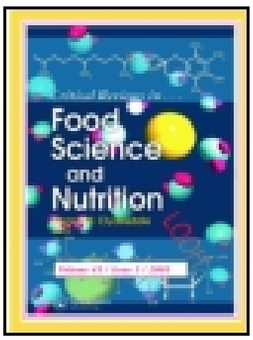

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

History, mechanism of action, and toxicity: a review of commonly used dough rheology improvers

Zhongxin Liang, Jihui Gao, Peixuan Yu & Dong Yang

To cite this article: Zhongxin Liang, Jihui Gao, Peixuan Yu & Dong Yang (2021): History, mechanism of action, and toxicity: a review of commonly used dough rheology improvers, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1956427

To link to this article: https://doi.org/10.1080/10408398.2021.1956427

Published online: 26 Jul 2021.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1956427

Taylor & Francis
Taylor & Francis Group

REVIEW

History, mechanism of action, and toxicity: a review of commonly used dough rheology improvers

Zhongxin Liang*, Jihui Gao*, Peixuan Yu, and Dong Yang

Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, China

ABSTRACT

Dough rheology improvers, which often are oxidative reagents in nature, have long been used in bread-making industry to enhance protein crosslinking and subsequently improve the dough rheological properties and bread qualities. Numerous studies were conducted to explore the effects of these oxidative agents on dough quality improving, however, the underlying mechanism of their action during dough development has not been fully understood. Due to the public health concerns, multiple oxidative reagents were banned in some countries across the world, while others are still permitted in accordance with regulations. Therefore, a comprehensive understanding of their application, significance, and safety in bread manufacturing is necessary. This review aims to provide a detailed information about the evolutionary history of several commonly used oxidants acting as dough rheology improvers, their mechanisms of action, as well as their potential toxicity.

KEYWORDS

Ascorbic acid; azodicarbonamide; dough rheology improvers; peroxides; potassium bromate; potassium iodate

Introduction

Bread, a baking product of the mixture of flour, water, and other ingredients, is very important dietary food for humans. The manual art of bread-making began long before the establishment of food industry. While an over-23,000-year history was found for the use of wheat as part of human diet, bread-making can be dated back to around 12,000 years ago in Mesopotamia or Ancient Egypt (Piperno et al. 2004; Mondal and Datta 2008). Nowadays, breads in different shapes and forms are still widely consumed throughout the world, especially in European countries and the Middle East (Dong and Karboune 2021). As one of the crucial sources of starch and other complex carbohydrates, bread is consumed as a typical staple food in many countries of the world. Hence, the bread industry has paid great attention on improving its commercial quality (Gellynck et al. 2009; Cappelli, Bettaccini, and Cini 2020; Parenti et al. 2021).

Early in the mid-20th century, the bread-making methodology has been changed from traditional bulk fermentation to new mechanical processes relying on exogenous oxidative additives (Zentner 1964). Thereafter, such additives have become an essential part of the bread-making procedures. They are added into flour before baking for bleaching, flour maturing, and dough conditioning (Cappelli et al. 2019). The additives for dough conditioning are usually termed as dough rheology improvers, improvers, enhancers, or strengtheners, which are used to improve the processing characteristics of dough and the overall quality of final baking products. These dough rheology improvers could be various natural, chemical, or microbial-derived additives, among which the oxidative agents are particularly important because of their oxidant effect on dough during mixing process, when the dough is developed via oxygen incorporation and protein network formation in gluten (Wieser 2012). Moreover, the oxidative reaction by the oxidants could also reinforce the disulfide bonds in glutenin, which function as the junctions of gluten protein network, and enhance the stable structure of gluten and dough (Li et al. 2020).

The use of oxidants as dough rheology improvers has a history of over 100 years since the beginning of last century. The most important ones include potassium bromate ($KBrO_3$), potassium iodate (KIO_3), calcium peroxide (CaO_2), acetone peroxides (AP), ascorbic acid (AA), and azodicarbonamide (ADA) (Joye, Lagrain, and Delcour 2009b; Wieser 2012; Shanmugavel et al. 2020; Dai and Tyl 2021), which have relatively low cost, high portability, and long shelf-life. However, their use is not permitted in several countries owing to their potential toxicity on human beings. These oxidants can improve dough/bread quality by modifying the rheological characteristics of dough, such as elasticity, viscosity, cohesiveness, adhesiveness, and extensibility, which greatly influence the status of dough during mixing and mechanical handling, and determine the quality of the final product (e.g., loaf volume, crumb structure, etc.) (Wieser 2012; Shanmugavel et al. 2020). These rheological properties can be examined by different apparatuses, so that the

CONTACT Dong Yang dyang@cau.edu.cn
*These authors contributed equally to this work.

beneficial effects of the oxidants can be quantitatively evaluated as well (Table 1). Whereas, the toxicity of consuming high dosage of these conditioning additives is one of the main concerns for human health. For instance, the use of $KBrO_3$ was banned in most countries, because the presence of its residues in the final baking products which possess high toxicity. To solve the potential toxicity problem, a trend in dough rheology improver development is to find natural molecules that are generally considered as safe. For example, an enzyme in wheat, protein disulfide isomerase (wPDI), was proven to improve dough rheological properties (Fu et al. 2020). Since wPDI was considered absolutely safe due to its natural essence, it has been a promising candidate as dough rheology improver (Pourmohammadi and Abedi 2021). On the other hand, a solid understanding on the context of the effects, underlying mechanisms, and potential toxicity of these oxidants is still necessary for a safer and more efficient application of the current dough rheology improvers in bread-making industry.

This article reviews the history, mechanisms of action, and potential toxicity of oxidative flour treatment agents used as dough rheology improvers. Relevant background information about gluten is also provided for demonstrating the detailed mechanisms of these oxidant agents.

The use of dough rheology improvers

Foreword

To get a better understanding of the mechanisms of the oxidants on improving dough, the importance of gluten for the properties of dough has to be introduced. Gluten, as an essential dough component accounting for approximately 6.5% of the total weight of dough (flour:water = 2:1), is a mixture of numerous diverse proteins, constituting 85%–90% of the endogenous proteins in flour (Biesiekierski 2017). Gliadin and glutenin, identified as the major proteins in gluten over a hundred years ago (Osborne 1907), were recognized as the foundations for the rheological structure of dough. Gliadin mainly contributes to viscosity and extensibility of dough, while glutenin is highly related to strength and elasticity (Wieser 2007). In practice, glutenins are bound by strong covalent force and disulfide bonds, subsequently forming aggregation, the process of which is also described as crosslinking. Being linked with gliadins through non-covalent bonds, they construct a unique and robust protein network in dough, thus shaping its distinct rheological properties (Joye, Lagrain, and Delcour 2009a). Therefore, gluten plays decisive role in the rheological quality of dough.

The beneficial effects of the oxidants are based on the direct or indirect oxidation of thiol groups and disulfide bonds. Disulfide bonds serve as junctions among glutenin protein network. In 1940, scientists recognized the vital roles of both thiol groups and disulfide bonds in determining the characteristics of dough (Sullivan et al. 1940). Frater et al. (1960) hypothesized that the rheological properties of dough are directly related to the following three factors: inner thiol groups, intermolecular disulfide bonds, and the rate of their interchanges. This hypothesis was supported by the study of Mauritzen and Stewart (1963). However, due to the extremely complex formation of large quaternary protein structure during the development of dough, the mechanism through which thiol groups contribute to the changes of the rheological properties of dough is still not well understood. Based on the fact that most of thiol groups are located in either N-terminal or C-terminal of glutenin (Shewry, Halford, and Tatham 1992; D'Ovidio and Masci 2004), the formation of gluten protein network can be roughly depicted as shown in Figure 1. Meanwhile, tyrosine crosslink (dityrosine) was also hypothesized to be present in dough protein matrix (Tilley et al. 2001). Since tyrosine residues are relatively abundant in the high M_r glutenin subunits ($3 \sim 5\%$) (Shewry, Halford, and Tatham 1989), the tyrosine crosslinks might also contribute to the stabilization of wheat gluten structure, similar to the function of disulfide linkages (Tilley et al. 2001). Nonetheless, this hypothesis was contradicted by Hanft and Koehler (2005) and Pena et al. (2006), who suggested that the formation of dityrosine between glutenins is barely able to affect the structure and rheological properties of wheat gluten.

The improving effects from oxidative dough rheology improvers are highly related to the changed inner protein network of dough. Even the use of sodium chloride (NaCl) was suggested to alter the glutenin structure to enhance the effects from rheology improvers (Guo et al. 2021). However, the extent to which rheology improvers can improve dough and bread quality varies, and this is dependent on the quantity used as well as the chemical properties of rheology improver itself. The beneficial effects and limitation of the oxidants commonly used as dough rheology improver are summarized in Tables 1 and 2, respectively.

Oxygen

Oxygen was the first exogenous substance used to facilitate the development of dough. It was revealed that the development of dough fails in the absence of oxygen during mixing process (Baker and Mize 1937). Freilich and Frey (1937) suggested that oxygen indirectly inhibits the proteolytic activity of proteases (papain type) that exert negative impacts on dough properties. However, these negative impacts were later reported to be attributed to glutathione. It was also demonstrated that oxygen could induce considerable changes on sulfhydryl and disulfide contents in dough during mixing procedure (Tsen 1963a). Moreover, oxygen plays an important role in flour maturation and improves the rheological properties of dough (Dempster, Hlynka, and Anderson 1954).

Oxygen from surrounding air is absorbed and incorporated into dough matrix. Xu (2001) demonstrated the detailed oxygen adsorption-desorption process during dough development. Once incorporated into dough matrix, oxygen interacts with dough components, such as pentosan, gluten, starch, fiber, lipid, enzymes, and glutathione, via radical chain reactions (Hird and Yates 1961a; Xu 2001), improving the viscoelasticity and stability of dough and enhancing its

Table 1. Beneficial effects of different additives on various rheological characteristics.[a]

Rheological characteristics	Effect[b]	$KBrO_3$	KIO_3	Peroxides[c]	AA	ADA	Reference
Loaf volume	+	nr (10–30)					Marais and D'appolonia 1981
						nr (2–30)	Joiner, Vidal, and Marks 1963
			nr (3.3–33)				Kohajdová and Karovičová 2010
		* (40)			*** (40)	** (40)	Pereira et al. 2009
			** (30)			* (30)	Yamada and Preston 1992
			* (50)			** (50)	
		* (30)			** (20)		El-Hady, El-Samahy, and Brümmer 1999
Elasticity	+	nr (10–50)			nr (50)		Dong and Hoseney 1995
		* (100)				** (300)	Attenburrow et al. 1990
					** (na)	* (na)	Miller and Hoseney 1999
				nr (1.5–12, C)			
		* (40)	*** (40)		** (100)		Indrani and Rao 2006
					nr (15–35)		Codina et al. 2007
Viscosity	+				** (na)	* (na)	Miller and Hoseney 1999
				nr (1.5–12, C)			
Tenacity	+				nr (15–35)		Codina et al. 2007
					* (60)	** (40)	Zhao et al. 2020
		* (40)	** (40)				Matsumoto et al. 1975
Cohesiveness	+	* (40)	*** (40)		** (100)		Indrani and Rao 2006
		** (1200)	* (1200)		*** (1200)		Tanaka, Endo, and Nagao 1980
						nr (7.5–15)	Joiner, Vidal, and Marks 1963
Adhesiveness		* (40)	*** (40)		** (100)		Indrani and Rao 2006
Extensibility	–	* (40)	*** (40)		** (100)		Indrani and Rao 2006
		* (40)	** (40)		*** (200)		
						nr (12–96)	Tsen 1963b
					nr (15–35)		Codina et al. 2007
				nr (268, A)			Tsen 1964
					* (60)	** (40)	Zhao et al. 2020
		* (40)			** (40)	*** (40)	Pereira et al. 2009
		* (30)			** (20)		El-Hady, El-Samahy, and Brümmer 1999
Hardness	+	* (40)	** (40)		** (100)		Indrani and Rao 2006
Strength	+	* (40)	*** (40)		** (100)		Indrani and Rao 2006
					nr (15–35)		Codina et al. 2007
					** (60)	* (40)	Zhao et al. 2020

[a]"*" in each cell indicates the relative degree of the beneficial effects of different additives, whereas the number indicates the weight of additives used in the experiment (weight unit in mg/kg); "nr" (no rank) indicates no comparative data available in the original article, and "na" indicates "not available." Only the studies employed the closest weight of additives were included for comparison.
[b]"+" indicates increasing effect; "–" indicates decreasing effect.
[c]"C" and "A" indicate calcium oxide and acetone peroxides, respectively.

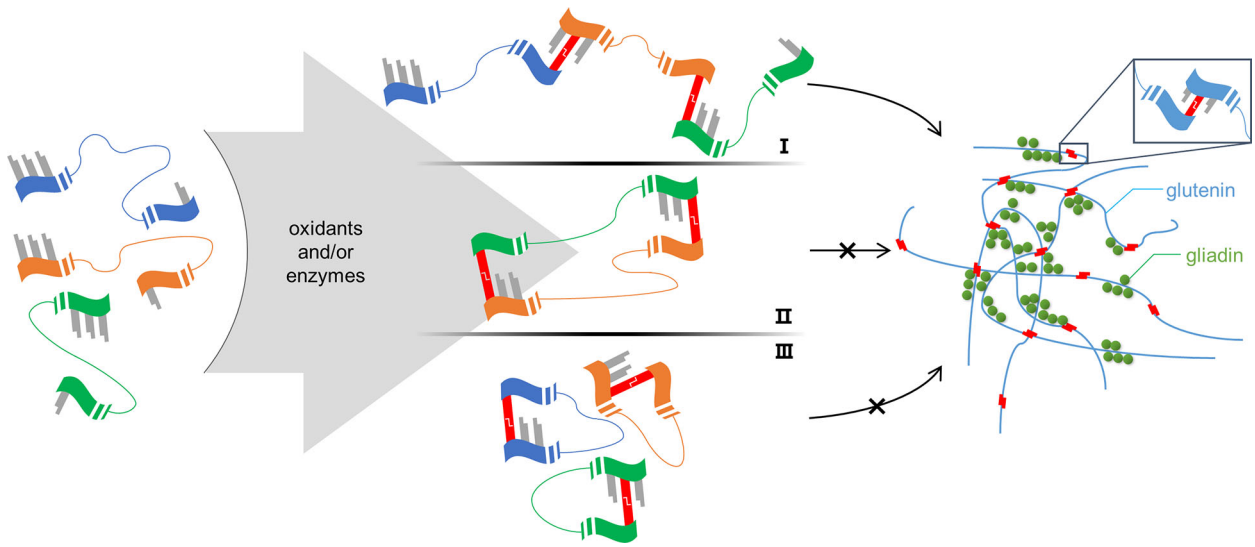

Figure 1. Formation patterns of disulfide bonds and gluten protein network. (I) Rheologically active intermolecular disulfide bonds. (II) Rheologically inactive intermolecular disulfide bonds. (III) Rheologically inactive intramolecular disulfide bonds. Only the rheologically active intermolecular disulfide bonds (I) are able to construct a cohesive protein network (Branlard and Dardevet 1985).

gas cell membrane and gas retention capability (Bloksma and Bushuk 1988). Oxygen can assist in promoting gluten development and improving its rheological properties (Dirndorfer, Kieffer, and Belitz 1986). On the other hand, oxygen is also consumed by exogenous yeast that participates in dough fermentation, which causes volume expansion and flavor change of bread. Moreover, a number of endogenous enzymes also compete for oxygen (Decamps

Table 2. Limitation and toxicity of dough rheology improvers.

Name of additives	Limit of usage (mg/kg)	Common usage in practice (mg/kg)	General quantities of residuals or metabolites in final baked products	LD_{50} (oral route)/ (mg/kg)	JECFA evaluation[a]	References
Potassium bromate	<75 in whole wheat flour (US) <50 in white flour (US) Not included in FAO/WHO Food Standards	15–50	Bromate: detectable levels when 50 mg/kg or above of $KBrO_3$ was add to flour Bromide: not exceed the amount of bromate used	Bromate: 157 for rats Bromide: 3070 for rats 3120 for mice	Not appropriate for use as a flour-treatment agent	21 CFR 137.155, 137.205; Cauvain and Young 2006; DrugFuture 2020; Thewlis 1974
Potassium iodate	<75 in whole wheat flour (US) Not included in FAO/WHO Food Standards	10–30	Iodine: nondetectable to 0.150 mg/slice or 0.063 mg/roll of commercial bakery products	Iodine: 14,000 for rats	Not recommended for use in flour treatment	Wieser 2012; London, Vought, and Brown 1965; Bürgi, Schaffner, and Seiler 2001; Cauvain and Young 2006; DrugFuture 2020
Peroxides	Calcium peroxide: <75 in flour (US) Acetone peroxide: not exceed the quantity of hydrogen peroxide equivalent necessary for the artificial maturing effect (US) Not included in FAO/WHO Food Standards	Calcium peroxide: 20–35 Acetone peroxide: 25	NA[b]	Calcium peroxide: >5000 for rats Calcium oxide: >2000 for rats Calcium hydroxide: 7340 for rats Acetone peroxide: 5230 ± 250 for rats	Not treatment level set	Compass Remediation Chemicals 2017; Oser and Morgareidge 1967; DrugFuture 2020
Ascorbic acid	<200 in flour (US, UK and China, etc.) <300 in flour (FAO/WHO Food Standards)	50–75	Ascorbic acid: Negligible levels	Ascorbic acid: 11,900 for rat 3367 for mice	ADI not specified	Wieser 2012; DrugFuture 2020
Azodicarbonamide	<45 in flour (most countries and FAO/WHO Food Standards)	5–25	ADA: Negligible levels Biurea: not exceed the amount of ADA used Semicarbazide: 0.300–0.400 mg/kg, when 45 mg/kg ADA was added to flour Urethane: 1–3 μg/kg, when 45 mg/kg ADA was added to flour	ADA: >6400 for rats Biurea: >2000 for rats Semicarbazide: 176 for mice Urethane: 1800 for rats 2500 for mice	Acceptable level of treatment: 0–45 mg/kg flour	Joiner, Vidal, and Marks 1963; Oser et al. 1965; Becalski et al. 2004; Noonan, Begley, and Diachenko 2008; Wu and Chen 2017; World Health Organization 1999; Cauvain and Young 2006

[a]JECFA = Joint FAO/WHO Expert Committee on Food Additives.
[b]NA = no data available.

et al. 2016), and several of them (e.g., ascorbic acid oxidase) were found to have influence on dough properties. Overall, during dough development, oxygen is absorbed but rapidly depleted by incorporating into the matrix, resulting in an anaerobic condition of dough quickly after the mixing process (Joye et al. 2012).

Based on the function of oxygen in dough development, various applications of atmospheric oxygen in bread-making have been established. The potential application of pure oxygen has also been proposed (Tsen 1963a), though it is barely portable and requires high costs. Most of the latest studies consider oxygen as redundant since it negatively affects the bread shelf life (Kütahneci and Ayhan 2021), and both scientists and manufacturers have been looking for liquid or solid additives that are both less expensive and more convenient to produce, store, and application in bread manufacturing.

Potassium bromate

$KBrO_3$ was worldwide used as a dough enhancer to improve the quality of bread. The structure of $KBrO_3$ is demonstrated in Figure 2a. The use of $KBrO_3$ in bread-making industry began from the last century (Kohman, Hoffman, and Godfrey 1915). In 1941, the US Food and Drug Administration (FDA) approved its use at a limited level of less than 75 parts per million (ppm) in bromated flour, and in 1952, its use in bread and rolls at the same level was

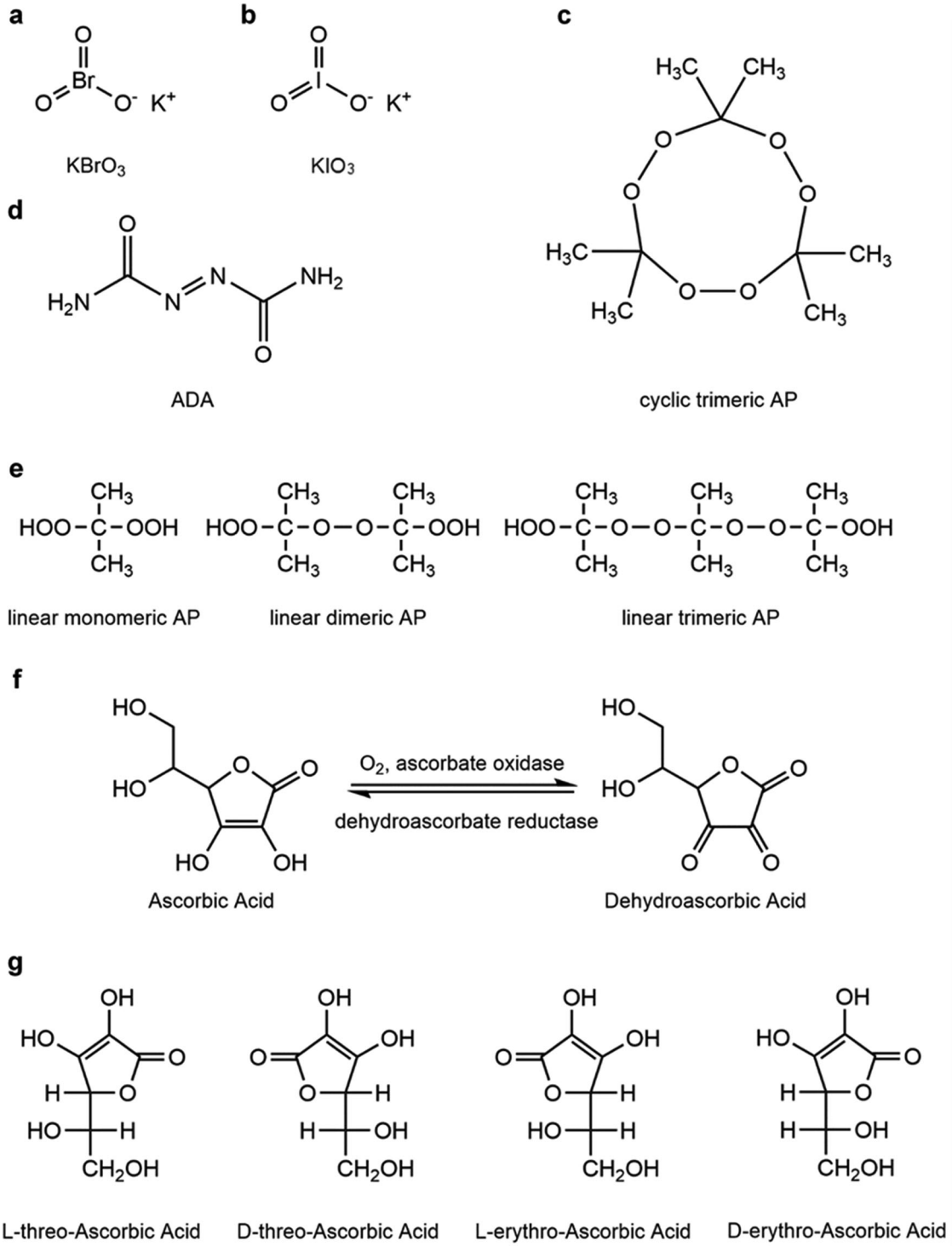

Figure 2. Molecular structures of chemicals as dough rheology improvers discussed in this review. (a) Potassium bromate (KBrO3); (b) Potassium iodate (KIO3); (c) Cyclic trimeric acetone peroxide (AP) as main product generated from the reaction between hydrogen peroxide and acetone; (d) Azodicarbonamide (ADA); (e) AP components used as food additives; (f) Ascorbic acid (AA) and dehydroascorbic acid (DAA) and they transformation pathway; (g) Fischer projection structures of four AA isomers.

further approved. Multiple previous studies have demonstrated that $KBrO_3$ can influence the loaf volume of bread and various rheological properties of dough (Table 1) (Tanaka, Endo, and Nagao 1980; Marais and D'appolonia 1981; Dong and Hoseney 1995). However, considering the detrimental impact of $KBrO_3$ on human health, it was classified as a potential human carcinogen and listed in the 2B group by the International Agency for Research on Cancer (IARC) at 1987. Later, the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) denied its use as an additive in bread at 1992. Whereas in contrast, $KBrO_3$ is still allowed to be used in bread-making by FDA at restricted levels.

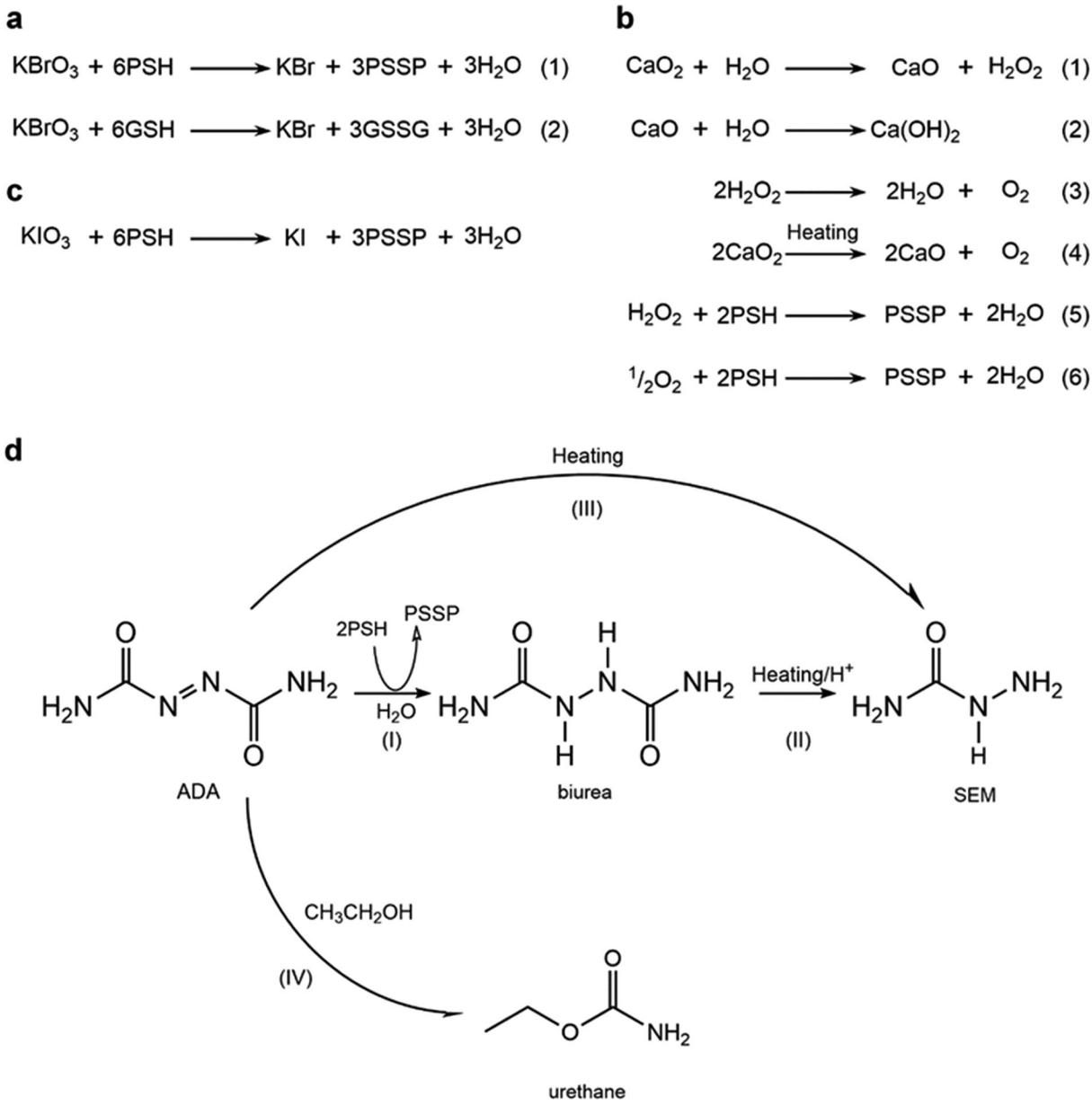

Figure 3. Dough improving reactions of four typical dough rheology improvers. (a) Equations of the reaction between KBrO3 and gluten protein (PSH and PSSP represent the reduced and oxidized forms, respectively) (1) or glutathione (GSH and GSSG represent the reduced and oxidized forms, respectively) (2). (b) Equations for the reactions of calcium oxide (CaO2) in dough. (c) Equations of the reaction between KIO3 and gluten protein. (d) Reaction pathways of azodicarbonamide (ADA) and its byproducts during bread-making process. The transformation of ADA into urethane in the presence of ethanol is speculative (IV).

Mechanism of action

$KBrO_3$ was originally considered as a yeast nutrient which can improve the quality of bread by activating yeasts in dough and then increasing gas retention of the bread (Bailey 1925). An early study by Geddes (1930) hypothesized that $KBrO_3$ could influence the solubility of endogenous phosphatides present in flour, the optimal quantity of which is essential for improving the bread-making quality. In 1936, a proteolytic mechanism was proposed by Jorgensen (1936), who suggested that $KBrO_3$, acting as an oxidant, can interact with the activators of flour innate proteases, thus inhibiting their activity and protecting the integrity of structural proteins in dough. However, several opposite views against this theory were proposed soon (Read and Haas 1937; Sullivan et al. 1940). It was suggested that glutathione (GSH), instead of protease, is responsible for the deleterious effects on the structural proteins in dough, and this GSH theory was confirmed and widely accepted since 1964 (Kuninori 1964).

Based on the GSH theory, GSH and other sulfhydryl compounds can inhibit the interchanges within the sulfhydryl-disulfide system in gluten, while the formation of sufficient disulfide bonds in the structural proteins was essential for the quality of bread-making (Bloksma and Bushuk 1988). The sulfhydryl groups in the reduced GSH can be oxidized by $KBrO_3$, forming disulfide bonds, and therefore, ensuring the baking quality. The equation of the reaction between glutathione and $KBrO_3$ is showed in Figure 3a (2). Moreover, further studies demonstrated that

gluten sulfhydryl-disulfide interchanges can be directly promoted by $KBrO_3$ as well (Figure 3a (1)) (Tsen 1963a), which is independent from GSH removal (Bloksma 1972).

Therefore, the beneficial effect of $KBrO_3$ on improving dough is twofold: it inhibits the detrimental effects of GSH on dough by oxidizing GSH in flour; it also directly oxidizes the sulfhydryl groups into disulfide bonds in dough, which is recognized as the main functional mechanism of $KBrO_3$. Besides, as a relatively slow-acting rheology improver, $KBrO_3$ is believed to have minimum impact on dough during the mixing process, while its reaction rate accelerates during fermentation and baking when pH is low and temperature is high (Himata et al. 2000; Wieser 2012). However, the slow action of $KBrO_3$ results in a lagged improvement on dough quality within a certain time period of reaction compared with other oxidative rheology improvers (Table 1).

Toxicity

$KBrO_3$ was originally thought to be converted into bromide (Br^-) during the baking process, ending with a non-detectable level in bread, whereas its presence in final baking products have been increasingly detected (Bushuk and Hlynka 1960; Thewlis 1974).

According to the first evaluation of the toxicity of $KBrO_3$ by JECFA in 1964, no significant toxic effect of $KBrO_3$ was shown on different animal models based on both short-term and long-term studies ($KBrO_3$ dosage in flour: 14–627 ppm). Hence, it was concluded that a treatment level of less than 75 ppm of $KBrO_3$ in flour is nontoxic for human health by WHO, which was reiterated again in the 27th meeting of JECFA in 1983. However, scientists have started to investigate the carcinogenic effect of $KBrO_3$ since 1974. At 1985, the outcomes from a further toxicological study in Japan based on rat model suggested that oral administration of $KBrO_3$ at levels of 250 and 500 ppm for 110 weeks could significantly increase the incidence of tumor formation in various organs of the rats (Kurokawa et al. 1986). Another study conducted based on Long-Evans rats illustrated that intraperitoneal injection of $KBrO_3$ can also considerably increase the possibility of chromosome aberrations in bone marrow cells of the rats (Fujie et al. 1988). Furthermore, clinical evidences indicated that $KBrO_3$ can induce complex symptoms of poisoning in human bodies, such as vomiting, diarrhea, renal failure, deafness, and central nervous system depression (Gradus et al. 1984). Kuwahara et al. (1984) suggested that $KBrO_3$ can cause acute tubular necrosis as well. Additionally, further observation revealed that $KBrO_3$ can also induce chronic renal failure, burning pain in feet, anuria, and cramping abdominal pain in patients. Histopathological analysis further revealed that these symptoms were highly related to the destruction of basement membrane, calcium deposition in tubules, and toxic tubulonecrosis, indicating severe damage on human renal functions induced by $KBrO_3$ ingestion (Kuwahara et al. 1984).

Based on the highly sensitive detective techniques, $KBrO_3$ residue was detected in bread with treatment levels above 75 ppm in flour, which forced the JECFA to revise the maximum acceptable treatment level of $KBrO_3$ as 60 ppm in flour at 1989. However, the acceptance of $KBrO_3$ treatment in flour was completely withdrawn in the 39th meeting of JECFA at 1992, and $KBrO_3$ was eventually concluded as a genotoxic carcinogen, which was reiterated again in the 44th meeting of JECFA. Thereafter, except for the United States (US), many countries around the world, including those in the European Union (EU), United Kingdom (UK), Canada, Brazil, China, Australia, New Zealand, etc., have banned the use of $KBrO_3$ as a flour treatment agent (Centre for Science and Environment 2016). According to the safety assessment of WHO, FDA and European Food Safety Authority (EFSA), as well as other information resources, the details about the toxicity and carcinogenicity of $KBrO_3$ are summarized in Table 2.

Potassium iodate

The use of KIO_3 in bread industry is highly parallel with that of $KBrO_3$, and their employment for improving bread quality was patented in 1915 (Kohman, Hoffman, and Godfrey 1915). Whereas, KIO_3 has been less widely applied by bakers since then, in comparison with $KBrO_3$. In 1943, the FDA authorized its addition to flour either solely or in combination with other oxidants such as $KBrO_3$, but its level was restricted to be less than 0.0075 part per 100 parts of flour (wt/wt). KIO_3 was also proved to be capable of enhancing gluten formation and adjusting the loaf volume and rheological characteristics of dough (Table 1) (Tanaka, Endo, and Nagao 1980; Indrani and Rao 2006; Kohajdová and Karovičová 2010); while KIO_3 is a stronger oxidant than $KBrO_3$, and its overdose can reversely impair the rheological properties of dough (Joye, Lagrain, and Delcour 2009a; Kohajdová and Karovičová 2010).

Although KIO_3 has been used to treat flour for many years, the intake of this compound at a high level was demonstrated to induce thyroid disorders in human bodies (Bürgi, Schaffner, and Seiler 2001). Therefore, many countries including those in the EU, UK, Australia, New Zealand, and China have already banned its use as a food additive, while it is still allowed in the US and India (Centre for Science and Environment 2016). Additionally, KIO_3 is also used as an iodine fortifier of table salt in several regions of the world.

Mechanism of action

KIO_3 has a similar structure as that of $KBrO_3$ (Figure 2b), as well as a similar mechanism of oxidizing to improve bread quality, which mainly functions on sulfhydryl groups to modulate the protein structure of gluten (Figure 3c) (Bloksma 1964). Whereas, a higher reaction rate is associated with KIO_3, for which it is reduced relatively faster and completely consumed during the mixing process (Bushuk and Hlynka 1960; Bloksma 1964), attributing to the higher oxidative property of iodate ($IO_3^- = +1.09$ V; $BrO_3^- = +0.61$ V) (Cauvain 2012). Therefore, KIO_3 is less preferred by bread manufactures since it cannot improve dough

quality during other stages of bread-making except for the mixing process, which provides less benefits on the final quality of the bread.

Based on the re-oxidation assay, previous studies have found that KIO_3 reacts significantly faster with high molecular weight-subunits isolated from glutenin, but it yields more intramolecular disulfide bonds that end up with low molecular weight-polymers and fewer aggregated proteins. In contrast, the reaction with $KBrO_3$ can generate more intermolecular disulfide linkages and form protein polymers with higher molecular weights (Figure 1) (Antes and Wieser 2001). However, KIO_3 and $KBrO_3$ can similarly react with low molecular weight-glutenin subunits.

Toxicity

Iodate is a strong oxidant and it can be rapidly reduced into iodide in the presence glutathione, cysteine, thioglycolate, and other sulfhydryl-containing compounds (Bürgi, Schaffner, and Seiler 2001). During bread-making, iodate is reduced by sulfhydryl groups at early stages, particularly the mixing process (Hird and Yates 1961b; Bloksma 1964), which eventually leads to iodide exposure to bread consumers. For this reason, research studies are focusing on investigating the toxicity of both iodate and iodide.

A previous study in the 1950s showed that high, single dose of iodate through oral, intraperitoneal, or intravenous route could lead to renal damage, hemoglobinuria, fatty visceral changes, degeneration in gastric parietal cells, and even death in mice (Webster et al. 1957). Oral administration of KIO_3 at 200–250 mg/kg caused hemoglobinuria, stomach mucosa injury, lobular pneumonia, other acute symptoms, and death in dogs (Webster, Stohlman, and Highman 1966). The corresponding subacute toxicity assay indicated that mice can tolerate less than 0.75% KIO_3 via drinking water for 15–16 weeks, showing no significant toxic symptoms except for increased hemolysis (Webster et al. 1959). Pigs that received less than 0.50% KIO_3 in drinking water for 4 weeks also remained in good health without toxic symptoms (Webster et al. 1959). Continuous intake of KIO_3 at doses of 6–100 mg/kg in milk or capsules for 68–192 days exhibited only limited adverse effects on dogs, such as hemosiderin deposition in organs, mucosal inflammation, and hematological changes (Webster, Stohlman, and Highman 1966). The first report on the toxicity of KIO_3 in human bodies was not until 1994 (Singalavanija, Dongosintr, and Dulayajinda 1994), in which the overdose of KIO_3 induced toxic effect on human retina—changes in pigment epithelium and photoreceptor cells. Other studies also reported the similar damage on retina caused by KIO_3 (Singalavanija, Ruangvaravate, and Dulayajinda 2000). At the same time, excessive iodide intake via oral route can also induce multiple negative effects including thyroid gland disorders (World Health Organization 1996). While according to Bürgi, Schaffner, and Seiler (2001), the normal exposure of iodate to humans is inadequate to cause toxic effect, due to its quick conversion into iodide during food preparation or in gut environment before it could be absorbed. The detailed quantitative information about KIO_3 toxicity is presented in Table 2.

Considering the adverse effects introduced by the excessive intake of iodine, JECFA recommended avoiding using KIO_3 as a flour treatment agent in 1965. However, in 1978, FDA affirmed KIO_3 as "generally recognized as safe (GRAS)." In 1991, JECFA suggested the continuing use of potassium iodate in salt (but not in flour/bread). Later in 2009, WHO claimed the potential health risk of both iodine deficiency and its over-absorption, and reaffirmed the recommendation of iodine intake (e.g., 150 μg/day for adults).

Peroxides

Four types of peroxides have been employed in bread-making industry: acetone peroxides (AP), benzoyl peroxide (BP), calcium peroxide (CaO_2), and hydrogen peroxide (H_2O_2). Among the inorganic peroxides, CaO_2 is mainly utilized as a dough rheology improver to reinforce the protein structure of gluten, while the use of H_2O_2 and BP in treating flour is rare, and BP is only supplemented to oxidize the carotenoid pigments in flour for bleaching. Based on the fact that the beneficial effects of CaO_2 and other general peroxides mostly rely on H_2O_2 generation (Wieser 2012), we also reviewed the background information of H_2O_2 and its effects on dough in the following contents.

The use of peroxides (H_2O_2 and CaO_2) as dough rheology improvers for bread manufacturing was first patented by Patterson (1921). The beneficial effect of H_2O_2 on the viscosity of wheat flour suspension was demonstrated by Durham (1925). Fiske (1930) reported the use of CaO_2 as an oxygen provider in bread-making process. In 1941, FDA approved the use of CaO_2 in treating flour with a limitation of less than 0.0075 part in 100 parts of flour (wt/wt). The supplementation of CaO_2 can modify the rheological properties of dough and promote the water absorption in dough (Table 1) (Tieckelmann and Steele 1991; Miller and Hoseney 1999), which facilitates the formation of a dry, elastic, stronger, and less sticky dough with better gas retention, shorter proof time, greater oven spring, and improved machining and handling characteristics (Dubois and Ash 1974; Tieckelmann and Steele 1991). CaO_2 is associated with low toxicity (Jakob et al. 2000), which is currently permitted to treat flour in the US and Canada, but not in China and European countries.

AP was introduced to bread manufacturing in 1961 as an optional flour maturing and bleaching agent, while it can also act as a dough rheology improver by altering the rheological properties of dough, generating final bread products with a high quality (Tsen 1964). AP is a mixture of the products formed by the reaction between H_2O_2 and acetone in a mild acid solution (Oser and Morgareidge 1967). These products chiefly consist of dominant AP cyclic trimer and minor AP monomer or dimer (Figure 2c, e) (Milas and Golubovic 1959). For bread-making, the AP mixture is pretreated with starch that acts as the carrier, through which the composition of AP could be modulated as dominant acyclic monomer (~90%) with residual linear dimer and

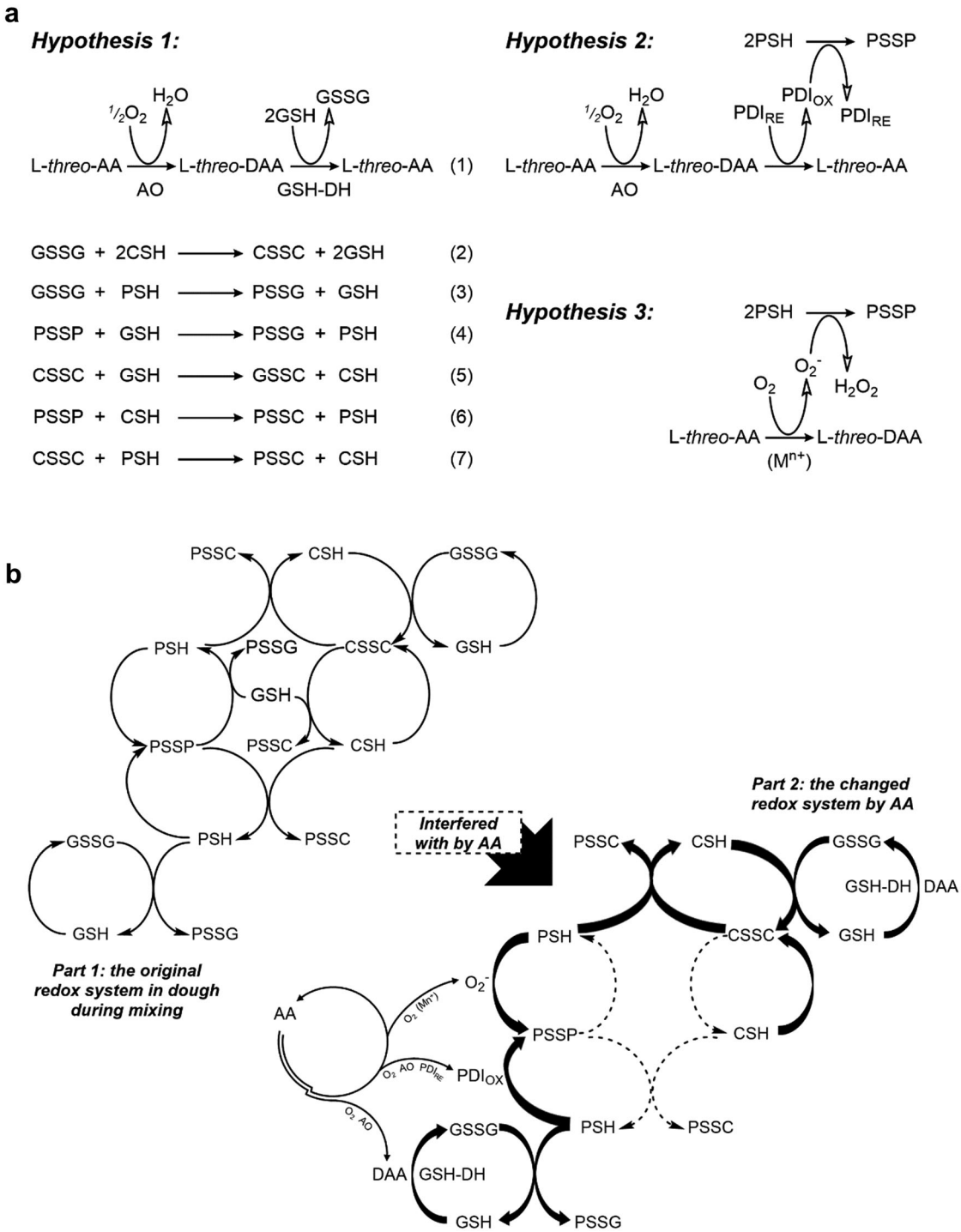

Figure 4. Mechanism of the improving effects of AA on dough and bread quality. (a) Three hypotheses for the mechanism of AA in improving bread quality. For Hypothesis 1, equation (1) was concluded from the previous studies by Grosch and Wieser (1999); equation (2) was given by Kieffer et al. (1990); equation (3–7) were proposed by Grosch and Wieser (1999). Hypothesis 2 was suggested by Every, Simmons, and Ross (2006). Hypothesis 3 was suggested by Nakamura and Kurata (1997a). (b) Model for the collective summary of Hypotheses 1–3. Dashed lines indicate the inhibitory effects of AA on reactions, while bold lines indicate the enhancing effects of AA on reactions. G(GSH) = glutathione; P(PSH) = gluten protein; C(CSH) = free cysteine or other low molecular weight-thiols.

trace (or no) linear trimer, but without cyclic trimer (Figure 2e) (Ferrari et al. 1963; Taylor 1964; Wieser 2012). However, because of the complex and dynamic composition of AP mixture, the precise prediction of its potential biological effects on food processing is extremely difficult. Thus, except for the US, only few countries allow the use of AP for bread-making.

Mechanism of action

H_2O_2 is released from peroxides during flour blending in the presence of water, which is presumably responsible for the beneficial effects on improving dough quality (Figure 3b, equations (1) (5)). Subsequently, oxygen is released from H_2O_2 during reaction, and it also participates in modifying dough conditions through the mechanism described

previously (Figure 3b, equations (3) (6)). According to a previous study of Manu and Prasada Rao (2011), mediated by peroxidase (EC 1.11.1), H_2O_2 can also facilitate the formation of dityrosine bonds in glutenin. Moreover, it was also proposed that H_2O_2 could foster the crosslinking between exogenous ferulic acid residues and water-extractable arabinoxylans or gluten tyrosine residues in dough. Such crosslinking was suggested to modulate the rheological properties of dough as well (Schofield 1996; Courtin and Delcour 2002), whereas, only tiny influence on dough was observed due to its formation in a small quantity (Piber and Koehler 2005).

CaO_2 is a very active oxidant, which can be used economically at low doses. The recommended dose range of CaO_2 is 20–35 mg/kg (Wieser 2012). Nevertheless, due to its low solubility in water, CaO_2 has to be mixed with flour under dry conditions. This drawback has limited its commercial use in bread-making. Moreover, the decomposition of CaO_2 can be accelerated by high temperature, therefore it is mainly effective before the baking process (Figure 3b, equation (4)).

AP is also an efficient sulfhydryl-oxidizing agent (Tsen 1964). Compared with other dough rheology improvers, AP is active in dry flour within 24 hours and it tolerates overtreatment. However, the same level of beneficial effect on dough/bread improvement requires a higher dose of AP, in comparison with iodate or ADA (Tsen 1964). Although the mechanism of AP in modulating dough might also include the release of H_2O_2, details in its reactions still remain to be further elaborated.

Toxicity

CaO and $Ca(OH)_2$ are two other crucial byproducts of CaO_2 from its reaction with water during baking process (Figure 3b, equations (1) (2) (4)) (Tieckelmann and Steele 1991), the toxicity of which is also important for evaluating the safety of CaO_2 application. JECFA declared nontoxicity of both CaO and $Ca(OH)_2$ and recommended no limitation on their allowable daily intake (Table 2). Thus, the employment of CaO_2 is regarded as completely safe for bread manufacturing.

FDA approved the use of AP as a food additive based on the safety evaluation carried out by a long-term animal study in 1959. According to Oser and Morgareidge (1967), rats and dogs were fed with diets comprised of breads that were made from flour treated with different levels of AP for 2 years and 1 year, respectively, and both animals appeared to be normal and healthy without any adverse responses. No death was found among the dogs. While the death of the rats, especially during the second year, was due to their advanced age and insignificantly different among different groups (Oser and Morgareidge 1967).

Ascorbic acid

AA, namely vitamin C, was first discovered to be potentially used as a dough enhancer in 1935 (Jorgensen 1935). AA possesses the capability of optimizing the protein structures and rheological properties of dough (Table 1) (Tanaka, Endo, and Nagao 1980; Dong and Hoseney 1995; Indrani and Rao 2006; Codina et al. 2007). In spite of its higher cost, an increasing number of countries have chosen AA as an alternative dough rheology improver for industrial bread-making. Especially in Europe, AA is currently the only permitted oxidative agent for manufacturing bread and other baked goods. Despite the fact that no limited maximum addition level of AA has been announced in most countries, its addition level is only limited to be less than 0.2 g/kg by the US and UK. In FAO/WHO Food Standards, however, the maximum level of AA is 0.3 g/kg. Although AA is naturally present in many plants and fruits, the commonly used one in food industry is mainly artificial by glucose fermentation (Sahi 2014).

Mechanism of action

AA has four forms: L-*threo*-AA, L-*erythro*-AA, D-*threo*-AA, and D-*erythro*-AA, among which L-*threo*-AA is the most effective one in improving the rheological properties of dough (Figure 2g) (Dong and Hoseney 1995). AA serves as an atypical chemical rheology improver in bread-making industry, which has a strong reductive activity, with a different mechanism of action from that of $KBrO_3$ and KIO_3.

Furthermore, AA has a high initial reaction rate compared with that of bromate (Stauffer and Beech 1990), and its beneficial effects on dough or bread are highly dependent on the presence of oxygen (Sandstedt and Hites 1945). AA competes with a plenty of other enzymes and yeasts in dough for oxygen to produce dehydroascorbic acid (DAA) (Elkassabany, Hoseney, and Seib 1980). The studies of Mair and Grosch (1979); Sarwin, Laskawy, and Grosch (1993) indicated that AA can only improve the quality of dough before the oxygen is exhausted in the system. Whereas, there was another point of view suggesting that such beneficial effects are long-lasting even after the mixing process, due to the cyclical nature of AA (Figure 4a, Hypothesis 1, equation (1)) (Stauffer and Beech 1990).

Hypothesis 1. It was proposed that AA could be oxidized by atmospheric oxygen and converted to DAA, which serves as the actual effector on dough improvement. The oxidation of AA into DAA is mediated by endogenous ascorbate oxidase (AO, EC 1.10.3.3) (Figure 2f) (Grant and Sood 1980). Meanwhile, it could also be catalyzed by metal ions through non-enzymatic routes (Every, Gilpin, and Larsen 1995). Due to its substrate specificity, AO can oxidize L-AA at a higher rate than D-AA (Every 1999). DAA was previously reported to inactivate endogenous proteinases that impact the quality of dough (Sandstedt and Hites 1945), but GSH was later demonstrated to be responsible for such activity. Further studies also suggested that DAA, once converted from AA, can oxidize several reductants (Kuninori 1963). One of these reductants was soon identified to be GSH (Kuninori 1964), and the reductive reaction was found to be carried out by glutathione dehydrogenase (GSH-DH, EC 1.8.5.1). GSH-DH, also designated as "dehydroascorbate reductase," specifically

catalyzes the reaction between DAA and GSH, through which the former could be converted back to AA (Figure 2f) (Walther and Grosch 1987). Besides, L-*threo*-DAA, among the four DAA isomers, is the best substrate for GSH-DH and have the highest rate of reaction (Walther and Grosch 1987). Furthermore, thiols with low molecular weights (e.g., cysteine [CSH]) that are endogenous in flour could also take part in the sulfhydryl-disulfide interchanges among gluten proteins (Sarwin, Laskawy, and Grosch 1993). Based on this, Grosch and Wieser (1999) proposed that GSH, CSH, sulfhydryl groups in peptide residues (PSH), and their redox products undergo multiple reactions during the mixing process of dough, and these reactions are greatly affected by the addition of AA. The presence of AA eventually antagonizes sulfhydryl groups and removes GSH, preventing the depolymerization of gluten proteins in dough (Figure 4a, Hypothesis 1).

Hypothesis 2. Despite the fact that AA improves bread-making mainly through removing free GSH, DAA can also directly oxidize other sulfhydryl groups in dough proteins and generate disulfide bonds (Tsen 1965). Every et al. (1999, 2000) revealed the presence of a DAA-reductase-typed enzyme that specifically catalyzes such oxidation process in flour. This enzyme, induced by AA or DAA, involves in the formation of disulfide linkages, and was recognized as the protein disulfide isomerase (PDI, EC 5.3.4.1) that is naturally present in wheat flour. It was hypothesized that PDI can catalyze the oxidation of sulfhydryl groups in dough proteins via the joint reaction with AA shown in Figure 4a, Hypothesis 2, thereby generating large glutenin polymers that are beneficial for dough quality (Every, Simmons, and Ross 2006). Even without the presence of AA, PDI itself can also directly improve the glutenin network formation, tough its underlying mechanism is still not fully understood (Fu et al. 2020).

Hypothesis 3. Nakamura and Kurata (1997a, 1997b) found that AA has a higher beneficial effect on dough texture than DAA and suggested that such advantage might be attributed to the extra process of AA oxidation. Considering that oxygen radicals are generated during AA oxidation and the effects of superoxide anion radical (O_2^-) on chain oxidation reactions (Von Sonntag et al. 1993), it was further hypothesized that several intermediate products, such as O_2^-, are produced during the oxidation of AA. Subsequently, O_2^- participates in the sulfhydryl-disulfide interchanges of gluten proteins, thereby modulating the rheological protein network in dough (Figure 4a, Hypothesis 3). This theory was later supported by Miyamoto and Nishimura (2006) and Nishimura (2013), suggesting that O_2^- generated during AA oxidation can induce the concomitant formation of thiyl radical in the gluten protein network of dough. Whereas, yeasts consume the majority of the absorbed oxygen in dough during their fermentation process, which inhibits the oxygen-mediated AA oxidation and the subsequent production of O_2^-. Therefore, this proposed mechanism only applies to the bread-making process without introducing yeasts (Miyamoto and Nishimura 2006).

Others. Another study conducted by Gerrard et al. (1998) showed that DAA can induce the crosslinking between dough proteins (e.g., glutenin) via disulfide bond-independent mechanism. For example, Maillard reaction, the complex reactions between reactive carbonyl groups and free amine groups in food constituents, can explain the browning on bread. Fayle et al. (2000) further demonstrated that these amine groups are free lysine residues.

Summary of hypotheses. Collectively, we have established a graphic model to illustrate the mechanisms of action of AA based on the widely recognized hypotheses (Hypothesis 1–3) that focus on the disulfide crosslinking theory (Figure 4b). Grosch and Wieser (1999) suggested that, during dough mixing process, the sulfhydryl-disulfide interchanges occur among GSH, PSH, CSH, and their oxidation products that include PSSP, PSSC, PSSG, GSSG, GSSC, and CSSC (Figure 4a, Hypothesis 1, equation (2–7); b, Part 1). Here the abbreviation "XSSX" was used to represent the disulfide bonds connecting gluten protein including gliadin and glutenin (P), free cysteine or other low molecular weight-thiols (C) or glutathione (G). This indicates the presence of a complicated redox system that is influenced by sulfhydryl/disulfide-involved reactions during the mixing and kneading processes of dough development. The endogenous GSH in dough is critical for Hypothesis 1, which directly breaks the disulfide linkages in PSSP, thus reducing the abundance of PSSP (Figure 4a, Hypothesis 1, equation (4); b, Part 1); GSH can also react with CSSC to produce CSH, which further diminishes PSSP through disulfide bond cleavage (Figure 4a, Hypothesis 1, equation (4) and (6); b, Part 1). When AA is mixed with dough, it reduces the quantity of GSH and thus prevents the damage of disulfide bonds in PSSP (Figure 4a, Hypothesis 1, equation (1); b, Part 2); AA, facilitated by PDI, also directly promotes the formation of PSSP via Hypothesis 2 and O_2^- via Hypothesis 3 (Figure 4a, Hypothesis 2, Hypothesis 3; b, Part 2). Overall, these complex reactions induced by AA eventually lead to increased amounts of GSSG, PSSG, PSSC, and PSSP (Figure 4b, Part 2), which serve as the foundation of glutenin polymerization and strengthen the protein structure of dough.

Safety

AA is a natural vitamin found in many plants and fruits. Unlike other rheology improvers that could pose a health threat, it mediates important physiological metabolism of human bodies, for instance, L-AA enhances the resistance to scurvy and participates in the formation and maintenance of cartilage, bones, gums, skin, teeth, etc., facilitating collagen synthesis. Moreover, AA is able to improve the activity of leucocytes and strengthen immune system (Davey et al. 2000). Whereas, L-AA is a very unstable compound because of its strong reductive ability, especially under heating environment such as baking condition. AA is fully decomposed during baking with little residues left in the final baked

goods. Although little is known about the decomposition products of AA after baking, as well as that of DAA, in consideration of the history of AA application in food processing, it is reasonable to believe that the intake of AA within the limited maximum dose for bread manufacturing is not toxic (Table 2).

Azodicarbonamide

Azodicarbonamide (ADA) is a synthetic compound with low molecular weight, which is generated from urea and hydrazine. Due to its capacity of releasing a large volume of gas under heating condition, ADA has been widely used for blowing and foaming plastics since 1940 (Chen et al. 2018). ADA was first used for treating milling cereal grains in 1956 (Marks, Joiner, and Parker 1959), when Wallace and Tiernan (New Jersey, USA) identified its potential in facilitating flour maturation. FDA approved the use of ADA as a food additive in cereal flour and as a dough rheology improver in 1962. Since then, ADA has been systematically introduced as a flour maturing agent around the world.

Owing to several of its favorable properties, ADA has been considered as an ideal substitute of chlorine dioxide and $KBrO_3$ for bread-making. For example, ADA is completely stable in the form of solid powder, which can be stored at room temperature in a long term. Compared with gas reagents, the utilization of ADA is convenient which requires no complex and costly apparatus; and ADA has no significant toxic effect, while its utilization requires relatively low cost (Joiner, Vidal, and Marks 1963; Wu and Chen 2017). In addition, it was also demonstrated that ADA can alter the loaf volume of bread and the rheological properties of dough (Table 1) (Joiner, Vidal, and Marks 1963; Tsen 1963b; Attenburrow et al. 1990; Miller and Hoseney 1999).

ADA is currently widely used for bread manufacture across the world, in countries such as America, Brazil, China, Canada, Korea, etc. (Wu and Chen 2017). Most of these countries limit the addition of ADA at a maximum level at 0.045 g/kg (e.g., the US and China), which was also documented in the food standards of FAO/WHO. However, considering the potential health risk associated with biurea and semicarbazide (SEM), the byproducts of ADA during bread-making, the European Union completely banned its application in food packaging at 2005. The use of ADA in food industry has also been forbidden in other countries including Australia, Singapore, Japan, and UK.

Mechanism of action

The mechanism of ADA in bread-making improvement is similar to that of $KBrO_3$. Once ADA is blended with flour, it can oxidize sulfhydryl groups, building disulfide linkages to maintain the stable protein structure in dough (Tsen 1963b). Moreover, in comparison with other oxidants like $KBrO_3$ and KIO_3, the oxidative reaction rate by ADA is relatively faster, for which the oxidization is mostly completed during the mixing period (Tsen 1963a, 1963b; Becalski et al. 2004); and no fermentation is required to trigger such oxidative reaction (Joiner, Vidal, and Marks 1963). ADA can react with flour components in the presence of water, rapidly oxidizing the sulfhydryl groups and completely transforming into biurea (Figure 3d, I) (Tsen 1963b; Becalski et al. 2004).

Toxicity

It was suggested that respiratory diseases, such as asthma and skin sensitization, can be caused by repeated or prolonged contact with ADA (Bonsall 1984; Normand et al. 1989). During food processing under high temperature (e.g., baking), ADA can transform into biurea and SEM (Figure 3d, II and III), both of which are toxic to humans (Pereira, Donato, and de Nucci 2004; Noonan, Begley, and Diachenko 2008). In addition, urethane (i.e., ethyl carbamate) is another toxic byproduct of ADA during baking process, the concentration of which in the final baking products is related with ethanol (Figure 3d, IV), toasting level, the type and quantity of yeasts used in fermentation, and the time length of fermentation (Zimmerli and Schlatter 1991; Sen et al. 1993; Cañas, Diachenko, and Nyman 1997). Therefore, the toxicity of ADA mainly relies on the three abovementioned byproducts instead of itself.

According to the safety evaluation conducted by JECFA in 1967, ADA, as a flour-treatment agent, is free from carcinogenic activity based on adequate animal studies (Food and Agriculture Organization of the United Nations 1967). It was also suggested that biurea, the primary residue of ADA in the final baking products, is stable and metabolically inert, which is associated with low toxicity and no carcinogenic potential. In 1985, the US FDA classified ADA as GRAS, declaring that its intended use in food production is safe. In 1999, WHO suggested that ADA and its byproduct biurea have low toxicity according to in vivo studies based on various dosage levels. As a matter of fact, both of them can be rapidly eliminated from human bodies, predominantly through urine, with only tiny retention of biurea (World Health Organization 1999).

The EFSA stated that the SEM residue in food products can hardly induce any genotoxicity or carcinogenicity on human bodies (Table 2). Similarly, JECFA claimed that human exposure to urethane through consuming foods is free of any potential health risk, though high-dose urethane has been shown to be toxic to rodents (Table 2), and it was categorized in group 2 A by the IARC, possessing carcinogenic potential on humans.

Wheat protein disulfide isomerase–a potential rheology improver in the future

wPDI is one of the endogenous enzymes encoded by wheat gene. It belongs to a big enzyme family, protein disulfide isomerases, that was found to be capable of catalyzing protein oxidative folding (Fu et al. 2020). In 1995, wPDI was found to present in the endoplasmic reticulum of wheat endosperm (Shimoni et al. 1995). In the later work of Ciaffi et al., the genes encoding wPDI in Chinese Spring wheat

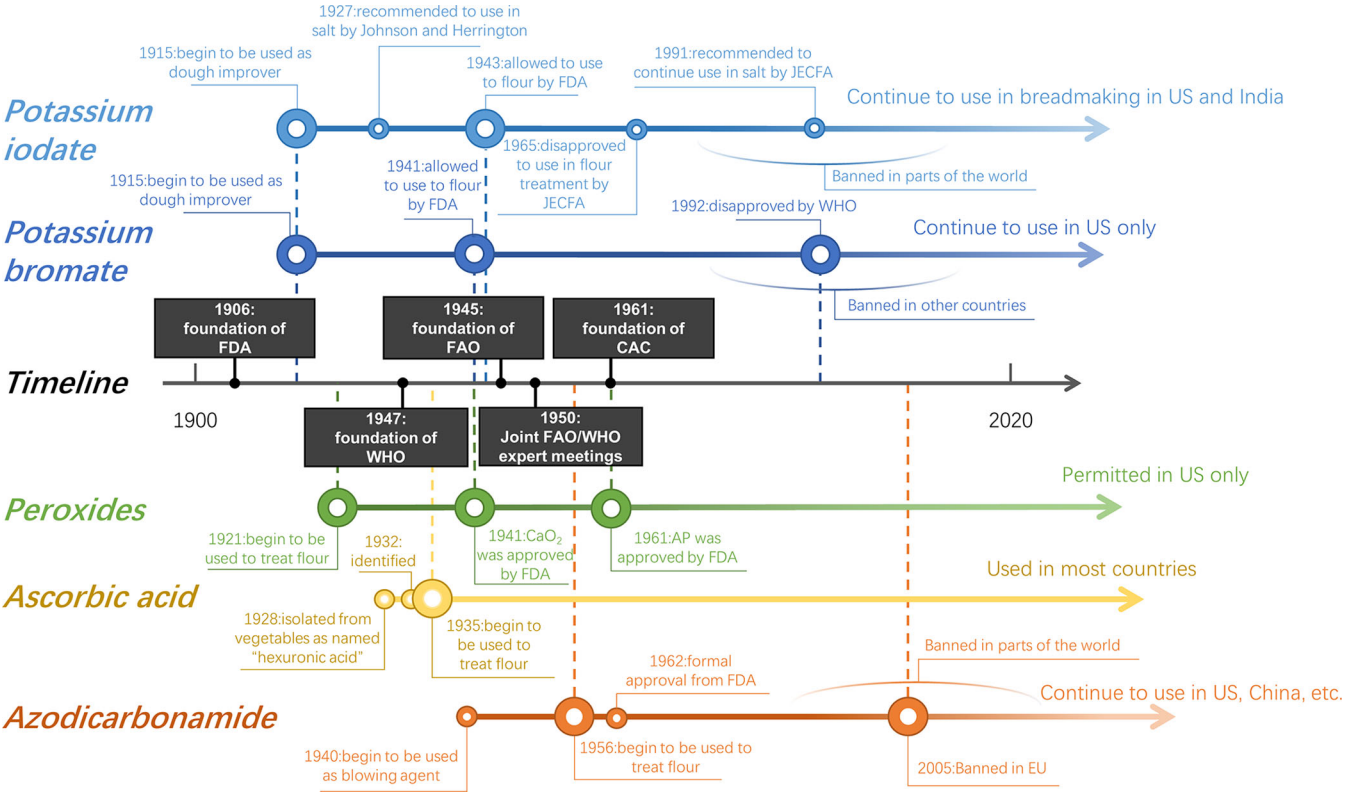

Figure 5. History of the use of dough rheology improvers. The circles in different lines represent the important related events happening in history, while the bigger circles represent the key events.

were found to locate on the 4A, 4B and 4D chromosomes (Ciaffi et al. 2006). And it was also showed that the wPDI gene contains 10 exons and 9 introns (Ciaffi et al. 2001).

It was believed that, during the wheat growth and development, wPDI family members have an important role in catalyzing and regulating the disulfide bond formation between glutenins (Dong et al. 2016). Through affecting the formation of higher glutenin macropolymers, the expression levels of wPDI significantly impact gluten quality. Besides, several studies also confirmed that adding purified wild type wPDI to flour can change the final rheological properties of dough or bread (Watanabe, Bell, and Brockway 1998; Liu et al. 2017; Zhao et al. 2020). However, whether adding the exogenous wPDI to flour can provide positive or deleterious effects on dough still retained controversial according to the varying results between these studies. For example, in the study of Liu et al. (2017), the addition of wild type wPDI weakened the processing quality of flour, but the modified wPDI with only chaperone activity retained can improve the flour quality. On the other hand, in the recent study of Zhao et al. (2020), wild type wPDI was found to improve dough and bread quality, even with a higher positive effect than other oxidative dough rheology improvers (e.g., AA and ADA). And this improving effect from wPDI was deemed as attributed to the ability of wPDI to form more rheologically active disulfide bonds, rather than simply increase the number of disulfide bonds. In addition, according to the study of Gao et al. (2020), another mechanism was also believed to contribute to the improving effect from wPDI. Namely, wPDI can enhance folding of the repetitive domain of 1Dx5, a high molecule weight glutenin subunit, and hence shorten the distance between two terminals of this domain. Since cysteines mainly present at terminals of glutenin, such effect subsequently improves the possibility of disulfide bond formation.

Although the exact effects of wPDI on dough or bread remain determination, wPDI itself has been attached great attention as a dough rheology improver, due to its well-defined chaperone activity. This activity, if properly developed, has helped wPDI become a promising substitute of other chemically-synthesized dough rheology improvers like ADA. Since it is also endogenous in wheat, wPDI is endowed superior safety compared to other dough rheology improvers, and this hence make it a new potential rheology improver.

Conclusions

The mechanism of action and toxicity of multiple oxidative dough rheology improvers are discussed in this review. Overall, their mechanisms of action are through either directly or indirectly oxidizing thiol groups into disulfide bonds in gluten proteins, which promotes the formation of a stronger protein network in dough. With the exception of $KBrO_3$, most of the dough rheology improvers exhibit low toxicity under normal bread-making conditions. The history of their uses as dough rheology improvers is summarized in Figure 5. Considering the toxicity of $KBrO_3$, AA and ADA have been widely applied as dough rheology improvers, either alone or in combination, in bread-making industry. However, exorbitant dosage of most of these additives can

also induce negative effect on dough, resulting in a final bread with reduced volume and overall poor quality. Due to the complexity and diversity of the chemical reactions involved in bread-making process, the complete mechanisms of action of these oxidants have not been fully demonstrated, and it is also difficult to predict the generated byproducts in the oxidative reactions. Therefore, continuous observation and assessment on the safety and toxicity of these dough rheology improvers are still necessary.

Now that the use of ADA is feasible and its cost is acceptable in breadmaking industry, there is no need for newly developed chemical dough rheology improver. But on the other hand, an obvious trend nowadays is to develop new natural dough rheology improvers. For instance, enzymes like wPDI were also proven to be capability to reinforce the glutenin crosslinking and thus improve dough quality. The unambiguous safety of wPDI make it a promising candidate as dough rheology improvers.

Acknowledgment

The authors are grateful to Chih-chen Wang in the Institute of Biophysics, Chinese Academy of Sciences, for her support and encouragement in our research.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China (31801482) and the National Key Research and Development Program of China (2019YFC1605000).

ORCID

Dong Yang http://orcid.org/0000-0001-5435-5905

References

Antes, S., and H. Wieser. 2001. Reoxidation behavior of wheat and rye glutelin subunits. *Cereal Chemistry Journal* 78 (1):8–13. doi: 10.1094/CCHEM.2001.78.1.8.

Attenburrow, G., D. J. Barnes, A. P. Davies, and S. J. Ingman. 1990. Rheological properties of wheat gluten. *Journal of Cereal Science* 12 (1):1–14. doi: 10.1016/S0733-5210(09)80152-5.

Bailey, C. H. 1925. *The chemistry of wheat flour*. New York, NY: The Chemical Catalogue Co., Inc.

Baker, J. C., and M. D. Mize. 1937. Mixing doughs in vacuum and in the presence of various gases. *Cereal Chemistry* 14:721–34.

Becalski, A., B. P. Y. Lau, D. Lewis, and S. W. Seaman. 2004. Semicarbazide formation in azodicarbonamide-treated flour: A model study. *Journal of Agricultural and Food Chemistry* 52 (18): 5730–4. doi: 10.1021/jf0495385.

Biesiekierski, J. R. 2017. What is gluten? *Journal of Gastroenterology and Hepatology* 32:78–81. doi: 10.1111/jgh.13703.

Bloksma, A. H. 1964. Oxidation by potassium iodate of thiol groups in unleavened wheat flour doughs. *Journal of the Science of Food and Agriculture* 15 (2):83–94. doi: 10.1002/jsfa.2740150204.

Bloksma, A. H. 1972. The relation between the thiol and disulfide contents of dough and its rheological properties. *Cereal Chemistry* 49: 104–18.

Bloksma, A. H., and W. Bushuk. 1988. Rheology and chemistry of dough. In *Wheat: Chemistry and technology*, Vol. 2, Chap. 4, ed. Y. Pomeranz. St. Paul, MN: American Association of Cereal Chemists.

Bonsall, J. L. 1984. Allergic contact dermatitis to azodicarbonamide. *Contact Dermatitis* 10 (1):42. doi: 10.1111/j.1600-0536.1984.tb00060.x.

Branlard, G, and M. Dardevet. 1985. Diversity of grain protein and bread wheat quality. *Journal of Cereal Science* 3 (4):345–54. doi:10.1016/S0733-5210(85)80007-2.

Bürgi, H., T. H. Schaffner, and J. P. Seiler. 2001. The toxicology of iodate: A review of the literature. *Thyroid* 11 (5):449–56. doi: 10.1089/105072501300176408.

Bushuk, W., and I. Hlynka. 1960. Disappearance of bromate during baking of bread. *Cereal Chemistry* 37:573–76.

Cañas, B. J., G. W. Diachenko, and P. J. Nyman. 1997. Ethyl carbamate levels resulting from azodicarbonamide use in bread. *Food Additives and Contaminants* 14 (1):89–94. doi: 10.1080/02652039709374501.

Cappelli, A., L. Bettaccini, and E. Cini. 2020. The kneading process: A systematic review of the effects on dough rheology and resulting bread characteristics, including improvement strategies. *Trends in Food Science & Technology* 104:91–101. doi: 10.1016/j.tifs.2020.08.008.

Cappelli, A., L. Guerrini, E. Cini, and A. Parenti. 2019. Improving whole wheat dough tenacity and extensibility: A new kneading process. *Journal of Cereal Science* 90:102852. doi: 10.1016/j.jcs.2019.102852.

Cauvain, S. P. 2012. *Breadmaking: Improving quality*. Cambridge, UK: Woodhead Publishing Limited.

Cauvain, S. P., and L. S. Young. 2006. *The chorleywood bread process*. Cambrige, UK: Woodhead Publishing Limited.

Centre for Science and Environment. 2016. Potassium bromate/iodate in bread and bakery products: A CSE Policy Brief. In *CSE study results and recommendations*, 1–12. New Delhi, India: Centre for Science and Environment.

Chen, Z., L. Chen, L. Lin, Y. Wu, and F. Fu. 2018. A colorimetric sensor for the visual detection of azodicarbonamide in flour based on azodicarbonamide-induced anti-aggregation of gold nanoparticles. *ACS Sensors* 3 (10):2145–51. doi: 10.1021/acssensors.8b00705.

Ciaffi, M., A. Paolacci, E. d'Aloisio, O. Tanzarella, and E. Porceddu. 2006. Cloning and characterization of wheat PDI (protein disulfide isomerase) homoeologous genes and promoter sequences. *Gene* 366 (2):209–18. doi: 10.1016/j.gene.2005.07.032.

Ciaffi, M., A. Paolacci, L. Dominici, O. Tanzarella, and E. Porceddu. 2001. Molecular characterization of gene sequences coding for protein disulfide isomerase (PDI) in durum wheat (Triticum turgidum ssp. durum). *Genes and Genomics* 265 (1–2):147–56. doi: 10.1016/S03708-1119(01)00348-1.

Codina, G. G., I. Cretu, V. Paslaru, and C. Arghire. 2007. Ascorbic acid influence on dough's behaviour. *Journal of Agroalimentary Processes and Technologies* 13 (2):299–302.

Compass Remediation Chemicals. 2017. Safety data sheet: Calcuim peroxide. https://compassremediation.com/wp-content/uploads/SDS/SDS-Calcium-Peroxide.pdf.

Courtin, C. M., and J. A. Delcour. 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science* 35 (3): 225–43. doi: 10.1006/jcrs.2001.0433.

D'Ovidio, R., and S. Masci. 2004. The low-molecular-weight glutenin subunits of wheat gluten. *Journal of Cereal Science* 39 (3):321–39. doi: 10.1016/j.jcs.2003.12.002.

Dai, Y., and C. Tyl. 2021. A review on mechanistic aspects of individual versus combined uses of enzymes as clean label-friendly dough conditioners in breads. *Journal of Food Science* 86 (5):1583–98. doi: 10.1111/1750-3841.15713.

Davey, M. W., M. V. Montagu, D. Inzé, M. Sanmartin, A. Kanellis, N. Smirnoff, I. J. J. Benzie, J. J. Strain, D. Favell, and J. Fletcher. 2000. Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and*

Agriculture 80 (7):825–60. doi: 10.1002/(SICI)1097-0010(20000515)80:7 < 825::AID-JSFA598 > 3.0.CO;2-6.

Decamps, K., I. J. Joye, D. E. de Vos, C. M. Courtin, and J. A. Delcour. 2016. Molecular oxygen and reactive oxygen species in bread-making processes: Scarce, but nevertheless important. *Critical Reviews in Food Science and Nutrition* 56 (5):722–36. doi: 10.1080/10408398.2013.795929.

Dempster, C. J., I. Hlynka, and J. A. Anderson. 1954. Extensograph studies of the improving action of oxygen in dough. *Cereal Chemistry* 31 (3):240–49.

Dirndorfer, M., R. Kieffer, and H. D. Belitz. 1986. Changes in the gluten of different wheat varieties due to oxidation. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 183 (1):33–38. doi: 10.1007/BF01027591.

Dong, L., N. Li, X. Lu, S. Prodanovic, Y. Xu, W. Zhang, and Y. Yan. 2016. Quality properties and expression profiling of protein disulfide isomerase genes during grain development of three spring wheat near isogenic lines. *Genetika* 48 (1):249–69. doi: 10.2298/GENSR1601249D.

Dong, W., and R. C. Hoseney. 1995. Effects of certain breadmaking oxidants and reducing agents on dough rheological properties. *Cereal Chemistry* 72 (1):58–63. doi: 10.1021/bp00031a017.

Dong, Y., and S. Karboune. 2021. A review of bread qualities and current strategies for bread bioprotection: Flavor, sensory, rheological, and textural attributes. *Comprehensive Reviews in Food Science and Food Safety* 20 (2):1937–81. doi: 10.1111/1541-4337.12717.

DrugFuture. 2020. Chemical toxicity database. https://www.drugfuture.com/toxic/.

Dubois, D. K., and D. J. Ash. 1974. Encapsulated calcium peroxide for continuous bread process. *Baker's Digest* 48 (3):40–41.

Durham, R. K. 1925. Effect of hydrogen peroxide on relative viscosity measurements of wheat and flour suspensions. *Cereal Chemistry* 2 (2):297–305.

El-Hady, E. A. A., S. K. El-Samahy, and J. M. Brümmer. 1999. Effect of oxidants, sodium-stearoyl-2-lactylate and their mixtures on rheological and baking properties of nonprefermented frozen doughs. *LWT - Food Science and Technology* 32 (7):446–54. doi: 10.1006/fstl.1999.0569.

Elkassabany, M., R. C. Hoseney, and P. A. Seib. 1980. Ascorbic acid as an oxidant in wheat flour dough. I: Conversion to dehydroascorbic acid. *Cereal Chemistry* 57:85–87.

Every, D. 1999. Purification and characterisation of ascorbate oxidase from flour and immature wheat kernels. *Journal of Cereal Science* 30 (3):245–54. doi: 10.1006/jcrs.1999.0282.

Every, D., M. J. Gilpin, and N. G. Larsen. 1995. Continuous spectrophotometric assay and properties of ascorbic acid oxidising factors in wheat. *Journal of Cereal Science* 21 (3):231–39. doi: 10.1006/jcrs.1995.0026.

Every, D., L. Simmons, M. Ross, P. E. Wilson, J. D. Schofield, S. S. Bollecker, and B. Dobraszczyk. 2000. Mechanism of the ascorbic acid improver effect on baking. In *Wheat gluten*, eds. P. R. Shewry, and A. S. Tatham, 277–82. Cambridge, UK: Royal Society of Chemistry.

Every, D., L. Simmons, K. H. Sutton, and M. Ross. 1999. Studies on the mechanism of the ascorbic acid improver effect on bread using flour fractionation and reconstitution methods. *Journal of Cereal Science* 30 (2):147–58. doi: 10.1006/jcrs.1999.0248.

Every, D., L. D. Simmons, and M. P. Ross. 2006. Distribution of redox enzymes in millstreams and relationships to chemical and baking properties of flour. *Cereal Chemistry Journal* 83 (1):62–8. doi: 10.1094/CC-83-0062.

Fayle, S. E., J. A. Gerrard, L. Simmons, S. J. Meade, E. A. Reid, and A. C. Johnston. 2000. Crosslinkage of proteins by dehydroascorbic acid and its degradation products. *Food Chemistry* 70 (2):193–98. doi: 10.1016/S0308-8146(00)00077-7.

Ferrari, C. G., K. Higashiuchi, J. A. Podliska, W. C. Schaefer, C. R. Russell, J. J. Maurice, C. E. Risto, D. H. Simmonds, P. M. Bell, and D. H. Sirnmonds. 1963. Flour maturing and bleaching with acyclic acetone peroxides. *Cereal Chemistry* 40:89.

Fiske, A. H. 1930. Baking powder. US Patent 1,775,037.

Food and Agriculture Organization of the United Nations. 1967. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids and bases. FAO Nutrition Meetings Report Series No. 40A,B,C WHO/Food Add./67.29

Frater, R., F. J. R. Hird, H. J. Moss, and J. R. Yates. 1960. A role for thiol and disulphide groups in determining the rheological properties of dough made from wheaten flour. *Nature* 186 (4723):451–54. doi: 10.1038/186451a0.

Freilich, J., and C. N. Frey. 1937. Effect of oxygen on proteolytic activity in bread dough. *Industrial and Engineering Chemistry* 15 (13):294. doi: 10.1021/cen-v015n013.p294.

Fu, J., J. Gao, Z. Liang, and D. Yang. 2020. PDI-regulated disulfide bond formation in protein folding and biomolecular assembly. *Molecules* 26 (1):171. doi: 10.3390/molecules26010171.

Fujie, K., H. Shimazu, M. Matsuda, and T. Sugiyama. 1988. Acute cytogenetic effects of potassium bromate on rat bone marrow cells *in vivo*. *Mutation Research/Genetic Toxicology* 206 (4):455–5. doi: 10.1016/0165-1218(88)90053-5.

Gao, J., P. Yu, H. Liang, J. Fu, Z. Luo, and D. Yang. 2020. The wPDI redox cycle coupled conformational change of the repetitive domain of the HMW-GS 1Dx5—A computational study. *Molecules* 25 (19):4393. doi: 103390/molecules25194393.

Geddes, W. F. 1930. Chemical and physico-chemical changes in wheat and wheat products induced by elevated temperatures: III. the influence of germ constituents on baking quality and their realation to improvement in flour induced by heat and chemical improvers. *Canadian Journal of Research* 2 (3):195–213. doi: 10.1139/cjr30-015.

Gellynck, X., B. Kühne, F. Van Bockstaele, D. Van de Walle, and K. Dewettinck. 2009. Consumer perception of bread quality. *Appetite* 53 (1):16–23. doi: 10.1016/j.appet.2009.04.002.

Gerrard, J. A., K. H. Sutton, S. E. Fayle, and A. J. Pratt. 1998. Dehydroascorbic acid mediated crosslinkage of proteins using maillard chemistry-relevance to food processing. In *The Maillard reaction in foods and medicine*, eds. J. O'Brien, H. E. Nursten, M. J. C. Crabbe, and J. M. Ames, 127–32. Cambridge, UK: Royal Society of Chemistry.

Gradus, D., M. Rhoads, L. B. Bergstrom, and S. C. Jordan. 1984. Acute bromate poisoning associated with renal failure and deafness presenting as hemolytic uremic syndrome. *American Journal of Nephrology* 4 (3):188–91. doi: 10.1159/000166804.

Grant, D. R., and V. K. Sood. 1980. Studies of the role of ascorbic acid in chemical dough development. II. Partial purification and characterization of enzyme oxidizing ascorbate in flour. *Cereal Chemistry* 57:46–49.

Grosch, W., and H. Wieser. 1999. Redox reactions in wheat dough as affected by ascorbic acid. *Journal of Cereal Science* 29 (1):1–16. doi: 10.1006/jcrs.1998.0218.

Guo, Y., C. Zhao, P. Yu, J. Gao, Z. Liang, R. Ji, H. Du, J. Fu, J. Liang, and D. Yang. 2021. Molecular basis of sodium chloride dominated glutenin interaction and bread properties. *LWT - Food Science and Technology* 142:111011. doi: 10.1016/j.lwt.2021.111011.

Hanft, F., and P. Koehler. 2005. Quantitation of dityrosine in wheat flour and dough by liquid chromatography-tandem mass spectrometry . *Journal of Agricultural and Food Chemistry* 53 (7):2418–23. doi: 10.1021/jf048005t.

Himata, K., M. Noda, S. Ando, and Y. Yamada. 2000. Measurement of bromate in bread by liquid chromatography with post-column flow reactor detection. *Journal of AOAC International* 83 (2):347–55. doi: 10.1134/S0015462809050020.

Hird, F. J. R., and J. R. Yates. 1961a. The oxidation of cysteine, glutathione and thioglycollate by iodate, bromate, persulphate and air. *Journal of the Science of Food and Agriculture* 12 (2):89–95. doi: 10.1002/jsfa.2740120201.

Hird, F. J. R., and J. R. Yates. 1961b. The oxidation of protein thiol groups by iodate, bromate and persulphate. *The Biochemical Journal* 80 (3):612–6. doi: 10.1042/bj0800612.

Indrani, D., and G. V. Rao. 2006. Effect of additives on rheological characteristics and quality of wheat flour parotta. *Journal of Texture Studies* 37 (3):315–38. doi: 10.1111/j.1745-4603.2006.00054.x.

Jakob, H., S. Leininger, T. Lehmann, S. Jacobi, and S. Gutewort. 2000. Peroxo compounds, inorganic. In *Ullmann's encyclopedia of industrial chemistry*, Vol. A19, eds. B. Elvers, S. Hawkins, and W. Russey, 177–98. Weinheim, Germany: Wiley-VCH.

Joiner, R. R., F. D. Vidal, and H. C. Marks. 1963. A new powdered agent for flour maturing. *Cereal Chemistry* 40:539–53.

Jorgensen, H. 1935. Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxydationsmitteln auf proteolytische Enzymaktivität; über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfahigkeit des Weizenmehles. *Biochemische Zeitschrift* 280 (1):1–37.

Jorgensen, H. 1936. On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chemistry* 13:346–54.

Joye, I. J., A. Draganski, J. A. Delcour, and R. Ludescher. 2012. Monitoring molecular oxygen depletion in wheat flour dough using Erythrosin B phosphorescence: A biophysical approach. *Food Biophysics* 7 (2):138–44. doi: 10.1007/s11483-012-9251-6.

Joye, I. J., B. Lagrain, and J. A. Delcour. 2009a. Endogenous redox agents and enzymes that affect protein network formation during breadmaking – A review. *Journal of Cereal Science* 50 (1):1–10. doi: 10.1016/j.jcs.2009.04.002.

Joye, I. J., B. Lagrain, and J. A. Delcour. 2009b. Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking – A review. *Journal of Cereal Science* 50 (1):11–21. doi: 10.1016/j.jcs.2009.04.001.

Kieffer, R., J.-J. Kim, C. Walther, G. Laskawy, and W. Grosch. 1990. Influence of glutathione and cysteine on the improver effect of ascorbic acid stereoisomers. *Journal of Cereal Science* 11 (2):143–52. doi:10.1016/S0733-5210(09)80116-1.

Kohajdová, Z., and J. Karovičová. 2010. Impact of potassium iodate on the quality of wheat-spelt baked goods. *Acta Scientiarum Polonorum Technologia Alimentaria* 9 (4):443–50.

Kohman, H. A., C. Hoffman, and T. M. Godfrey. 1915. Manufacture of bread. US Patent 1,148,328.

Kuninori, T. 1963. L-Ascorbic acid oxidizing system in dough and dough improvement. *Cereal Chemistry* 40:647–57.

Kuninori, T. 1964. Glutathione in wheat and wheat flour. *Cereal Chemistry* 41:252–59.

Kurokawa, Y., S. Takayama, Y. Konishi, Y. Hiasa, S. Asahina, M. Takahashi, A. Maekawa, and Y. Hayashi. 1986. Long-term in vivo carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan. *Environmental Health Perspectives* 69:221–35. doi: 10.1289/ehp.8669221.

Kütahneci, E., and Z. Ayhan. 2021. Applications of different oxygen scavenging systems as an active packaging to improve freshness and shelf life of sliced bread. *Journal of Consumer Protection and Food Safety* 2021:1–13. doi: 10.1007/s00003-021-01331-3.

Kuwahara, T., Y. Ikehara, K. Kanatsu, T. Doi, H. Nagai, H. Nakayashiki, T. Tamura, and C. Kawai. 1984. 2 cases of potassium bromate poisoning requiring long-term hemodialysis therapy for irreversible tubular damage. *Nephron* 37 (4):278–80. doi: 10.1159/000183265.

Li, Y., J. Fu, Q. Shen, and D. Yang. 2020. High-molecular-weight glutenin subunits: Genetics, structures, and relation to end use qualities. *International Journal of Molecular Sciences* 22 (1):184. doi: 10.3390/ijms22010184.

Liu, G., J. Wang, Y. Hou, Y.-B. Huang, C.-Z. Li, L. Li, and S.-Q. Hu. 2017. Improvements of modified wheat protein disulfide isomerases with chaperone activity only on the processing quality of flour. *Food and Bioprocess Technology* 10 (3):568–81. doi: 10.1007/s11947-016-1840-9.

London, W. T., R. L. Vought, and F. A. Brown. 1965. Bread—A dietary source of large quantities of iodine. *The New England Journal of Medicine* 273 (7):381–81. doi: 10.1056/NEJM196508122730708.

Mair, G., and W. Grosch. 1979. Changes in glutathione content (reduced and oxidised form) and the effect of ascorbic acid and potassium bromate on glutathione oxidation during dough mixing. *Journal of the Science of Food and Agriculture* 30 (9):914–20. doi: 10.1002/jsfa.2740300914.

Manu, B. T., and U. J. S. Prasada Rao. 2011. Role of peroxidase and H_2O_2 in cross-linking of gluten proteins. *Journal of Food Biochemistry* 35 (6):1695–702. doi: 10.1111/j.1745-4514.2010.00494.x.

Marais, G. F., and B. L. D'appolonia. 1981. Factors contributing to baking quality differences in hard red spring wheat. I. Bases for different loaf volume potentials. *Cereal Chemistry* 58 (5):444–47.

Marks, H. C., R. R. Joiner, and H. K. Parker. 1959. Procedures and compositions for the treatment of flour. US Patent 2,903,361.

Matsumoto, H., J. Nishiyama, T. Mita, and T. Kuninori. 1975. Rheology of fermenting dough [Wheat]. *Cereal Chemistry* 52 (3): 82–88.

Mauritzen, C. M., and P. Stewart. 1963. Disulphide-sulphydryl exchange in dough. *Nature* 197 (4862):48–9. doi: 10.1038/197048a0.

Milas, N. A., and A. Golubovic. 1959. Studies in organic peroxides. XXVI. Organic peroxides derived from acetone and hydrogen peroxide. *Journal of the American Chemical Society* 81 (24):6461–62. doi: 10.1021/ja01533a033.

Miller, K. A., and R. C. Hoseney. 1999. Effect of oxidation on the dynamic rheological properties of wheat flour-water doughs. *Cereal Chemistry Journal* 76 (1):100–4. doi: 10.1094/CCHEM.1999.76.1.100.

Miyamoto, Y., and K. Nishimura. 2006. Production of thiyl radical on a peptide derived from wheat protein by superoxide anion radical. *Cereal Chemistry Journal* 83 (5):472–77. doi: 10.1094/CC-83-0472.

Mondal, A., and A. K. Datta. 2008. Bread baking – A review. *Journal of Food Engineering* 86 (4):465–74. doi: 10.1016/j.jfoodeng.2007.11.014.

Nakamura, M., and T. Kurata. 1997a. Effect of L-ascorbic acid and superoxide anion radical on the rheological properties of wheat flour-water dough. *Cereal Chemistry Journal* 74 (5):651–55. doi: 10.1094/CCHEM.1997.74.5.651.

Nakamura, M., and T. Kurata. 1997b. Effect of L-ascorbic acid on the rheological properties of wheat flour-water dough. *Cereal Chemistry Journal* 74 (5):647–50. doi: 10.1094/CCHEM.1997.74.5.647.

Nishimura, K. 2013. Production of thiyl radical on soluble glutenin by superoxide anion radical. *Food Science and Technology Research* 19 (1):117–22. doi: 10.3136/fstr.19.117.

Noonan, G. O., T. H. Begley, and G. W. Diachenko. 2008. Semicarbazide formation in flour and bread. *Journal of Agricultural and Food Chemistry* 56 (6):2064–67. doi: 10.1021/jf073198g.

Normand, J. C., F. Grange, C. Hernandez, A. Ganay, P. Davezies, A. Bergeret, and G. Prost. 1989. Occupational asthma after exposure to azodicarbonamide: Report of four cases. *Occupational and Environmental Medicine* 46 (1):60–2. doi: 10.1136/oem.46.1.60.

Osborne, T. B. 1907. *The proteins of the wheat kernel*. Sacramento, CA: Creative Media Partners.

Oser, B. L., and K. Morgareidge. 1967. Safety evaluation of flour treated with acetone peroxides. *Food and Cosmetics Toxicology* 5 (3):309–19. doi: 10.1016/S0015-6264(67)83056-6.

Oser, B. L., M. Oser, K. Morgareidge, and S. S. Sternberg. 1965. Studies of the safety of azodicarbonamide as a flour-maturing agent. *Toxicology and Applied Pharmacology* 7 (3):445–72. doi: 10.1016/0041-008X(65)90146-8.

Parenti, O., L. Guerrini, S. B. Mompin, M. Toldrà, and B. Zanoni. 2021. The determination of bread dough readiness during kneading of wheat flour: A review of the available methods. *Journal of Food Engineering* 309:110692. doi: 10.1016/j.jfoodeng.2021.110692.

Patterson, C. J. 1921. Process of bread-making. US Patent 1,385,842.

Pena, E., A. Bernardo, C. Soler, and N. Jouve. 2006. Do tyrosine cross-links contribute to the formation of the gluten network in common wheat (*Triticum aestivum* L.) dough? *Journal of Cereal Science* 44 (2):144–53. doi: 10.1016/j.jcs.2006.05.003.

Pereira, A. S., J. L. Donato, and G. de Nucci. 2004. Implications of the use of semicarbazide as a metabolic target of nitrofurazone contamination in coated products. *Food Additives and Contaminants* 21 (1): 63–9. doi: 10.1080/02652030310001647217.

Pereira, E. P. R., E. O. C. Amorim, H. C. I. Ambiel, Y. K. Chang, and C. J. Steel. 2009. Influence of oxidizing agents on the rheological properties of doughs prepared from white flour and whole-grain flour and on the specific volume of french rolls. *Brazilian Journal of Food Technology* 12 (3):161–71. doi: 10.4260/BJFT2009800900009.

Piber, M., and P. Koehler. 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 (13):5276–84. doi: 10.1021/jf050395b.

Piperno, D. R., E. Weiss, I. Holst, and D. Nadel. 2004. Processing of wild cereal grains in the Upper Palaeolithic revealed by starch grain analysis. *Nature* 430 (7000):670–3. doi: 10.1038/nature02734.

Pourmohammadi, K., and E. Abedi. 2021. Enzymatic modifications of gluten protein: Oxidative enzymes. *Food Chemistry* 356:129679. doi: 10.1016/j.foodchem.2021.129679.

Read, J. W., and L. W. Haas. 1937. Bakingquality of flour as affected by certain enzyme actions. III. Purified amylase and the relative proteolytic activity of anxiolytic agents. *Cereal Chemistry* 14:58–73.

Sahi, S. S. 2014. Ascorbic acid and redox agents in bakery systems. In *Bakery products science and technology*, ed. W. Zhou, 183–97. Hoboken, NJ: Wiley Blackwell.

Sandstedt, R. M., and B. D. Hites. 1945. Ascorbic acid and some related compounds as oxidizing agents in doughs. *Cereal Chemistry* 22:161–87.

Sarwin, R., G. Laskawy, and W. Grosch. 1993. Changes in the levels of glutathione and cysteine during the mixing of doughs with L-threo- and D-erythro-ascorbic acid. *Cereal Chemistry* 70 (5):553–57. doi: 10.1007/978-94-007-4923-8_5.

Schofield, J. D. 1996. Non-starch polysaccharides and enzymic improvement of bread quality. In *Wheat structure: Biochemistry and functionality*, ed. J. P. Schofield, 341–75. Cambridge, UK: Royal Society of Chemistry.

Sen, N. P., S. W. Seaman, M. Boyle, and D. Weber. 1993. Methyl carbamate and ethyl carbamate in alcoholic beverages and other fermented foods. *Food Chemistry* 48 (4):359–66. doi: 10.1016/0308-8146(93)90318-A.

Shanmugavel, V., K. Komala Santhi, A. H. Kurup, S. Kalakandan, A. Anandharaj, and A. Rawson. 2020. Potassium bromate: Effects on bread components, health, environment and method of analysis: A review. *Food Chemistry* 311:125964. doi: 10.1016/j.foodchem.2019.125964.

Shewry, P. R., N. G. Halford, and A. S. Tatham. 1992. High molecular weight subunits of wheat glutenin. *Journal of Cereal Science* 15 (2): 105–20. doi: 10.1016/S0733-5210(09)80062-3.

Shewry, R. P., N. G. Halford, and A. S. Tatham. 1989. The high molecular weight subunits of wheat, barley and rye: Genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. *Oxford Surveys of Plant Molecular and Cell Biology* 6:163–219.

Shimoni, Y., G. Segal, X. Zhu, and G. Galili. 1995. Nucleotide sequence of a wheat cDNA encoding protein disulfide isomerase. *Plant Physiology* 107 (1):281. doi: 10.1104/pp.107.1.281.

Singalavanija, A., N. Dongosintr, and D. Dulayajinda. 1994. Potassium iodate retinopathy. *Acta Ophthalmologica* 72 (4):513–19. doi: 10.1111/j.1755-3768.1994.tb02806.x.

Singalavanija, A., N. Ruangvaravate, and D. Dulayajinda. 2000. Potassium iodate toxic retinopathy: A report of five cases. *Retina (Philadelphia, Pa.)* 20 (4):378–83. doi:10.1097/00006982-200007000-00010.

Stauffer, C. E., and G. Beech. 1990. *Functional additives for bakery foods*. New York, NY: AVI Book, Van Nostrand Reinhold.

Sullivan, B., M. Howe, F. D. Schmalz, and G. R. Astleford. 1940. The action of oxidizing and reducing agents on flour. *Cereal Chemistry* 17:507–28.

Tanaka, K., S. Endo, and S. Nagao. 1980. Effect of potassium bromate, potassium iodate, and L-ascorbic acid on the consistency of heated dough. *Cereal Chemistry* 57 (3):169–74.

Taylor, J. T. 1964. Analysis of acetone peroxide premixes for bleaching and maturing flour. *Journal of AOAC INTERNATIONAL* 47 (2): 363–6. doi: 10.1093/jaoac/47.2.363.

Thewlis, B. H. 1974. The fate of potassium bromate when used as a breadmaking improver. *Journal of the Science of Food and Agriculture* 25 (12):1471–5. doi: 10.1002/jsfa.2740251207.

Tieckelmann, R. E., and R. E. Steele. 1991. Higher-assay grade of calcium peroxide improves properties of dough. *Food Technology* 45 (1):106–12.

Tilley, K. A., R. E. Benjamin, K. E. Bagorogoza, B. M. Okot-Kotber, O. Prakash, and H. Kwen. 2001. Tyrosine cross-links: Molecular basis of gluten structure and function. *Journal of Agricultural and Food Chemistry* 49 (5):2627–32. doi: 10.1021/jf010113h.

Tsen, C. C. 1963a. Changes in sulfhydryl and disulfide contents of doughs during mixing under various conditions. *Cereal Chemistry* 40:399–408.

Tsen, C. C. 1963b. The reaction mechanism of azodicarbonamide in dough. *Cereal Chemistry* 40 (6):638–46.

Tsen, C. C. 1964. Comparactive study on reactions of iodate, azodicarbonamide and acetone peroxides in simple chemical system in dough. *Cereal Chemistry* 41:20–3.

Tsen, C. C. 1965. The improving mechanism of ascorbic acid. *Cereal Chemistry* 42:86–97.

Von Sonntag, C., D. J. Deeble, M. Hess, H. P. Schuchmann, and M. N. Schuchmann. 1993. Superoxide radical anion in some unexpected chain reactions. In *Active oxygens, lipid peroxides and antioxidants*, ed. K. Yagi, 127–38. Tokyo, Japan: Japan Scientific Societies Press.

Walther, C., and W. Grosch. 1987. Substrate specificity of the glutathione dehydrogenase (dehydroascorbate reductase) from wheat flour. *Journal of Cereal Science* 5 (3):299–305. doi: 10.1016/S0733-5210(87)80030-9.

Watanabe, E., A. Bell, and B. Brockway. 1998. The effect of protein disulphide isomerase on dough rheology assessed by fundamental and empirical testing. *Food Chemistry* 61 (4):481–6. doi: 10.1016/S0308-8146(97)00095-2.

Webster, S. H., M. E. Rice, B. Highman, and E. F. Stohlman. 1959. The toxicology of potassium and sodium iodates: II. Subacute toxicity of potassium lodate in mice and guinea pigs. *Toxicology and Applied Pharmacology* 1 (1):87–96. doi: 10.1016/0041-008X(59)90152-8.

Webster, S. H., M. E. Rice, B. Highman, and W. F. Von Oettingen. 1957. The toxicology of potassium and sodium iodates: Acute toxicity in mice. *Journal of Pharmacology and Experimental Therapeutics* 120 (2):171–8.

Webster, S. H., E. F. Stohlman, and B. Highman. 1966. The toxicology of potassium and sodium iodates: III. Acute and subacute oral toxicity of potassium iodate in dogs. *Toxicology and Applied Pharmacology* 8 (2):185–92. doi: 10.1016/S0041-008X(66)80002-9.

Wieser, H. 2007. Chemistry of gluten proteins. *Food Microbiology* 24 (2):115–9. doi: 10.1016/j.fm.2006.07.004.

Wieser, H. 2012. The use of redox agents in breadmaking. In *Breadmaking: Improving quality*, ed. S. P. Cauvain, 447–69. Cambridge, UK: Woodhead Publishing Limited.

World Health Organization. 1996. *WHO/UNICEF/ICCIDD Joint Consultation: Recommended iodine levels in salt and guidelines for monitoring their adequacy and effectiveness*. Geneva, Switzerland: WHO/NUT 96/13, p. 4.

World Health Organization. 1999. Azodicarbonamide. Concise International Chemical Assessment Document 16.

Wu, H., and B. Chen. 2017. Effects of azodicarbonamide on human health. *Shanghai Journal of Preventive Medicine* 29 (10):813–8. doi: 10.3969/j.issn.1004-9231.2017.10.017.

Xu, F. 2001. Adsorption of oxygen gas by hydrated wheat flour. *LWT - Food Science and Technology* 34 (2):66–70. doi: 10.1006/fstl.2000.0726.

Yamada, Y., and K. R. Preston. 1992. Effects of individual oxidants on oven rise and bread properties of Canadian short process bread. *Journal of Cereal Science* 15 (3):237–51. doi: 10.1016/S0733-5210(09)80122-7.

Zentner, H. 1964. The oxidation of mechanically developed doughs. *Journal of the Science of Food and Agriculture* 15 (9):629–34. doi: 10.1002/jsfa.2740150910.

Zhao, C., Z. Luo, M. Li, J. Gao, Z. Liang, S. Sun, X. Wang, and D. Yang. 2020. Wheat protein disulfide isomerase improves bread properties via different mechanisms. *Food Chemistry* 315:126242. doi: 10.1016/j.foodchem.2020.126242.

Zimmerli, B., and J. Schlatter. 1991. Ethyl carbamate: Analytical methodology, occurrence, formation, biological activity and risk assessment. *Mutation Research/Genetic Toxicology* 259 (3-4):325–50. doi: 10.1016/0165-1218(91)90126-7.