O, E.C. 1.1.3.10) (Decamps et al., 2012a). However, overoxidation can lead to too strong doughs in which gas cell expansion is hampered, as is the case when using too high levels of some oxidative chemicals or enzymes.

This review focuses on O₂ and reactive oxygen species (ROS) in breadmaking. More in particular, their fate in the breadmaking process and the probability of different (non-) enzymic reactions involving O₂ and ROS taking place is critically assessed.

1. Oxygen species

1.1 Molecular oxygen

O₂ is produced by cyanobacteria, algae and plants and used in cellular respiration by all forms of complex life (Kerfeld and Krogmann, 1998). Its molecular orbitals are ($\sigma 1s$)² ($\sigma^* 1s$)² ($\sigma 2s$)² ($\sigma^* 2s$)² ($\sigma 2p$)² ($\pi 2p$)⁴ ($\pi^* 2p$)² (**Figure 1**). The most abundant form of O₂ is 3O_2 . It is quite stable and has two unpaired electrons, which occupy the two $\pi^* 2p$ orbitals, and have a same spin orientation (**Figure 1**). 3O_2 is paramagnetic and has diradical properties. It can react with radicals and propagates radical chain reactions. Although most food compounds are of non-radical nature, the oxidation reactions in food systems are mainly initiated by formation of radicals, which then react with 3O_2 (Min and Boff, 2002).

Excitation of 3O_2 by UV irradiation in the presence of a photosensitizer such as chlorophyll which absorbs energy from light and transfers it to 3O_2 results in the formation of 1O_2 (Choe and Min, 2006). In a first form of 1O_2 (**Figure 1**), the electrons are coupled in one orbital. In this state, electronic repulsion between the two electrons results in a highly reactive molecule (Van Dyck, 2007). This 1O_2 rapidly reacts, *e.g.* with other electron rich compounds, which can fill the empty orbital (Girotti, 1998; Min and Boff, 2002; Van Dyck, 2007). The second form of 1O_2 (**Figure 1**) has two electrons with opposite spin divided over the two $\pi^* 2p$ orbitals. This 1O_2 has a much shorter lifetime than the first and plays no significant role in the oxidation of fats and oils

(Belitz et al., 2009), and presumably also not in the oxidation of other food constituents. The abundance of sensitizers in some food products results in ${}^1\text{O}_2$ initiating oxidative reactions during food production and storage (Korycka-Dahl and Richardson, 1980). ${}^1\text{O}_2$ likely reacts in two-electron rather than in radical reactions. The reactions occurring evidently depend on the medium and the conditions and can lead to products different from those formed in radical reactions (Min and Boff, 2002).

1.2 Reactive oxygen species

The term ROS covers a variety of radicals, such as O_2^\bullet , $\cdot\text{OH}$, HOO^\bullet and ROO^\bullet radicals, and non-radical derivatives of ${}^3\text{O}_2$, such as H_2O_2 , O_3 and ${}^1\text{O}_2$ (Choe and Min, 2006). ROS can be formed during food processing and storage and, as their name implies, readily react with proteins, carbohydrates and vitamins (Choe and Min, 2006). Furthermore, ROS with a high reduction potential can oxidize polyunsaturated fatty acids (PUFA) (Koppenol, 1990).

In what follows, we give a short overview of those ROS which are or might be relevant in dough and bread production, and of several reactions in which ROS are formed (**Figure 2**).

1.2.1 Superoxide anion radical

O_2^\bullet is formed when ${}^3\text{O}_2$ takes up an electron (Choe and Min, 2006). HOO^\bullet , the protonated form of O_2^\bullet , can be produced in a reaction between H_2O_2 and $\cdot\text{OH}$ (Choe and Min, 2006). HOO^\bullet is more reactive than O_2^\bullet because it has a higher standard reduction potential (Choe and Min, 2006). Whether O_2^\bullet is present as such or in its protonated form, depends on the pH of the reaction medium (as will be discussed in Section 2.3.1).

Superoxide dismutase (SOD) catalyzes the dismutation of O_2^- into O_2 and H_2O_2 and plays an important role in cellular defense systems against oxidative damage, as the formed oxygen species are substantially less reactive than O_2^- (and HOO^\bullet) (Halliwell, 1989; McCord and Fridovich, 1969).

1.2.2 Hydrogen peroxide

H_2O_2 can be formed by sequential univalent reduction of 3O_2 in which some amino acids serve as electron donors. It can also be formed by dismutation of O_2^- in the presence of SOD, or in a reaction between HOO^\bullet and hydroperoxides (Choe and Min, 2006).

H_2O_2 can be fully reduced to water or to water and O_2 in a reaction catalyzed by POD or catalase (CAT, E.C. 1.11.1.6), respectively, or can be converted to $\cdot OH$ in the presence of transition metal ions (Turrens, 2003). H_2O_2 can oxidize sulphydryl (SH) groups to SS bonds (Manu and Rao, 2011) or, in principle, also to higher oxidation forms such as sulfenic, sulfinic or sulfonic acids [Figure 3, reaction 1 (Winterbourn and Metodiewa, 1999)]. Sulfenic acid may then react with protein SH groups to form SS bonds [Figure 3, reaction 2 (Packer, 1974; Winterbourn and Metodiewa, 1999)]. Furthermore, and again in principle, H_2O_2 can oxidize the thioether group on methionine to methionine sulfoxide (Liang et al., 2012) [Figure 3, reaction 3 (Chu and Trout, 2004)]. If occurring, it is unclear whether such reactions would be ionic or radical (see below).

1.2.3 Hydroxyl radical

3O_2 , O_2^- and H_2O_2 are important precursors of $\cdot OH$ (Choe and Min, 2006; Korycka-Dahl and Richardson, 1980). $\cdot OH$ is one of the strongest oxidants in Nature (Turrens, 2003). It readily

reacts with many inorganic and organic compounds. $\cdot\text{OH}$ generally abstracts a hydrogen atom from a carbon-hydrogen bond in saturated compounds with the concomitant production of a free radical that is more stable and has a longer lifetime than $\cdot\text{OH}$ itself. The latter can then, *e.g.* in a reaction with $^3\text{O}_2$, create ROO^\bullet which propagates other free radical reactions by subsequent abstraction of hydrogen atoms from *e.g.* fatty acids. $\cdot\text{OH}$ reacts with the double bond of unsaturated compounds such as PUFA to yield hydroxylated fatty acid radicals, which in turn can react with $^3\text{O}_2$ to form ROO^\bullet (Choe and Min, 2006; Korycka-Dahl and Richardson, 1980).

1.2.4 Ozone

While low concentrations of O_3 are released in the troposphere by plants and soil, high tropospheric O_3 concentrations result from photochemical reactions involving *e.g.* nitrogen oxides, hydrocarbons or volatile organic compounds present in the air. O_3 can oxidize amino acids such as histidine to 2-amino-4-oxo-4-(3-formylureido)-butanoic acid, methionine to methionine sulfoxide, tryptophan to *N*-formyl kynurenine or tyrosine (Tyr) to dihydroxyphenyl alanine (Kotiaho et al., 2000). Furthermore, O_3 can also oxidize peptides and proteins (Kotiaho et al., 2000; Sharma and Graham, 2010) and react with PUFA to form H_2O_2 and aldehydes (Pryor et al., 1991).

2. Molecular oxygen and reactive oxygen species in wheat flour and breadmaking

2.1 Molecular oxygen in wheat flour

During wheat milling, flour is exposed to air. Indeed, several processing steps such as aspiration and removal of dust, segregation of materials during wheat cleaning and conveying of raw,

intermediate and final materials, are performed in or using air (Posner, 2009). Evidently, air and, hence, O₂ is present in the interstitial space of flour particles. Ameille et al (2000) estimated the O₂ level in flour to be 10.8 mmol per kg (or 0.3 g per kg) flour. Atmospheric O₂ plays an important role in flour maturation (Campbell, 2003; Yoneyama et al., 1970). It is believed that hydrolysis of flour lipids and subsequent oxidation of the free fatty acids formed as well as (coupled) oxidation of SH groups into SS bonds contribute to flour maturation (Wieser, 2003).

2.2 Molecular oxygen in dough

2.2.1 Solubility of molecular oxygen in water and dough liquor

The solubility of O₂ in water at 1.0 atm pressure of O₂ and 25 °C is approximately 40 mg O₂ per L. As the partial pressure of O₂ in air is only 0.2 atm, the maximum concentration of O₂ in water in contact with air is about 8 mg O₂ per L (Ji et al., 2007; Pătulea et al., 2012). Hence, approximately 4.8 mg or 0.2 mmol O₂ per kg of wheat flour is imported by the water added during doughmaking when assuming an optimal water addition level of 60% on flour base.

As the liquid phase of dough contains some (added) sugar and salt, the concentration of dissolved O₂ in this phase is probably much lower than that in pure water. For instance, in the breadmaking protocol of Shogren and Finney (1984), the dough recipe contains both added sucrose [5.1% of flour (dry matter basis)] and salt [1.3% of flour (dry matter basis)]. Assuming that all added sucrose and salt dissolves in the dough liquor, of which the volume equals half of the added water volume (Salt et al., 2006), the concentrations of sucrose and salt in the cited protocol are typically 0.6 mol/L and 0.8 mol/L, respectively. At the same time, predictions can be made about the solubility of O₂ in the dough's liquid phase. Ji and co-workers (2007) reported

that the solubility of O₂ in 0.5 mol/L sucrose is about 20% lower than that in pure water. Salt probably has a larger impact on the solubility of O₂ than sucrose as Jamnongwong and co-workers (2010) reported a 56% decrease in O₂ solubility in water at 20 °C containing 0.86 mol/L salt. Moreover, as the liquid phase of dough not only contains salt and sucrose but also other (organic) compounds, the real solubility of O₂ in the dough's liquid phase is presumably substantially different from that predicted here. On the basis of the above, one could hypothesize that addition of sucrose and salt in solution results in a substantially lower O₂ level imported by the water than when sucrose and salt are added separately and as solids. However, confirming or negating this hypothesis would need experimental underpinning, as one might argue that dough's liquid phase also contains components which bind O₂.

2.2.2 Incorporation of molecular oxygen in dough

During doughmaking, air is mixed in dough. Tsen and Bushuk (1963) and Chin and co-workers (2005) have reported on the impact of O₂ incorporation on dough rheology. They found that mixing in air produces dough with higher mixing resistance and lower extensibility than dough obtained by mixing in N₂ atmosphere. According to Graveland and co-workers (1980), no overmixing occurs when mixing dough in N₂ atmosphere. Junge and co-workers (1981) monitored dough density as a function of mixing time and found that air inclusion occurs slowly when the flour-water mixture hydrates and then speeds up as the dough matrix develops. During overmixing, dough further incorporates air. Levavasseur and co-workers (2006) reported total O₂ uptake levels of about 4.1 mmol per kg flour in yeastless dough. Under normal doughmaking conditions (about 0.60 L water per kg wheat flour), the quantity of O₂ in flour (10.8 mmol per kg

flour, Section 2.1) is approximately 54 times that provided by the water (approximately 0.2 mmol per kg flour). When doughmaking water is enriched with O₂, the quantity imported by it increases from 4.8 mg or 0.2 mmol/kg wheat flour to 0.2 g or 6.3 mmol/kg wheat flour (Lösche et al., 2011). This increase in O₂ can be expected to have consequences for the O₂ consuming reactions in dough. Indeed, when enriching dough water with O₂, the contribution of the O₂ in the mixing water increases as its quantity is only 1.7 times lower than that provided by wheat flour.

The total O₂ level of freshly mixed dough can be estimated from the O₂ levels in the ingredients and imported by mixing air into dough (**Figure 4**).

At the point of optimum mixing, typically about half of the maximum level of air has been incorporated (Junge et al., 1981). This corresponds to 5% (v/v), if we assume a maximum incorporation level of 10% (v/v) (Campbell, 2003). Assuming that 5% (v/v) air [*i.e.* approximately 0.6 mmol or 19.2 mg O₂, assuming the densities of dough and air to be 1.10 g/cm³ and 1.20 mg/cm³, respectively (Campbell et al., 1993; Fan et al., 1999)] is incorporated in dough from 1 kg of flour (~1.6 kg dough), freshly mixed dough would contain approximately 11.6 mmol O₂ per kg wheat flour, and, hence, 7.2 mmol O₂ per kg dough, which shows the O₂ level in wheat flour to be substantially higher than that incorporated during mixing. However, this calculation does not take into account conversions or losses of O₂ during mixing. Indeed, we can assume that a substantial level of air is ‘squeezed out’ during flour hydration, while, later during mixing, air is incorporated in the dough structure as the gluten network starts to form. Air, and hence O₂, incorporation in dough is further highly related to the energy input during mixing (Eyoun et al., 2003) and thus to mixer design and blade speed (Campbell et al., 1993).

The air incorporated during mixing contributes to oxidation of dough components and affects dough development and rheological properties. Inclusion of air creates very small gas cells that act as nucleation sites for the CO₂ that moves into the preexisting cells during fermentation. Mixing dough under high vacuum eliminates these nucleation sites, decreases retention of CO₂ gas and results in poorly structured bread loaves of low volume (Dobraszczyk, 2003). The incorporation of air (and thus mainly N₂ and O₂), the rheology of the dough matrix and the level of CO₂ produced are all important factors contributing to the overall bread quality (Campbell, 2003; Delcour and Hoseney, 2010).

2.3 Reactive oxygen species in dough

ROS appear in radical or non-radical form (Section 1.2). The literature contains only little data on the formation and role of ROS in wheat flour dough. This is probably due to the lack of straightforward methods for their analysis. Hence, there is substantial uncertainty about the occurrence, actual level and conversion of these compounds in dough. Furthermore, wheat contains a large variety of antioxidants which can scavenge free radicals, such as phenolic acids or flavonoids (Dykes and Rooney, 2007). While, in principle, these can affect the level and reactivity of ROS in wheat processing, most of these bioactive compounds are principally present in the bran and germ fractions, rather than in wheat flour itself (Dykes and Rooney, 2007). Furthermore, gluten proteins contain some Tyr. Tyr is an antioxidant (Van Overveld et al., 2000).

Four decades ago, Dronzek and Bushuk (1968) suggested the presence/formation of free radicals in dough. They reported that, when added to dough, methyl methacrylate polymerizes and,

hence, hypothesized the presence of free radicals because methyl methacrylate only polymerizes by a free radical mechanism. Furthermore, there is also evidence for formation of ROS in enzymic reactions.

2.3.1 Superoxide anion radical

Several studies (Hoseney et al., 1980; MacRitchie, 1975; Sidhu et al., 1980) indicate that SS bonds are cleaved during dough mixing. Indeed, MacRitchie (1975) suggested that shear forces, such as occurring during dough mixing, can result in cleavage of SS bonds with the concomitant production of thiyl radicals (**Figure 3, reaction 4**). However, according to Graveland and co-workers (1980), cleavage of SS bonds into thiyl radicals and subsequent reformation of SS bonds takes place in the presence of radicals, such as $O_2^{\cdot-}$ (**Figure 3, reaction 4**). Involvement of $O_2^{\cdot-}$ was deduced from a lower level of cleavage of SS bonds into thiyl radicals when dough was made with SOD (Graveland et al., 1980). Upon supplementation of the dough recipe with xanthine and xanthine oxidase in order to increase the $O_2^{\cdot-}$ level, the rate of SS bond cleavage increased (Graveland et al., 1980). More recently, Miyamoto and Nishimura (2006) reported that $O_2^{\cdot-}$, produced upon photo-activation of riboflavin in dough, in the presence of gluten peptides, removes hydrogen radicals from SH groups. Their results suggest that $O_2^{\cdot-}$ is indeed generated in dough and that it results in formation of thiyl radicals from SH compounds such as GSH [**Figure 3, reaction 4** (Packer, 1974)].

As the pK_a of the $O_2^{\cdot-}/HOO^{\cdot}$ system is 4.8 (Belitz et al., 2009), one can calculate that, in freshly mixed dough with a pH of about 6.0, $O_2^{\cdot-}$ is about 15 times more predominant than HOO^{\cdot} . However, upon fermentation, dough acidifies and its pH drops to about 5.2 [120 min

fermentation (Jayaram et al., 2013)], resulting in a concentration of O_2^- which is only 2.5 times higher than that of HOO^\bullet . HOO^\bullet can create thiyl radicals from SH groups [Figure 3, reaction 4 (Nakamura and Kurata, 1997; Packer, 1974)].

Two isoenzymes of SOD have been detected in wheat seedlings. They have optimal activity at pH 8.0 and over a wide temperature range [from 5 to 50 °C (Lai et al., 2008)]. In addition, three SOD isoforms have been found in wheat germ. These are optimally active at pH 10.0 (Beauchamp and Fridovich, 1973). As it is not uncommon that wheat flour is contaminated with germ tissue, traces of these SODs may also be present in wheat flour. However, the activity of the enzymes is presumably low due to the high pH values required for optimal activity.

2.3.2 Hydrogen peroxide

Although neither intensively investigated nor described, it has been hypothesized that yeast produces radicals and H_2O_2 during respiration (Boveris, 1978; Perrone et al., 2008). While according to Liao and co-workers (1998) yeast produces H_2O_2 during fermentation, in-house experimental data allowed concluding that their method to determine the exact level of H_2O_2 is very sensitive to interference by components such as glucose, 2-keto-D-glucose and D-glucono-1,5-lactone. Moreover, to date, to the best of our knowledge, there is no clear evidence for H_2O_2 release by yeast in dough. However, if produced, H_2O_2 very likely would initiate oxidation reactions.

Manu and Rao (2011) suggested that, in dough, H_2O_2 can oxidize SH groups, hence promoting formation of links within and between proteins. H_2O_2 can also crosslink Tyr residues in protein (Tilley et al., 2001) (Figure 3, reaction 5). However, Hanft and Koehler (2005) only found very

limited di-Tyr formation in yeastless dough even when high levels of H₂O₂ were generated by use of hexose oxidase (HO, E.C. 1.1.3.5).

Furthermore, there is evidence for *in vitro* crosslinking of AX molecules (Figueroa-Espinoza and Rouau, 1998; Neukom and Markwalder, 1978; Oudgenoeg et al., 2001; Schooneveld-Bergmans et al., 1999). Such AX crosslinking occurs via oxidation of ester bound ferulic acid (FA) by H₂O₂ in an enzymic reaction catalyzed by POD (Garcia et al., 2002) or laccase (LAC, E.C. 1.10.3.2) (Figueroa-Espinoza and Rouau, 1998; Selinheimo et al., 2007) (**Figure 3, reaction 6**). The effect of both enzymes will be discussed later in this review (Sections 3.4 and 3.6, respectively). The above *in vitro* experiments, however, do not necessarily prove that AX crosslinking occurs in yeasted dough.

While more work is clearly needed in this area, it is useful to speculate here on its potential outcome. Assuming that AX do crosslink in yeasted dough, this could result in several effects: (i) crosslinking of water extractable AX to water extractable AX would increase their molecular weight and the viscosity of dough liquor; (ii) crosslinking of water extractable AX to water unextractable AX would render them unextractable and lower both the AX concentration in dough liquor and dough liquor viscosity; and (iii) crosslinking of water unextractable AX to water unextractable AX would change the properties of this AX population as well. The impact that such different crosslinking components would have on bread quality would then be difficult to predict.

In this context, it is of interest that the addition of POD to yeastless dough recipe has been suggested to form a covalently crosslinked AX network (Dunnewind et al., 2002). Whether such network would be formed in yeasted dough remains to be explored. Also, the relative importance

of such network when compared to that of the gluten network may well be very limited when one considers the low AX and FA contents in wheat flour.

Furthermore, Oudgenoeg and co-workers (2001) suggested the possibility of covalent crosslinking between gluten proteins and FA of AX based on *in vitro* evidence in which the authors demonstrated crosslinking of a Tyr containing peptide to FA molecules (**Figure 3, reaction 7**). Earlier, Hoseney and Faubion (1981) speculated on the formation of protein radicals upon adding H₂O₂ to a flour water extract. In their view, these protein radicals can react with the double bond of FA. Piber and Koehler (2005), who detected such crosslinks in dough even when no H₂O₂ was added, but hypothesized that they are only of minor importance to the functionality of dough.

At the right dosage, H₂O₂ improves bread dough stability. Indeed, dough made with oxidases producing H₂O₂ shows less collapse and higher bread loaf volumes than reference breads in a breadmaking protocol in which stress was imposed on the proofed dough (Decamps et al., 2012a). However, addition of high levels of H₂O₂ to a dough recipe decreases bread loaf volumes, probably as a result of overoxidation (Decamps et al., 2012a). Furthermore, dough made with addition of 3.4 nkat GO/g flour feels very dry and strong (Vemulapalli et al., 1998). The increased dryness and strength is probably caused by the production of H₂O₂ which has been presumed to cause the water-extractable pentosans in dough to gel as a result of a reaction catalyzed by POD. Such oxidative gelation limits water mobility, resulting in increased dough dryness.

2.3.3 Ozone

Mixing dough in O₃ atmosphere substantially impacts dough properties. It decreases mixing time and improves dough ‘machinability’ and CO₂ retention during fermentation (Coste and Dubois, 2008; Lullien-Pellerin, 2012). Several studies report on the beneficial effects of ozonation of wheat flour or grain (Sandhu et al., 2011; Violleau et al., 2012). Sandhu and co-workers (2011) found that O₃ treatment of flour not only improves color characteristics, but also leads to a higher level of SS bonds in proteins. Furthermore, ozonation of wheat prior to milling yields flour that produces dough with improved rheological properties (Violleau et al., 2012).

3. Molecular oxygen and reactive oxygen species consumption and production in dough

Figure 5 gives an overview of ways in which O₂ or ROS can be consumed or produced during breadmaking.

Wheat flour contains a number of enzymes which use O₂ as substrate. Even in yeastless dough, the O₂ level decreases steeply (Joye et al., 2012). Most of the O₂ in freshly mixed dough is consumed by wheat LOX (Eyoum et al., 2003; Graveland, 1970). Other important O₂ consuming enzymes are AH₂ oxidase and PPO, the latter term covering two different enzyme types, *i.e.* monophenol monooxygenase [also referred to as tyrosinase (TYR, E.C. 1.14.18.1)] and diphenol oxidase (catechol oxidase, E.C. 1.10.3.1) (Mayer, 1987). Furthermore, POD can consume H₂O₂ and CAT catalyzes the dismutation of H₂O₂ into O₂ and H₂O.

As they (potentially) impact the functionality of wheat gluten proteins, the use of several exogenous enzymes such as TYR, LAC or oxidases [GO, HO, P₂O or sulphhydryl oxidase (SO, E.C. 1.8.3.2)] in dough recipes has also been studied. By including such enzymes in dough formulas, one targets to more efficiently use O₂ for changing gluten functionality and dough

characteristics, although the competition for O₂ as a substrate logically increases. In addition, yeast also consumes O₂. We here discuss the literature data on the different O₂ and ROS consuming or producing (enzyme) systems.

3.1 Lipoxygenase

LOX, a non-heme iron containing dioxygenase, catalyzes the oxidation of PUFA containing a *cis,cis*-1,4-pentadiene system, and thus linoleic (C18:2, *cis,cis*-9,12-octadecadienoic acid) and linolenic (C18:3, *cis,cis,cis*-9,12,15-octadecatrienoic acid) acid. In Nature, two types of LOX activity can be distinguished. The first LOX type attacks both free and esterified PUFA, while the second LOX type only shows activity towards free PUFA. While soybean LOX belongs to the first LOX group, wheat and durum wheat enzymes more closely match the second LOX type (Christopher et al., 1970). Wheat flour lipid content ranges from 2.0-2.5% on flour weight basis (Pareyt et al., 2011), of which 0.07 to 0.08% are PUFA [calculation based on in-house experimental data and data by Chung and Ohm (2009) and Morrison (1978)].

The rate of PUFA oxidation in dough increases upon mixing under pure O₂ or upon adding (purified) wheat LOX (Graveland, 1968). To date, four different LOX isoforms have been extracted from wheat (Wallace and Wheeler, 1975). The pH optimum of most isolated wheat isoforms is in the neutral pH range. LOX from wheat and soybean bleaches carotenoids (Faubion and Hoseney, 1981). As soybean contains high LOX activity, supplementation of dough recipe with soybean LOX increases mixing tolerance and improves dough rheology (Faubion and Hoseney, 1981), and whitens dough and bread crumb (Junqueira et al., 2007). LOX activity produces fatty acid hydroperoxides. The hydroperoxide group can subsequently be cleaved to

generate $\cdot\text{OH}$. The latter can then form radicals from SH groups and, hence, promote formation of SS bonds (**Figure 3, reaction 4**).

3.2 Ascorbic acid oxidase

Wheat flour contains an AH₂ oxidase which catalyzes oxidation of vitamin C (L-threo-AH₂) by O₂ (Grosch and Wieser, 1999). The catalytic cycle of AH₂ oxidase is assumed to proceed by a sequential mechanism in which single electrons are abstracted from AH₂ in one of the catalytic sites of AH₂ oxidase (Farver and Pecht, 1992). Wheat AH₂ oxidase shows optimal activity in slightly acidic conditions (pH 5.4-6.0) and at a temperature of about 40 °C (Pfeilsticker and Roeung, 1982). AH₂ oxidase levels in flour from different wheat cultivars vary threefold (Every et al., 1996). Upon storage of wheat flour for 300 days at 20 °C, AH₂ oxidase activity decreased by 48% and wheat kernels stored for two or more years showed no residual AH₂ oxidase activity (Every et al., 1996).

L-Threo-AH₂ is quickly oxidized to L-threo-dehydroascorbic acid (DHA). The latter accepts electrons in the oxidation of endogenous glutathione (GSH) catalyzed by wheat flour GSH dehydrogenase (E.C. 1.8.5.1) (Grosch and Wieser, 1999). The oxidation of GSH lowers its concentration and prevents protein depolymerization (dough softening) by SH-SS interchange reactions (Koehler, 2003).

Nakamura and Kurata (1997) reported an effect of AH₂ on the rheological properties of dough and found it to be more pronounced when dough was mixed in air than in N₂. These authors hypothesized that the observed dough improving effect is due to O₂ $^{\cdot}$ generation during oxidation of AH₂ as O₂ $^{\cdot}$ can affect SH/SS-interchange reactions in gluten molecules. Production of O₂ $^{\cdot}$ in

dough was (indirectly) deduced from dough hardness experiments using O_2^\bullet scavenging (SOD, **Figure 2**) and O_2^\bullet producing (xanthine and xanthine oxidase) systems and detecting O_2^\bullet using nitro blue tetrazolium.

3.3 Polyphenol oxidase

While TYR catalyzes hydroxylation of mono- to diphenols and oxidation of *o*-diphenols to *o*-quinones, catechol oxidase only catalyzes the oxidation of *o*-diphenols to *o*-quinones (Mayer, 1987). Catechol oxidase and TYR both transfer two electrons to O_2 . Previous studies have indicated the presence of as many as nine to twelve isoforms of PPO in wheat (Kruger, 1976; Taneja et al., 1974). Wheat PPO enzymes have an optimum pH for activity of 6.0 to 7.0 (Marsh and Galliard, 1986).

Compared to the activities of wheat LOX and AH₂ oxidase, the activity of PPO naturally present in wheat flour is negligible (Honold and Stahmann, 1968). Marsh and Galliard (1986) found PPO activity in four white flour samples to vary threefold. Only about 10% of wheat kernel PPO activity is present in flour, as PPO activity is mainly associated with bran (Marsh and Galliard, 1986).

TYR addition to dough recipes triggers crosslinking of gluten proteins but not FA oxidation (Selinheimo et al., 2007). TYR catalyzes the formation of *e.g.* di-Tyr (**Figure 3, reaction 5**), Tyr-cysteine (**Figure 3, reaction 8**) and Tyr-lysine [**Figure 3, reaction 9** (Wang et al., 2012)] protein crosslinks. Such protein crosslinking increases dough strength and improves bread quality (Selinheimo et al., 2007).

Most TYRs isolated from various organisms and used as bread recipe supplements have a pH optimum in the neutral or slightly acidic range (Faccio, 2011). Furthermore, the optimum temperature for TYR activity is about 25-30 °C (Faccio, 2011). These conditions correspond to the temperature and pH values prevalent in dough immediately after mixing and during fermentation. Although TYR can affect protein crosslinking in dough, this enzyme is not used in industrial practice.

3.4 Peroxidase

POD, a heme containing enzyme, catalyzes the oxidation of a wide variety of phenolic or SH containing substrates with H₂O₂. Billaud and co-workers (1999) reported the presence of four isoforms of POD in wheat which showed optimal activity in a pH range varying from 5.3 to 6.3. Delcros and co-workers (1998) observed no effect of mixing on POD activity.

Takasaki and co-workers (2005) reported no effect of horseradish POD neither on dough mixing characteristics nor on total gas production during fermentation. Garcia and co-workers (2002) reported *in vitro* oxidation of free or esterified [5-*O*-(trans-feruloyl)-L-arabinose] FA upon addition of wheat POD and H₂O₂. Furthermore, these authors stated that SH groups, *in vitro*, can regenerate FA out of phenoxy radicals, produced by added wheat POD and H₂O₂, in a reaction in which the SH groups are oxidized to SS bonds. If such reactions would also occur in breadmaking, this could affect SH/SS-interchange reactions in the process. Hilhorst and co-workers (2002) attributed the decreased extractability of AX upon supplementation of yeasted dough recipe with soy POD to crosslinking of AX molecules. Although wheat flour itself

contains POD, it seems from their data that addition of exogenous POD is needed to obtain AX crosslinking in yeasted dough.

3.5 Oxidases

GO, HO and P₂O are (sugar) oxidases using O₂ as electron acceptor. Although these enzymes catalyze oxidation of β-D-glucose by O₂ and thereby produce H₂O₂, they are very different. GO is a homodimeric flavoprotein which very selectively oxidizes β-D-glucose into D-glucono-1,5-lactone, showing optimal activity in a pH range from 5.0 to 6.5 and at 35 °C (Bankar et al., 2009; Decamps et al., 2012b). HO is a homodimeric flavoprotein catalyzing the oxidation of C₁ of several mono- and disaccharides (Poulsen and Hostrup, 1998). The temperature and pH values for optimal activity of HO are 25 °C and pH 6.3, respectively (Hansen and Stougaard, 1997; Sullivan and Ikawa, 1973). P₂O is a homotetrameric flavoprotein which catalyzes the oxidation of C₂ or C₃ of a range of mono- and disaccharides to their corresponding dicarbonyl derivatives, showing optimal activity in a pH range from 5.5 to 6.5 and at 30 °C (Decamps et al., 2012b; Leitner et al., 2001). Furthermore, P₂O and HO show higher affinity towards glucose (and O₂) than GO does (Decamps et al., 2012b; Poulsen and Hostrup, 1998). The temperature and pH optima for these oxidizing enzymes correspond to the values prevalent in dough immediately after mixing and during fermentation. GO (Decamps et al., 2012a; Gul et al., 2009), P₂O (Decamps et al., 2012a) and HO (Gul et al., 2009) can improve (dough stability and) bread quality.

SO oxidizes free SH containing molecules to the corresponding SS containing compounds, thereby reducing O₂ to H₂O₂. Typical substrates for SO are small SH compounds such as

cysteine, GSH and cysteine-containing peptides (Faccio, 2011). As SO also produces H₂O₂, its activity may trigger a range of oxidation reactions including that of SH groups (Faccio et al., 2012). Faccio and co-workers (2012) reported that supplementation of dough recipe with SO has no impact on dough rheological properties, although it intensifies the effect of AH₂. Indeed, simultaneous inclusion of AH₂ and SO in a dough recipe reduces dough extensibility. Faccio and co-workers (2012) attributed such effect on dough rheology to removal of reduced GSH and production of H₂O₂ which then oxidizes AH₂ into DHA. Other researchers observed significant effects of SO neither on bread loaf volume nor on mixograph properties (Kaufman and Fennema, 1987). Furthermore, Kaufman and Fennema (1987) found no effect of SO addition on the level of free SH groups in flour-buffer suspensions. To date, only GO and HO are used in industrial breadmaking practice.

3.6 Laccase

LAC, a multicopper oxidoreductase, catalyzes the oxidation of several phenolic and non-phenolic compounds (Necochea et al., 2005). LAC shows high affinity over broad temperature and pH ranges with optima at a temperature of 50 °C and pH 3.0 (Han et al., 2005).

LAC catalyzes the oxidation of Tyr residues in proteins and FA esters linked to AX (**Figure 3, reactions 5 and 6**). The formed FA radicals react non-enzymically to produce di-FA in a yeastless dough (Labat et al., 2000). Addition of LAC to dough recipes decreases dough development time, increases dough consistency and decreases dough tolerance to overmixing (Labat et al., 2000). Primo-Martin and co-workers (2003) reported changes in dough rheology and improved gluten quality when adding LAC to yeastless dough recipes. Later on, Selinheimo

and co-workers (2007) speculated that LAC promotes crosslinking of high molecular weight AX which further reinforces the gluten network. Flander and co-workers (2008) reported increased resistance to stretching and decreased extensibility of dough. However, whether LAC has an impact on dough is not so clear as other researchers found LAC to neither affect dough viscoelastic properties nor bread quality (Caballero et al., 2007). While LAC seems to be an interesting enzyme in the context of breadmaking, it is not used in industrial practice.

3.7 Catalase

CAT is a heme containing enzyme, catalyzing the dismutation of H₂O₂ into O₂ and H₂O. It is naturally present in wheat flour (Honold and Stahmann, 1968; Kruger, 1977). To date, two isoforms with optimal activity in the neutral pH range have been purified from wheat germ (Garcia et al., 2000). The level of O₂ produced by CAT in dough is, however, low as the enzyme is not very stable under conditions mimicking those appearing during breadmaking. *In vitro*, one isoform of CAT shows an activity loss of 70% at pH 5.4 upon five minutes of incubation at 30 °C, while activity loss was less than 30% for the other (Garcia et al., 2000). Delcros and co-workers (1998) found that supplementation of dough recipes with 5.7 µmol H₂O₂ per g flour decreases the activity of CAT with 20% during the first minutes of mixing. They attributed this to an enzymic self-destruction mechanism. Furthermore, Liao and co-workers (1998) stated that CAT is inhibited by peroxides in the lipid fraction of wheat flour.

In addition, in dough, there is a severe competition for H₂O₂ (Decamps et al., 2012a). As H₂O₂ is believed to contribute to dough stability, high CAT activity is undesirable. However, dismutation

of H₂O₂ produces O₂ which serves as a substrate for the above described enzymes beneficial to breadmaking quality.

3.8 Yeast

Baker's yeast (*Saccharomyces cerevisiae*), a single-cell eukaryotic organism, under aerobic conditions consumes O₂ and converts sugar to water and CO₂ while, under subsequent anaerobic conditions, it converts sugar to CO₂ and ethanol in the fermentation phase. During mixing, yeasted dough steadily consumes O₂ (Marston, 1986). Joye and co-workers (2012) reported O₂ consumption rate in mixed dough to increase with yeast level. During fermentation, yeast produces CO₂ and ethanol, both of which dissolve in the dough's aqueous phase. When the latter is saturated with CO₂, the produced gas mainly diffuses into the gas cells. As a result, dough volume increases and the typical foam structure is formed (Delcour and Hoseney, 2010).

Higher yeast concentrations reduce the level of oxidative enzymes needed in breadmaking (Finney et al., 1976). Indeed, yeast and oxidation exert similar dough strengthening effects (Decamps et al., 2012a; Hoseney et al., 1979). Furthermore, yeast can increase the viscosity of the water-extractable fraction of wheat flour (Hoseney and Faubion, 1981). These authors explained the increase in viscosity as a result of the addition of a protein thiyl radical to the activated double bond of FA esterified to AX (**Figure 3, reaction 7**). This reaction and crosslinking of AX molecules through di-FA bridges increase the viscosity (**Figure 3, reaction 6**). According to Liao and co-workers (1998), the oxidizing power of yeast probably stems from production of H₂O₂ during fermentation. However, we hypothesized (Section 2.3.2) that yeast releases little if any H₂O₂ into dough.

3.9 Calcium and benzoyl peroxide

CaO_2 can be used in dough formulae to improve dough handling (Tieckelmann and Steele, 1991). In aqueous environments, it decomposes into $\text{Ca}(\text{OH})_2$ and O_2 . However, at lower pH values, such as occurring in dough, CaO_2 produces Ca^{2+} and H_2O_2 . The latter may subsequently be converted to O_2 and water by CAT activity or may trigger (oxidation) reactions as described above (Tieckelmann and Steele, 1991). Dibenzoyl peroxide, although banned in many countries, can be used as bleaching agent for flour and dough (Gelinas et al., 1998; Sievert et al., 2008). It has no effect on dough's baking properties (Sievert et al., 2008).

3.10 Other systems

O_2 is also consumed in reactions catalyzed by some metal ions such as those modifying protein amino acid residues and resulting in formation of carbonyl derivatives (Stadtman, 1990). It is, however, very difficult to estimate the effective O_2 consumption by and the effect of such systems in flour based dough.

4. Competition for and concentration of molecular oxygen and reactive oxygen species during breadmaking

4.1 Yeastless dough

During mixing, O_2 levels in dough are reasonably high and several O_2 consuming reactions compete for the O_2 present in freshly mixed dough (Levavasseur et al., 2006). After mixing, most likely no additional O_2 is incorporated in dough (Joye et al., 2012). Furthermore, O_2 in

dough is quickly consumed after dough mixing, rapidly turning dough into a, for all practical purposes, anaerobic system (Joye et al., 2012; Levavasseur et al., 2006). However, according to Xu (2001), O₂ depletion in dough is only apparent and a mere physical phenomenon as O₂ would adsorb on hydrated starch granules and fibers. In this view, upon baking, O₂ is quickly desorbed from dough and contributes to oven spring. At the same time, O₂ would trigger reactions that do not occur when there is a lack of free accessible O₂ in dough at this stage of breadmaking.

As mentioned above, AH₂ oxidase, LOX and PPO are naturally present in wheat flour. LOX, which has a very high affinity towards O₂, accounts for about 60% of the O₂ consumption in yeastless dough (Potus and Nicolas, 2010). Tsen and co-workers (1962) described that, during mixing, oxidation of PUFA by LOX and of SH, whether or not catalyzed by metal ions, competes for the available O₂. Thus, enzyme affinity for O₂ also plays a major role in determining the activity of the enzymes.

Wheat flour POD, LOX and CAT exhibit different behavior during mixing. While the activity of POD remains almost unaffected when mixing dough, LOX and CAT activities are significantly reduced (Delcros et al., 1998). For LOX, a self-destruction mechanism has been postulated as being at the basis of this activity loss. Upon oxidation, LOX synthesizes transitory (or secondary) products which irreversibly rescind its activity. For CAT, the activity decrease is probably due to non-reversible denaturation because of shear effects or the low dough pH. However, the residual activity of both enzymes strongly depends on the mixing conditions and time (Delcros et al., 1998).

4.2 Yeasted dough

As mentioned above, yeast respiration also consumes O₂. Taken together, the oxidation of PUFA by endogenous LOX and the respiration by yeast are estimated to account for more than 70% of the O₂ consumption in yeasted dough (Potus and Nicolas, 2010).

4.3 Yeasted dough containing added oxidizing enzymes

When GO, HO, P₂O, SO, LAC or TYR are added to dough formulae, they also compete for O₂ in dough. As dough quickly turns anaerobic during fermentation, the effect of these enzymes greatly depends on their activity during mixing and first minutes of fermentation. Their affinity for O₂ hence determines whether and to what extent the enzymes can affect dough properties. It is clear that enzymes having high affinity for O₂ and ‘easy’ access to their substrates have a chance to exert a more pronounced effect on dough characteristics. Obviously, the rates of the O₂ consuming reactions are also important.

The oxidation of PUFA, the respiration by yeast and the oxidation of glucose by GO are estimated to account for more than 74% of the O₂ consumption in yeasted dough (Potus and Nicolas, 2010). Potus and Nicolas (2010) also reported that, upon addition of soybean LOX, the balance of O₂ consumption clearly shifts to oxidation of PUFA, reducing the quantitative importance of the other oxidation pathways.

4.4 An integrated view on molecular oxygen and reactive oxygen species in breadmaking

Table 1 contains a schematic overview of the O₂ and ROS consuming and producing systems in dough. Their relative contribution to the O₂ and ROS consumption and production has been estimated and their potential effect on dough properties during breadmaking is shown.

No other researchers have supported the O₂ physical adsorption theory described by Xu (2001). Since, on the contrary, other authors (Joye et al., 2012; Levavasseur et al., 2006) observed that O₂ is quickly consumed after dough mixing, we postulate that dough turns anaerobic during the first minutes of fermentation. Obviously, O₂ and ROS consuming and producing reactions occur in that admittedly short time frame. Therefore, we suggest that only those enzymes having high affinity for O₂ and having access to it can use it as a substrate. Furthermore, reaction conditions and availability of the other substrates also play a role in determining the exact reactions taking place.

Furthermore, we agree with Levavasseur and co-workers (2006) that the activity of enzymes using O₂ in dough largely depends on the efficiency of O₂ incorporation during mixing. The latter, in turn, is largely determined by the type of mixer used (Levavasseur et al., 2006). It is reasonable to assume that already during the first minutes of mixing conversions (or losses) of O₂ occur.

According to Potus and Nicolas (2010), the activity of LOX contributes to 60% of the O₂ consumption in yeastless dough. Supplementation of dough recipe with yeast increases the consumption with another 10% and further addition of GO to the dough formula further intensifies the O₂ consumption to 74%. The residual O₂ (26%) is then available to be consumed by other reactions. Although one is bound to introduce errors when allocating a certain level of O₂ consumption to specific (enzyme) systems, it is reasonable to assume that part of this O₂ consumption will induce oxidation of SH groups. It has been found that the SH concentration in wheat flour is about 1.0 mmol/kg flour (Belitz et al., 2009). Furthermore, oxidation of SH compounds affects SH/SS-interchange reactions and, hence, alters dough properties. At the same

time, only 0.5 mmol O₂ per kg flour is needed to oxidize the SH level in flour. Taken together, it is clear that O₂ and ROS can significantly contribute to SH oxidation in gluten proteins of dough. In addition, the little literature data available suggest that supplementation of dough recipe with exogenous oxidative enzyme appears to be necessary if one seeks to crosslink AX molecules. Further research is nevertheless needed to investigate the extent of such AX crosslinking and its potential impact on bread quality.

However, as the concentration of O₂ and ROS is limiting and as they serve as substrates for several added or native (enzyme) systems, a severe competition for O₂ and ROS exists in dough. Further research focusing on O₂ consuming or producing systems in the breadmaking context is, hence, of utmost importance. The insights obtained will be useful in modeling physical and chemical processes in dough, allow more accurate prediction of the effect of the O₂ consuming improvers and their appropriate supplementation level, and contribute to a more targeted search and development of dough stabilizing agents.

Acknowledgements

Karolien Decamps and Iris Joye gratefully acknowledge financial support from the ‘Fonds voor Wetenschappelijk Onderzoek – Vlaanderen’ (FWO, Brussels, Belgium). This research is also part of the Methusalem program “Food for the future” at the KU Leuven. Jan A. Delcour is W.K. Kellogg Chair in Cereal Science and Nutrition at KU Leuven.

References

- Ameille, V., Davidou, S., Drapron, R., Potus, J., Nicolas, J., 2000. Continuous measurement of oxygen consumption during mixing of unyeasted wheat flour dough. *Sciences Des Aliments* **20**: 221-235.
- Bankar, S.B., Bule, M.V., Singhal, R.S., Ananthanarayan, L., 2009. Glucose oxidase: An overview. *Biotechnology Advances* **27**: 489-501.
- Beauchamp, C.O., Fridovich, I., 1973. Isozymes of superoxide dismutase from wheat germ. *Biochimica Et Biophysica Acta* **317**: 50-64.
- Belitz, H.-D., Grosch, W., Schieberle, P., 2009. Food Chemistry (4th revised Edition). Springer-Verlag, Berlin, 1070 p.
- Billaud, C., Louarme, L., Nicolas, J., 1999. Comparison of peroxidases from barley kernel (*Hordeum vulgare L.*) and wheat germ (*Triticum aestivum L.*): Isolation and preliminary characterization. *Journal of Food Biochemistry* **23**: 145-172.
- Boveris, A., 1978. Production of superoxide anion and hydrogen peroxide in yeast mitochondria. In: Bacila, M., Hokerer, B.L., Stoppani, A.O.M. (Eds.), *Biochemistry and Genetics of Yeast*. Academic Press, NY, USA, pp. 65-80.
- Caballero, P.A., Gomez, M., Rosell, C.M., 2007. Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. *Journal of Food Engineering* **81**: 42-53.
- Campbell, G.M., 2003. Bread aeration. In: Cauvain, S.P. (Ed.) *Bread making: Improving quality*. Woodhead Publishing Limited, Cambridge, UK, pp. 352-374.

ACCEPTED MANUSCRIPT

Campbell, G.M., Rielly, C.D., Fryer, P.J., Sadd, P.A., 1993. Measurement and interpretation of dough densities. *Cereal Chemistry* **70**: 517-521.

Chin, N.L., Martin, P.J., Campbell, G.M., 2005. Dough aeration and rheology: Part 3. Effect of the presence of gas bubbles in bread dough on measured bulk rheology and work input rate. *Journal of the Science of Food and Agriculture* **85**: 2203-2212.

Choe, E., Min, D.B., 2006. Chemistry and reactions of reactive oxygen species in foods. *Critical Reviews in Food Science and Nutrition* **46**: 1-22.

Christopher, J., Pistorius, E., Axelrod, B., 1970. Isolation of an isozyme of soybean lipoxygenase. *Biochimica Et Biophysica Acta* **198**: 12-19.

Chu, J.W., Trout, B.L., 2004. On the mechanisms of oxidation of organic sulfides by H₂O₂ in aqueous solutions. *Journal of the American Chemical Society* **126**: 900-908.

Chung, O.K., Ohm, J.-B., 2009. Wheat lipids. In: Khan, K., Shewry, P.R. (Eds.), *Wheat: Chemistry and Technology*. AACC International, St. Paul, MN, USA, pp. 363-399.

Coste, C., Dubois, M., 2008. Use of ozone for improving kneading. United States Patent 0044519.

Decamps, K., Joye, I.J., Courtin, C.M., Delcour, J.A., 2012a. Glucose and pyranose oxidase improve bread dough stability. *Journal of Cereal Science* **55**: 380-384.

Decamps, K., Joye, I.J., Haltrich, D., Nicolas, J., Courtin, C.M., Delcour, J.A., 2012b. Biochemical characteristics of *Trametes multicolor* pyranose oxidase and *Aspergillus niger* glucose oxidase and implications for their functionality in wheat flour doughs. *Food Chemistry* **131**: 1485-1492.

ACCEPTED MANUSCRIPT

- Delcour, J.A., Hoseney, R.C., 2010. Principles of Cereal Science and Technology (3rd Edition). AACC International, St. Paul, MN, USA, 270 p.
- Delcros, J.F., Rakotozafy, L., Boussard, A., Davidou, S., Porte, C., Potus, J., Nicolas, J., 1998. Effect of mixing conditions on the behavior of lipoxygenase, peroxidase, and catalase in wheat flour doughs. *Cereal Chemistry* **75**: 85-93.
- Dobraszczyk, B.J., 2003. Measuring the rheological properties of dough. In: Cauvain, S.P. (Ed.) Bread making: Improving quality. Woodhead Publishing Limited, Cambridge, UK, pp. 375-400.
- Dronzek, B., Bushuk, W., 1968. A note on formation of free radicals in dough during mixing. *Cereal Chemistry* **45**: 286.
- Dunnewind, B., van Vliet, T., Orsel, R., 2002. Effect of oxidative enzymes on bulk rheological properties of wheat flour doughs. *Journal of Cereal Science* **36**: 357-366.
- Dykes, L., Rooney, L.W., 2007. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World* **52**: 105-111.
- Every, D., Gilpin, M., Larsen, N.G., 1996. Ascorbate oxidase levels in wheat and their relationship to baking quality. *Journal of Cereal Science* **23**: 145-151.
- Eyoun, A., Celhay, F., Néron, S., El Amrani, F., Boussard, A., Poiffait, A., Potus, J., Baret, J.L., Nicolas, J., 2003. Biochemical factors of importance in the oxygen consumption on unyeasted and yeasted wheat flours during mixing. In: Courtin, C.M., Veraverbeke, W.S., Delcour, J.A. (Eds.), Recent Advances in Enzymes in Grain Processing. Katholieke Universiteit Leuven, Leuven, Belgium, pp. 303-309.
- Faccio, G., 2011. Discovery of oxidative enzymes for food engineering. VTT, University of Helsinki, Finland, 105 p.

ACCEPTED MANUSCRIPT

- Faccio, G., Flander, L., Buchert, J., Saloheimo, M., Nordlund, E., 2012. Sulfhydryl oxidase enhances the effects of ascorbic acid in wheat dough. *Journal of Cereal Science* **55**: 37-43.
- Fan, J.T., Mitchell, J.R., Blanshard, J.M.V., 1999. A model for the oven rise of dough during baking. *Journal of Food Engineering* **41**: 69-77.
- Farver, O., Pecht, I., 1992. Low activation barriers characterize intramolecular electron-transfer in ascorbate oxidase. *Proceedings of the National Academy of Sciences of the United States of America* **89**: 8283-8287.
- Faubion, J.M., Hoseney, R.C., 1981. Lipoxygenase: Its biochemistry and role in breadmaking. *Cereal Chemistry* **58**: 175-180.
- Figueroa-Espinoza, M.C., Rouau, X., 1998. Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidase: Mechanism of linkage between feruloylated arabinoxylans. *Cereal Chemistry* **75**: 259-265.
- Finney, P.L., Magoffin, C.D., Hoseney, R.C., Finney, K.F., 1976. Short-time baking systems. 1. Interdependence of yeast concentration, fermentation time, proof time, and oxidation requirement. *Cereal Chemistry* **53**: 126-134.
- Flander, L., Rouau, X., Morel, M.H., Autio, K., Seppanen-Laakso, T., Kruus, K., Buchert, J., 2008. Effects of laccase and xylanase on the chemical and rheological properties of oat and wheat doughs. *Journal of Agricultural and Food Chemistry* **56**: 5732-5742.
- Garcia, R., Kaid, N., Vignaud, C., Nicolas, J., 2000. Purification and some properties of catalase from wheat germ (*Triticum aestivum L.*). *Journal of Agricultural and Food Chemistry* **48**: 1050-1057.

ACCEPTED MANUSCRIPT

- Garcia, R., Rakotozafy, L., Telef, N., Potus, J., Nicolas, J., 2002. Oxidation of ferulic acid or arabinose-esterified ferulic acid by wheat germ peroxidase. *Journal of Agricultural and Food Chemistry* **50**: 3290-3298.
- Gelinas, P., Poitras, E., McKinnon, C.M., Morin, A., 1998. Oxido-reductases and lipases as dough-bleaching agents. *Cereal Chemistry* **75**: 810-814.
- Girotti, A.W., 1998. Lipid hydroperoxide generation, turnover, and effector action in biological systems. *Journal of Lipid Research* **39**: 1529-1542.
- Graveland, A., 1968. Combination of thin layer chromatography and gas chromatography in analysis on a microgram scale of lipids from wheat flour and wheat flour doughs. *Journal of the American Oil Chemists Society* **45**: 834-840.
- Graveland, A., 1970. Enzymatic oxidations of linoleic acid and glycerol-1-monolinoleate in doughs and flour-water suspensions. *Journal of the American Oil Chemists Society* **47**: 352-361.
- Graveland, A., Bosveld, P., Lichtendonk, W.J., Moonen, J.H.E., 1980. Superoxide involvement in the reduction of disulfide bonds of wheat gel proteins. *Biochemical and Biophysical Research Communications* **93**: 1189-1195.
- Grosch, W., Wieser, H., 1999. Redox reactions in wheat dough as affected by ascorbic acid. *Journal of Cereal Science* **29**: 1-16.
- Gul, H., Ozer, M.S., Dizlek, H., 2009. Improvement of the wheat and corn bran bread quality by using glucose oxidase and hexose oxidase. *Journal of Food Quality* **32**: 209-223.
- Halliwell, B., 1989. Tell me about free radicals, doctor: A review. *Journal of the Royal Society of Medicine* **82**: 747-752.

ACCEPTED MANUSCRIPT

- Han, M.J., Choi, H.T., Song, H.G., 2005. Purification and characterization of laccase from the white rot fungus *Trametes versicolor*. *Journal of Microbiology* **43**: 555-560.
- Hanft, F., Koehler, P., 2005. Quantitation of dityrosine in wheat flour and dough by liquid chromatography-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* **53**: 2418-2423.
- Hansen, O.C., Stougaard, P., 1997. Hexose oxidase from the red alga *Chondrus crispus*: Purification, molecular cloning, and expression in *Pichia pastoris*. *Journal of Biological Chemistry* **272**: 11581-11587.
- Hilhorst, R., Gruppen, H., Orsel, R., Laane, C., Schols, H.A., Voragen, A.G.J., 2002. Effects of xylanase and peroxidase on soluble and insoluble arabinoxylans in wheat bread dough. *Journal of Food Science* **67**: 497-506.
- Honold, G.R., Stahmann, M.A., 1968. The oxidation-reduction enzymes of wheat. IV. Qualitative and quantitative investigations of oxidases. *Cereal Chemistry* **45**: 99-108.
- Hoseney, R.C., Faubion, J.M., 1981. A mechanism for the oxidative gelation of wheat flour water soluble pentosans. *Cereal Chemistry* **58**: 421-424.
- Hoseney, R.C., Hsu, K.H., Junge, R.C., 1979. Simple spread test to measure the rheological properties of fermenting dough. *Cereal Chemistry* **56**: 141-143.
- Hoseney, R.C., Rao, H., Faubion, J., Sidhu, J.S., 1980. Mixograph Studies. 4. The mechanism by which lipoxygenase increases mixing tolerance. *Cereal Chemistry* **57**: 163-166.
- Jamnongwong, M., Loubiere, K., Dietrich, N., Hebrard, G., 2010. Experimental study of oxygen diffusion coefficients in clean water containing salt, glucose or surfactant: Consequences on the liquid-side mass transfer coefficients. *Chemical Engineering Journal* **165**: 758-768.

ACCEPTED MANUSCRIPT

- Jayaram, V.B., Cuyvers, S., Lagrain, B., Verstrepen, K.J., Delcour, J.A., Courtin, C.M., 2013. Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. *Food Chemistry* **136**: 301-308.
- Ji, P.J., Feng, W., Tan, T.W., Zheng, D.X., 2007. Modeling of water activity, oxygen solubility and density of sugar and sugar alcohol solutions. *Food Chemistry* **104**: 551-558.
- Joye, I.J., Draganski, A., Delcour, J.A., Ludescher, R.D., 2012. Monitoring molecular oxygen depletion in wheat flour dough using Erythrosin B phosphorescence: A biophysical approach. *Food Biophysics* **7**: 138-144.
- Junge, R.C., Hoseney, R.C., Varrianomarston, E., 1981. Effect of surfactants on air incorporation in dough and the crumb grain of bread. *Cereal Chemistry* **58**: 338-342.
- Junqueira, R.M., Rocha, F., Moreira, M.A., Castro, I.A., 2007. Effect of proofing time and wheat flour strength on bleaching, sensory characteristics, and volume of French breads with added soybean lipoxygenase. *Cereal Chemistry* **84**: 443-449.
- Kaufman, S.P., Fennema, O., 1987. Evaluation of sulphhydryl oxidase as a strengthening agent for wheat flour dough. *Cereal Chemistry* **64**: 172-176.
- Kerfeld, C.A., Krogmann, D.W., 1998. Photosynthetic cytochromes c in cyanobacteria, algae, and plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 397-425.
- Koehler, P., 2003. Effect of ascorbic acid in dough: Reaction of oxidized glutathione with reactive thiol groups of wheat glutelin. *Journal of Agricultural and Food Chemistry* **51**: 4954-4959.
- Koppenol, W.H., 1990. Oxyradical reactions: From bond-dissociation energies to reduction potentials. *Febs Letters* **264**: 165-167.

ACCEPTED MANUSCRIPT

- Korycka-Dahl, M., Richardson, T., 1980. Initiation of oxidative changes in foods. *Journal of Dairy Science* **63**: 1181-1198.
- Kotiaho, T., Eberlin, M.N., Vainiotalo, P., Kostiainen, R., 2000. Electrospray mass and tandem mass spectrometry identification of ozone oxidation products of amino acids and small peptides. *Journal of the American Society for Mass Spectrometry* **11**: 526-535.
- Kruger, J.E., 1976. Changes in polyphenol oxidases of wheat during kernel growth and maturation. *Cereal Chemistry* **53**: 201-213.
- Kruger, J.E., 1977. Changes in the catalases of wheat during kernel growth and maturation. *Cereal Chemistry* **54**: 820-826.
- Labat, E., Morel, M.H., Rouau, X., 2000. Effects of laccase and ferulic acid on wheat flour doughs. *Cereal Chemistry* **77**: 823-828.
- Lai, L.S., Chang, P.C., Chano, C.T., 2008. Isolation and characterization of superoxide dismutase from wheat seedlings. *Journal of Agricultural and Food Chemistry* **56**: 8121-8129.
- Leitner, C., Volc, J., Haltrich, D., 2001. Purification and characterization of pyranose oxidase from the white rot fungus *Trametes multicolor*. *Applied and Environmental Microbiology* **67**: 3636-3644.
- Levavasseur, L., Rakotozafy, L., Manceau, E., Louarme, L., Robert, H., Baret, J.L., Potus, J., Nicolas, J., 2006. Discrimination of wheat varieties by simultaneous measurements of oxygen consumption and consistency of flour dough during mixing. *Journal of the Science of Food and Agriculture* **86**: 1688-1698.

ACCEPTED MANUSCRIPT

- Liang, X., Kaya, A., Zhang, Y., Le, D.T., Hua, D., Gladyshev, V.N., 2012. Characterization of methionine oxidation and methionine sulfoxide reduction using methionine-rich cysteine-free proteins. *Bmc Biochemistry* **13**: 1-10.
- Liao, Y.E., Miller, R.A., Hoseney, R.C., 1998. Role of hydrogen peroxide produced by Baker's yeast on dough rheology. *Cereal Chemistry* **75**: 612-616.
- Lösche, K., von Bargen, M., Weissbach, A., 2011. Method for making dough. Patent EP 2340715 A2.
- Lullien-Pellerin, V., 2012. Ozone in grain processing. In: O'Donnell, C., Tiwari, B., Cullen, P.J., Rice, R.G. (Eds.), *Ozone in Food Processing*. Wiley-Blackwell, Chichester, West-Sussex, UK, pp. 81-96.
- MacRitchie, F., 1975. Mechanical degradation of gluten proteins during high-speed mixing of doughs. *Journal of Polymer Science: Polymer Symposia* **49**: 85-90.
- Manu, B.T., Rao, U.J.S.P., 2011. Role of peroxidase and H_2O_2 in cross-linking of gluten proteins. *Journal of Food Biochemistry* **35**: 1695-1702.
- Marsh, D.R., Galliard, T., 1986. Measurement of polyphenol oxidase activity in wheat-milling fractions. *Journal of Cereal Science* **4**: 241-248.
- Marston, P.E., 1986. Dough development for breadmaking under controlled atmospheres. *Journal of Cereal Science* **4**: 335-344.
- Mayer, A.M., 1987. Polyphenol oxidases in plants: Recent progress. *Phytochemistry* **26**: 11-20.
- McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry* **244**: 6049-6055.

ACCEPTED MANUSCRIPT

- Min, D.B., Boff, J.M., 2002. Chemistry and reaction of singlet oxygen in foods. *Comprehensive Reviews of Food Science and Food Safety* **1**: 58-72.
- Miyamoto, Y., Nishimura, K., 2006. Production of thiyl radical on a peptide derived from wheat protein by superoxide anion radical. *Cereal Chemistry* **83**: 472-477.
- Morrison, W.R., 1978. Wheat lipid composition. *Cereal Chemistry* **55**: 548-558.
- Nakamura, M., Kurata, T., 1997. Effect of L-ascorbic acid and superoxide anion radical on the rheological properties of wheat flour water dough. *Cereal Chemistry* **74**: 651-655.
- Necochea, R., Valderrama, B., Diaz-Sandoval, S., Folch-Mallol, J.L., Vazquez-Duhalt, R., Iturriaga, G., 2005. Phylogenetic and biochemical characterisation of a recombinant laccase from *Trametes versicolor*. *Fems Microbiology Letters* **244**: 235-241.
- Neukom, H., Markwalder, H.U., 1978. Oxidative gelation of wheat-flour pentosans: New way of cross-linking polymers. *Cereal Foods World* **23**: 374-376.
- Oudgenoeg, G., Hilhorst, R., Piersma, S.R., Boeriu, C.G., Gruppen, H., Hessing, M., Voragen, A.G.J., Laane, C., 2001. Peroxidase-mediated cross-linking of a tyrosine-containing peptide with ferulic acid. *Journal of Agricultural and Food Chemistry* **49**: 2503-2510.
- Packer, J.E., 1974. The radiation chemistry of thiols. In: Patai, S. (Ed.) *The chemistry of the thiol group*. John Wiley & Sons, Bristol, United Kingdom, pp. 481-517.
- Pareyt, B., Finnie, S.M., Putseys, J.A., Delcour, J.A., 2011. Lipids in bread making: Sources, interactions, and impact on bread quality. *Journal of Cereal Science* **54**: 266-279.
- Pătulea, A., Băran, N., Călușaru, I.M., 2012. Measurements of dissolved oxygen concentration in stationary water. *World Environment* **2**: 104-109.

ACCEPTED MANUSCRIPT

- Perrone, G.G., Tan, S.X., Dawes, I.W., 2008. Reactive oxygen species and yeast apoptosis. *Biochimica et Biophysica Acta - Molecular Cell Research* **1783**: 1354-1368.
- Pfeilsticker, K., Roeung, S., 1982. Characterization of L-ascorbic acid oxidase (EC 1.10.3.3) from wheat flour. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* **174**: 306-308.
- Piber, M., Koehler, P., 2005. Identification of dehydro-ferulic acid-tyrosine in rye and wheat: Evidence for a covalent cross-link between arabinoxylans and proteins. *Journal of Agricultural and Food Chemistry* **53**: 5276-5284.
- Posner, E.S., 2009. Wheat flour milling. In: Khan, K., Shewry, P.R. (Eds.), *Wheat: Chemistry and Technology*. AACC International, St. Paul, MN, USA, pp. 119-152.
- Potus, J., Nicolas, J., 2010. L'oxygène, un ingrédient oublié de la pâte à pain. *Industries des Céréales* **166**: 3-10.
- Poulsen, C., Hostrup, P.B., 1998. Purification and characterization of a hexose oxidase with excellent strengthening effects in bread. *Cereal Chemistry* **75**: 51-57.
- Primo-Martin, C., Valera, R., Martinez-Anaya, M.A., 2003. Effect of pentosanase and oxidases on the characteristics of doughs and the glutenin macropolymer (GMP). *Journal of Agricultural and Food Chemistry* **51**: 4673-4679.
- Pryor, W.A., Das, B., Church, D.F., 1991. The ozonation of unsaturated fatty acids: Aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. *Chemical Research in Toxicology* **4**: 341-348.
- Salt, L.J., Wilde, P.J., Georget, D., Wellner, N., Skeggs, P.K., Mills, E.N.C., 2006. Composition and surface properties of dough liquor. *Journal of Cereal Science* **43**: 284-292.

ACCEPTED MANUSCRIPT

- Sandhu, H.P.S., Manthey, F.A., Simsek, S., Ohm, J.B., 2011. Comparison between potassium bromate and ozone as flour oxidants in breadmaking. *Cereal Chemistry* **88**: 103-108.
- Schooneveld-Bergmans, M.E.F., Dignum, M.J.W., Grabber, J.H., Beldman, G., Voragen, A.G.J., 1999. Studies on the oxidative cross-linking of feruloylated arabinoxylans from wheat flour and wheat bran. *Carbohydrate Polymers* **38**: 309-317.
- Selinheimo, E., Autio, K., Krijus, K., Buchert, J., 2007. Elucidating the mechanism of laccase and tyrosinase in wheat bread making. *Journal of Agricultural and Food Chemistry* **55**: 6357-6365.
- Sharma, V.K., Graham, N.J.D., 2010. Oxidation of amino acids, peptides and proteins by ozone: A review. *Ozone - Science & Engineering* **32**: 81-90.
- Shewry, P.R., Popineau, Y., Lafiandra, D., Belton, P., 2001. Wheat glutenin subunits and dough elasticity: Findings of the Eurowheat project. *Trends in Food Science & Technology* **11**: 433-441.
- Shogren, M.D., Finney, K.F., 1984. Bread-making test for 10 grams of flour. *Cereal Chemistry* **61**: 418-423.
- Sidhu, J.S., Nordin, P., Hoseney, R.C., 1980. Mixograph Studies. 3. Reaction of fumaric acid with gluten proteins during dough mixing. *Cereal Chemistry* **57**: 159-163.
- Sievert, D., Hoseney, R.C., Delcour, J.A., 2008. Bread and other baked products. In: Ullman's Encyclopedia of Industrial Chemistry. John Wiley & Sons, Inc., NY, pp. 259-316.
- Stadtman, E.R., 1990. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. *Free Radical Biology and Medicine* **9**: 315-325.

ACCEPTED MANUSCRIPT

- Sullivan, J.D., Ikawa, M., 1973. Purification and characterization of hexose oxidase from red alga *Chondrus crispus*. *Biochimica Et Biophysica Acta* **309**: 11-22.
- Takasaki, S., Kato, Y., Murata, M., Homma, S., Kawakishi, S., 2005. Effects of peroxidase and hydrogen peroxide on the dityrosine formation and the mixing characteristics of wheat-flour dough. *Bioscience Biotechnology and Biochemistry* **69**: 1686-1692.
- Taneja, S.R., Abrol, Y.P., Sachar, R.C., 1974. Modulation of o-diphenolase and monophenolase enzymes during wheat development. *Cereal Chemistry* **51**: 457-465.
- Tieckelmann, R.E., Steele, R.E., 1991. Higher-assay grade of calcium peroxide improves properties of dough. *Food Technology* **45**: 106-108.
- Tilley, K.A., Benjamin, R.E., Bagorogoza, K.E., Okot-Kotber, B.M., Prakash, O., Kwen, H., 2001. Tyrosine cross-links: Molecular basis of gluten structure and function. *Journal of Agricultural and Food Chemistry* **49**: 2627-2632.
- Tsen, C.C., Bushuk, W., 1963. Changes in sulfhydryl and disulfide contents of doughs during mixing under various conditions. *Cereal Chemistry* **40**: 399-408.
- Tsen, C.C., Hlynka, I., 1962. The role of lipids in oxidation of doughs. *Cereal Chemistry* **39**: 209-219.
- Turrens, J.F., 2003. Mitochondrial formation of reactive oxygen species. *Journal of Physiology - London* **552**: 335-344.
- Van Dyck, S., 2007. The impact of singlet oxygen on lipid oxidation. *Lipid Technology* **19**: 278-280.

ACCEPTED MANUSCRIPT

- Van Overveld, F.W.P.C., Haenen, G.R.M.M., Rhemrev, J., Vermeiden, H.P.W., Bast, A., 2000. Tyrosine as important contributor to the antioxidant capacity of seminal plasma. *Chemico-Biological Interactions* **127**: 151-161.
- Vemulapalli, V., Miller, K.A., Hoseney, R.C., 1998. Glucose oxidase in breadmaking systems. *Cereal Chemistry* **75**: 439-442.
- Veraverbeke, W.S., Delcour, J.A., 2002. Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition* **42**: 179-208.
- Violleau, F., Pernot, A.-G., Surel, O., 2012. Effect of Oxygreen wheat ozonation process on bread dough quality and protein solubility. *Journal of Cereal Science* **55**: 392-396.
- Wallace, J.M., Wheeler, E.L., 1975. Lipoxygenase from wheat: Examination of its reaction characteristics. *Journal of Agricultural and Food Chemistry* **23**: 146-150.
- Wang, Z.T., Zhu, L., Yin, F., Su, Z.Q., Li, Z.D., Li, C.Z., 2012. Silver-catalyzed decarboxylative chlorination of aliphatic carboxylic acids. *Journal of the American Chemical Society* **134**: 4258-4263.
- Wieser, H., 2003. The use of redox agents in breadmaking. In: Cauvain, S.P. (Ed.) *Bread making: Improving quality*. Woodhead Publishing Limited, Cambridge, UK, pp. 424-446.
- Winterbourn, C.C., Metodiewa, D., 1999. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radical Biology and Medicine* **27**: 322-328.
- Wrigley, C.W., Békés, F., Bushuk, W., 2006. Gluten: A balance of gliadin and glutenin. In: Wrigley, C.W., Békés, F., Bushuk, W. (Eds.), *Gliadin and Glutenin: The unique balance of wheat quality*. AACC International, St. Paul, MN, USA, pp. 3-32.

ACCEPTED MANUSCRIPT

Xu, F., 2001. Adsorption of oxygen gas by hydrated wheat flour. Lebensmittel-Wissenschaft und -Technologie - Food Science and Technology **34**: 66-70.

Yoneyama, T., Suzuki, I., Murohash, M., 1970. Natural maturing of wheat flour. I. Changes in some chemical components and in farinograph and extensigraph properties. Cereal Chemistry **47**: 19-26.

Figure 1.

Ordering of electrons in different molecular orbitals of ${}^3\text{O}_2$ and the two forms of ${}^1\text{O}_2$ (A, B).

Adapted from Choe and Min (2006).

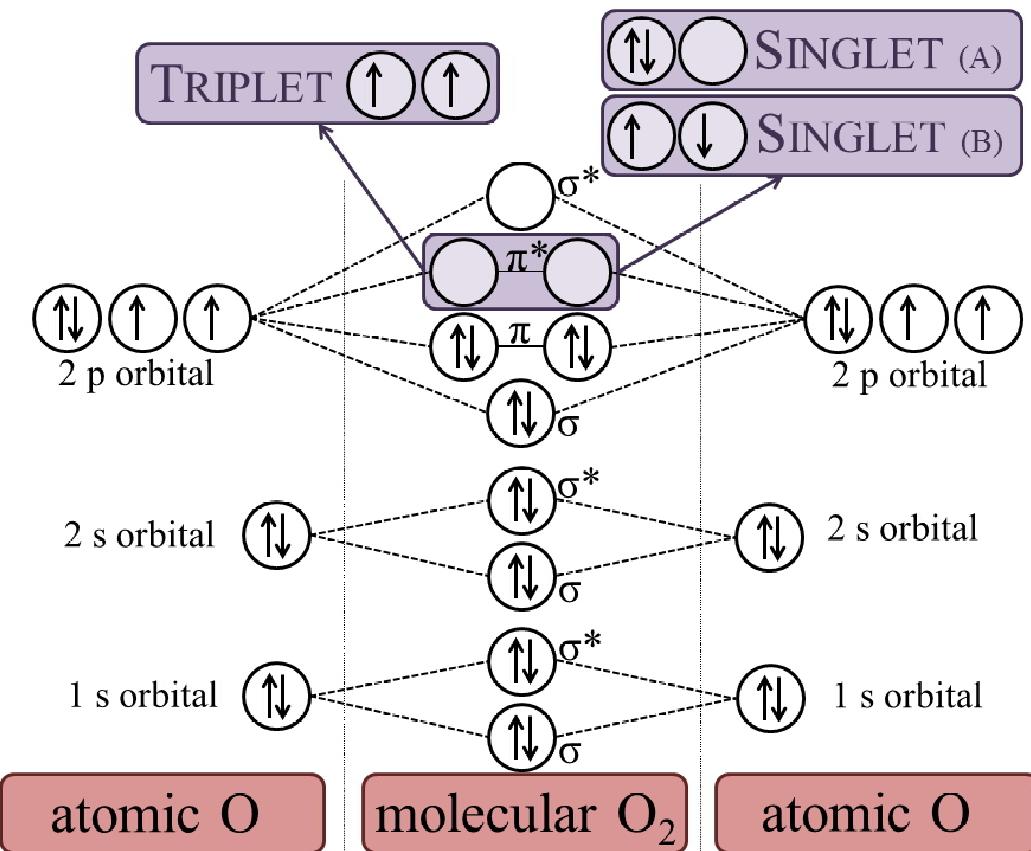


Figure 2.

Reaction pathways of ${}^3\text{O}_2$ and reactive oxygen species.

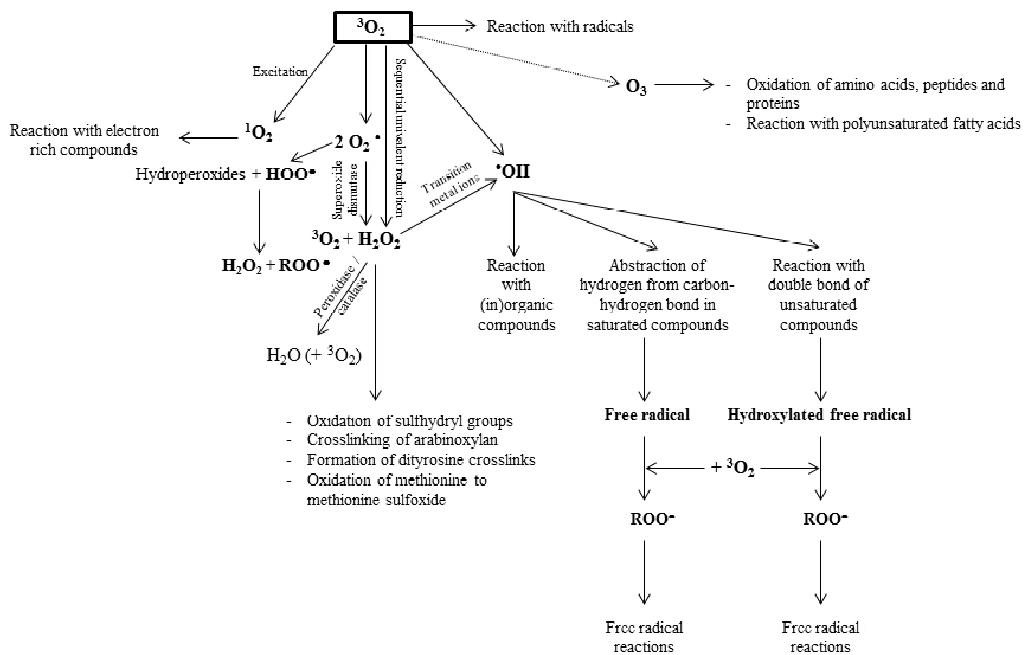
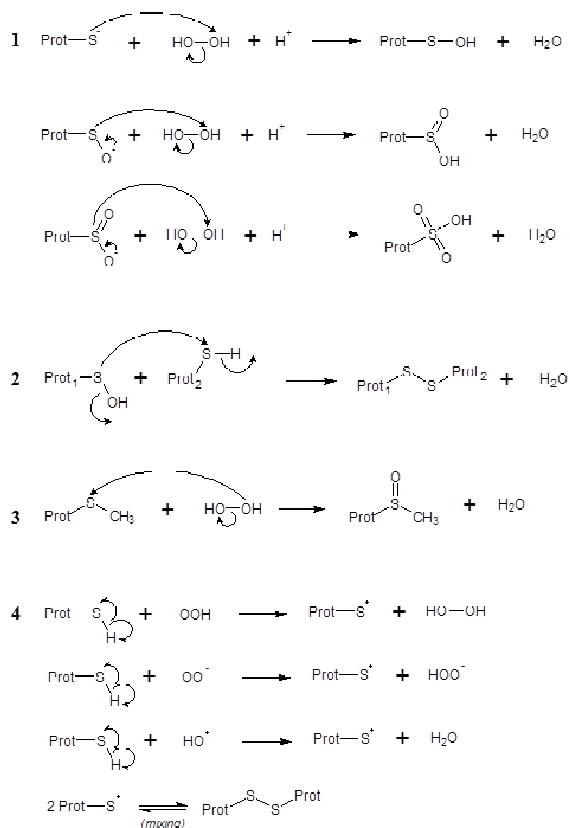
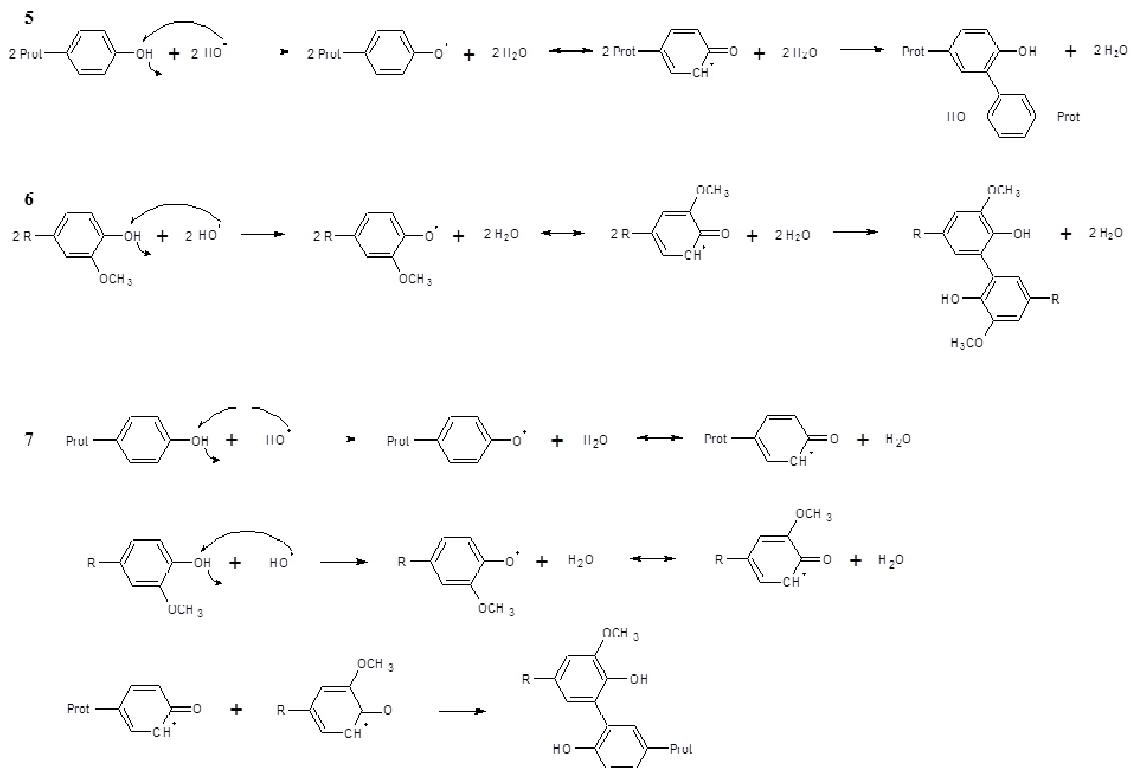


Figure 3.

Overview of some reactions with reactive oxygen species (potentially) occurring in wheat flour dough.





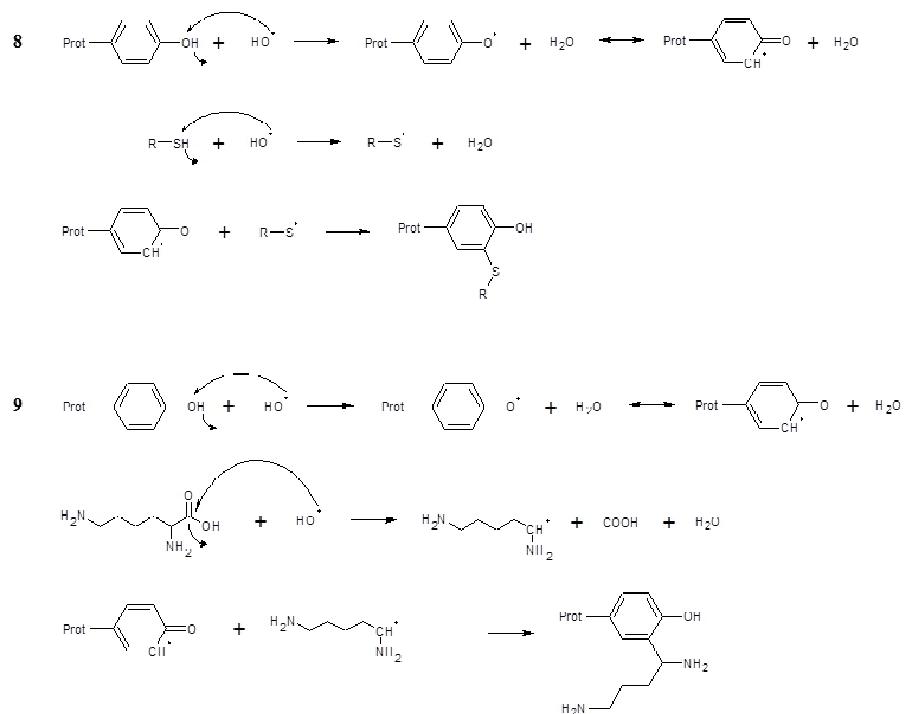


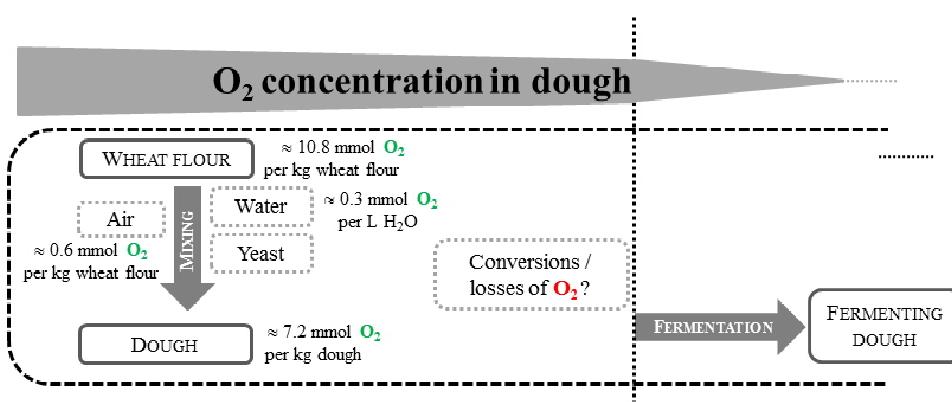
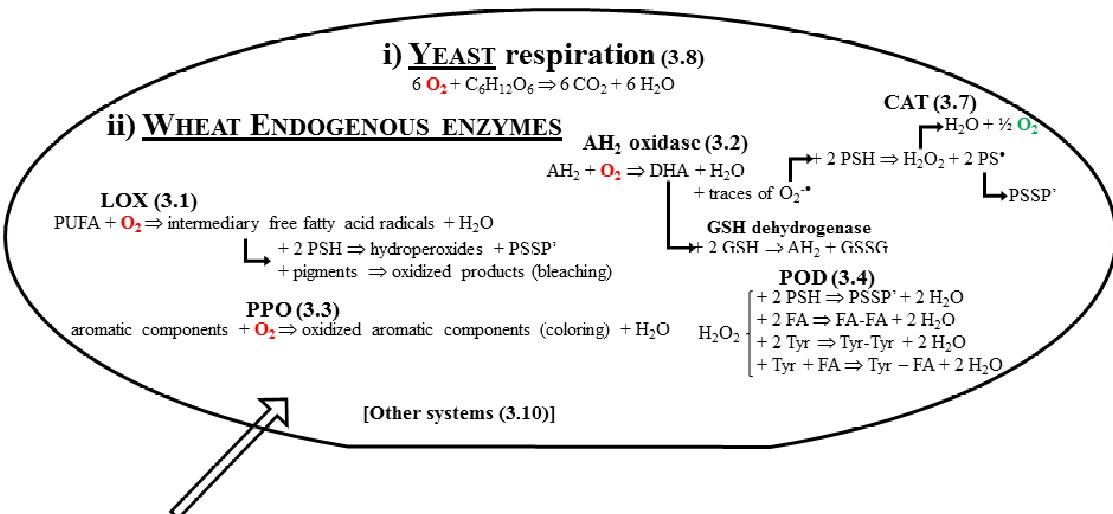
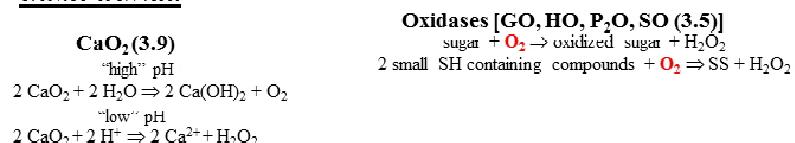
Figure 4.Sources of O₂ in dough.

Figure 5.

Reactions consuming or producing O₂ during breadmaking (with indication of the corresponding section): (i) consumption by yeast respiration; (ii) consumption or production by wheat endogenous enzymes such as lipoxygenase [LOX, oxidation of polyunsaturated fatty acids (PUFA)], ascorbic acid (AH₂) oxidase [oxidation of AH₂ to dehydroascorbic acid (DHA) and traces of O₂[•] and the subsequent oxidation of glutathione (GSH) to oxidized GSH (GSSG) by GSH dehydrogenase], polyphenol oxidase (PPO, oxidation of aromatic components), peroxidase [POD, oxidation of sulphydryl (SH) groups in proteins (PSH) to disulfide bonds in proteins (PSSP')], ferulic acid (FA) to di-FA, tyrosine (Tyr) to di-Tyr or Tyr and FA to Tyr-FA] and catalase (CAT, dismutation of H₂O₂ to H₂O and O₂) or by other systems; (iii) consumption by exogenous enzymes or compounds such as tyrosinase (TYR, oxidation of aromatic components), laccase (LAC, oxidation of aromatic components), oxidases [glucose oxidase (GO), hexose oxidase (HO), pyranose oxidase (P₂O) or sulphydryl oxidase (SO), oxidation of sugar substrates or small SH containing compounds] and CaO₂ (production of O₂ or H₂O₂).



**iii) EXOGENOUS ENZYMES/
COMPOUNDS**



Tables**Table 1.**

Overview of the different O₂ or reactive oxygen species (ROS) consuming or producing systems in dough. Their relative contribution to O₂ and ROS consumption and production and their potential effect on dough properties during breadmaking are also indicated.

Ascorbic acid (AH₂), catalase (CAT), disulfide (SS), glucose oxidase (GO), laccase (LAC), lipoxygenase (LOX), peroxidase (POD), polyphenol oxidase (PPO), pyranose oxidase (P₂O), sulfhydryl (SH), tyrosinase (TYR).

<u>System</u>	<u>O₂ or ROS consuming?</u>	<u>O₂ or ROS producing?</u>	<u>Relative contribution to O₂ and ROS consumption or production</u>	<u>Potential effect on dough properties during breadmaking</u>	<u>References</u>
LOX	x		60% of O ₂ consumption in yeastless dough	Increased mixing tolerance and improved dough rheology	(Faubion and Hoseney, 1981) (Potus and Nicolas, 2010)
Yeast	x		70% of O ₂ consumption of yeasted dough can be explained by LOX and yeast activity	Dough strengthening effect Increased viscosity of water extract of wheat flour upon addition of yeast	(Liao et al., 1998) (Hoseney and Faubion, 1981) (Potus and Nicolas, 2010)
Oxidases	x		74% of O ₂ consumption of yeasted dough supplemented with GO can be explained by LOX, yeast and GO activity	Improved bread dough stability and final quality (for P ₂ O and GO)	(Decamps et al., 2012a) (Potus and Nicolas, 2010)
AH₂ oxidase	x		No information	Effect on dough rheology and final bread quality	(Nakamura and Kurata, 1997)
TYR	x		No information	Increased dough strength and improved bread quality	(Selinheimo et al., 2007)
LAC	x		No information	Effect on dough rheology and improved gluten quality No effect on dough properties or final quality	(Labat et al., 2000; Selinheimo et al., 2007) (Caballero et al., 2007)
PPO	x		Negligible activity	Bleaching effect Improved dough rheology	(Honold and Stahmann, 1968) (Faubion and Hoseney, 1981)
POD	x		Consumption of H ₂ O ₂ , if present (produced) in dough	Effect on SH/SS-interchange No effect on dough mixing characteristics or on total gas production during fermentation (added POD)	(Garcia et al., 2002) (Takasaki et al., 2005)
Metal-ion catalyzed	x		No information	No information	(Stadtman, 1990)

oxidation reactions					
CAT		x	Limited production of O ₂ due to low activity of CAT and low level of H ₂ O ₂	No effect on dough rheology	(Liao et al., 1998)
CaO₂		x	No information	Improved dough handling	(Tieckelmann and Steele, 1991)
Dibenzoyl peroxide		x	No information	No effect on dough properties	(Sievert et al., 2008)