

# Oxido-Reductases and Lipases as Dough-Bleaching Agents

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## ABSTRACT

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To replace benzoyl peroxide as a bread dough-bleaching agent, pure and commercial oxido-reductases (peroxidases, catalases, glucose oxidases, lipoxygenase, and laccase) were screened based on degradation of  $\beta$ -carotene in a liquid system (5  $\mu$ g of  $\beta$ -carotene/mL of 0.1M citrate phosphate buffer at pH 5.5 or 6.5) or dough. Peroxidases had the best bleaching activity;

some catalases also showed bleaching potential in a liquid system but not in bread dough, suggesting that screening enzymes in liquid media has limited application for dough. In 100 g of flour, combinations of peroxidase (3,000 U), lipase (815–1,630 U), and linoleic acid (0–300 mg) completely bleached bread dough.

Bread crumb color partially results from natural yellowish pigments present in flour, the carotenoids, which include  $\beta$ -carotene, xanthophyll (e.g., lutein) and flavones (e.g., triclin) (Kruger and Reed 1988). One kilogram of wheat flour contains  $\approx$ 3 mg of carotenoids, mainly lutein (Farre-Rovira and Costes 1974). Benzoyl peroxide is the most common flour-bleaching agent, but it is permitted only in certain countries, such as Canada and the United States. Benzoyl peroxide mainly affects flour lipophilic pigments, and its action is complete within 24 hr, depending on concentration.

Lipoxygenase is the only enzymatic bleaching agent permitted in most European countries. In North America, some bakers use lipoxygenase as a complementary bleaching agent to benzoyl peroxide. As enzyme-active soy flour, lipoxygenase is recommended at 0.5–1.0% (flour basis) in bread formulations, but in practice, it would be used at lower concentrations (Stauffer 1994). Soy flour is the major source of lipoxygenase in North America; lipoxygenase also is present in several plants, and alternative sources, such as lentil flour, have been proposed to the baking industry (Chang and McCurdy 1985). Although it is less enzyme-active than soy flour, broad bean (*Vicia faba*) flour is a regular source of lipoxygenase in European countries; at 0.5% (flour basis) high bleaching potential has been reported (Calvel 1984). In contrast to benzoyl peroxide, lipoxygenase action takes place in the bread mixer because it needs water and oxygen to become active.

A major drawback of full-fat enzyme-active flours containing lipoxygenase is their potentially negative effect on bread flavor (Wolf 1975). Although the flavor may be acceptable for some types of bread, Calvel (1990) condemned the use of adjunct flours containing lipoxygenase because they would be deleterious to the flavor of French bread at levels as low as 0.2% (soy flour) or 0.5% (broad bean flour). Off-flavor development (rancidity) also limits the use of lipoxygenase in bread and may explain why it is only a complementary bleaching agent to benzoyl peroxide in North America.

In essence, the presence of unsaturated lipids that may be attacked by lipoxygenase produces both off-flavor and indirect bleaching action. Among the three lipoxygenase isozymes in soy flour, isozyme 2 is the most effective bleaching agent but also the most potent off-flavor precursor (Grosch et al 1976, van Ruth et al 1992). Isozyme 2 can be removed by defatting (Addo et al 1993), but its bleaching activity diminishes proportionally. Lipoxygenase modifies lipids present in soy flour and other sources into hydroperoxides that are readily transformed into a wide range of carbonyl compounds that contribute to food flavor deterioration (Frankel 1991).

Linoleic (C<sub>18:2</sub>) and linolenic (C<sub>18:3</sub>) acids are the main substrates of lipoxygenase, but monoglycerides are not transformed.  $\beta$ -Carotene protects cell membranes from injuries naturally by quenching reactive oxygen species (Krinsky 1979). Flour pigments are bleached by cooxidation, and as a result, the bleaching effect is obtained from the action of free radicals and other reactive oxygen species formed during fatty acid oxidation, not directly from lipoxygenase action. Other hydroperoxide-reactive enzymes, such as hydroperoxide isomerase, may cleave fatty acid hydroperoxides into shorter chains and form off-flavor compounds (e.g., *trans*-2-nonenal, hexanal, and hexanol) (Gardner 1988).

To avoid the problems described above, an alternative solution would be to use bleaching enzymes that do not rely as much as lipoxygenase does on unsaturated lipids to produce free radicals. For example, these enzymes might partially or totally substitute for lipoxygenase without impairing bread flavor. Two types of oxido-reductases are capable of some bleaching action on flour pigments: catalases (Hawthorn and Todd 1955a,b) and peroxidases (Ben-Aziz et al 1971, Nicolas 1978, Ekstrand and Björck 1986). Catalases help transform hydrogen peroxide into water and oxygen; peroxidases catalyze the oxidation of a number of aromatic amines and phenols by hydrogen peroxide.

The general objective of this study was to screen enzymes to replace the most popular chemical bleaching agent, benzoyl peroxide. Various types of oxido-reductases, such as catalases and peroxidases, were screened in liquid media and tested in dough. Lipases also were tested in dough, because they have been reported to have some bleaching action in dough (Si and Hansen 1994).

## MATERIALS AND METHODS

### Screening of Oxido-Reductases in Liquid Media

Table I presents a list of the oxido-reductases tested. Amano Enzymes USA Co. Ltd. (Lombard, IL), Biopole SA (Brussels, Belgium), Danisco Ingredients USA (New Century, KS), Enzyme Development Corporation (New York), Genencor (Rochester, NY), Novo Nordisk (Danbury, CT), Rhône-Poulenc, ABM Brewing & Enzyme Group (Stockport, UK), Sigma Chemical Co. (St. Louis, MO), Solvay Enzymes (Elkhart, IN), STC Laboratories (Winnipeg, MB), and Stern-Enzyme GmbH & Co. (Ahrensburg, Germany) provided enzyme samples.

For buffered  $\beta$ -carotene solutions,  $\beta$ -carotene and linoleic acid solutions were prepared inside a dark glove box under nitrogen atmosphere to protect them from light and oxygen. A stock solution of  $\beta$ -carotene (solution A) was prepared by dissolving 25 mg of synthetic  $\beta$ -carotene (Sigma) in 25 mL of chloroform and 0.9 mL of Tween 80; solution A was stored at  $-18^{\circ}\text{C}$  under nitrogen. On a daily basis, 1 mL of solution A was evaporated to dryness under nitrogen and immediately dissolved in 10 mL of ethylenediamine-tetraacetic acid (EDTA) (0.25%, w/v) (solution B). Solution C was 0 or 100  $\mu$ L of linoleic acid, 600  $\mu$ L of Tween 80 (25%, v/v), and 10.8 mL of distilled water, to which 378  $\mu$ L of 1.0N NaOH was

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added drop by drop with constant stirring (magnetic bar stirrer). pH was adjusted to 8.6 with 1.0N HCl (162  $\mu$ L), and water was added to a final volume of 16.2 mL. Solution D was 500  $\mu$ L of solution B and 1 mL of solution C brought to 10 mL with 0.1M citrate phosphate buffer (pH 5.5 or 6.5).

The method used to screen enzymes was based on procedures described by Ben-Aziz et al (1971) and McDonald (1979). Degradation of  $\beta$ -carotene (5  $\mu$ g/mL) was followed at 450 nm with a spectrophotometer (Lambda Reader Automated Microplate Reader, Perkin-Elmer Corp., Norwalk, CT). The reaction was performed at room temperature in 96-well assay plates with flat bottoms (Dow Corning, Midland, MI). A standard curve of  $\beta$ -carotene (0–5  $\mu$ g/mL) was read at the same time. Enzyme solution (10  $\mu$ L at 2,000 or 10,000 U/mL) was mixed with 200  $\mu$ L of solution D (with or without linoleic acid; equivalent to 2 mM or 557  $\mu$ g/mL/assay plate) and 0–5  $\mu$ L of hydrogen peroxide at 0.2% (v/v) (equivalent to 1.37 mM or 46.5  $\mu$ g/mL/assay plate).

For confirmation assays, experiments were randomized with 15 enzymes tested at two concentrations with four substrates and two pH's. All 240 treatments were performed during the same day, and three repetitions were done on different days.

### Standardization of Oxido-Reductases Activity

Except for lipoxygenase, enzyme units were standardized according to Sigma procedures for enzymatic assays.

For catalase, 0.036% (w/w) hydrogen peroxide solution was prepared with 50 mM potassium phosphate buffer (pH 7.0, 25°C); the  $A_{240\text{nm}}$  of this solution was 0.52–0.55 U. Hydrogen peroxide solution (2.9 mL) was mixed with 0.1 mL of catalase solution (50–100 U/mL of 50 mM potassium phosphate buffer, pH 7.0, 4°C). Time elapsed for the linear decrease of absorbance from 0.45 to 0.40  $A_{240\text{nm}}$ , corresponding to a decrease from 10.3 to 9.2 mM  $\text{H}_2\text{O}_2$ . One unit of catalase transformed 1  $\mu$ mol  $\text{H}_2\text{O}_2$ /min at pH 7.0 and 25°C.

For glucose oxidase, 2.4 mL of 0.21 mM *o*-dianisidine solution in deionized water, 0.5 mL of 10%  $\beta$ -D-(+)-glucose solution in 50 mM sodium acetate buffer (pH 5.1, 25°C), and 0.1 mL of fresh peroxidase solution (60 U/mL of cold deionized water) were mixed in a cup,

and 0.1 mL of fresh glucose oxidase solution (0.4–0.8 U/mL of cold acetate buffer) was added. Immediately after mixing, the increase in absorbance was recorded at 500 nm for  $\approx$ 5 min. One unit of glucose oxidase oxidized 1  $\mu$ mol/min of  $\beta$ -D-(+)-glucose to D-glucuronic acid and hydrogen peroxide (equivalent to an oxygen uptake of 22.4  $\mu$ L/min) at pH 5.1 and 25°C.

For laccase, 2.2 mL of 100 mM potassium phosphate buffer (pH 6.5, 25°C) and 500  $\mu$ L of laccase solution (25–50 U/mL of cold deionized water) were mixed in a cup. A 0.216M syringaldazine solution (300  $\mu$ L) in absolute methanol was added. After mixing, the increase in absorbance was recorded at 530 nm for 10 min. One unit of laccase corresponded to a change of 0.001 A/min in 3 mL (final volume) of syringaldazine solution at pH 6.5 and 30°C.

For peroxidase, 0.3% (w/w) hydrogen peroxide solution was prepared with deionized water and hydrogen peroxide (30%, w/w). ABTS (2.9 mL of 9.1 mM +2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) in 100 mM potassium phosphate buffer (pH 5.0, 25°C) was mixed with 0.1 mL of peroxidase solution at 0.2–0.8 U/mL

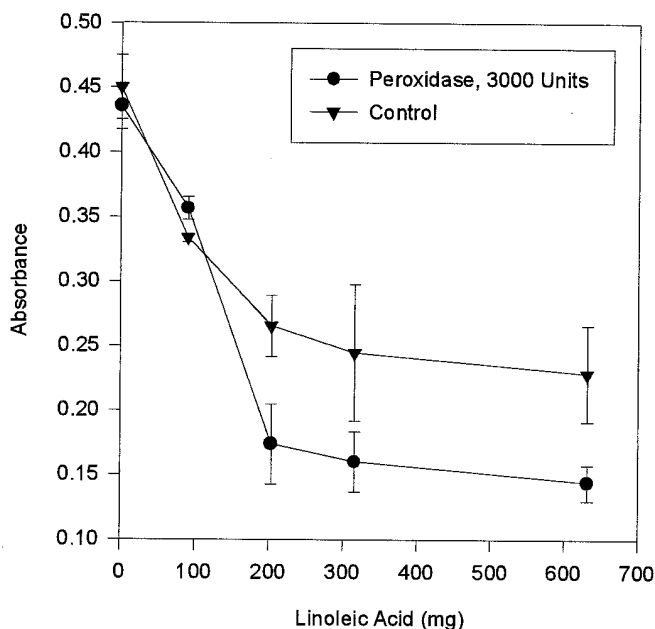


Fig. 1. Combined effects of soybean peroxidase and linoleic acid on degradation of  $\beta$ -carotene after mixing dough for 8 min. Complete bleaching obtained at 0.18 A.

TABLE I  
Oxido-Reductases Tested in Liquid Media

Enzyme	Source	Manufacturer	Commercial Name	
Catalase	Beef liver	Sigma	...	
	<i>Aspergillus niger</i>	Sigma	...	
	<i>A. niger</i>	Rhône-Poulenc	Catalase L43	
	<i>A. niger</i>	Amano	Catalase NL	
	<i>A. niger</i>	Amano	Hidelase <sup>a</sup>	
	<i>A. niger</i>	Genencor	Fermcolase 1000	
	<i>A. niger</i>	Novo Nordisk	Catazyme 50L	
	<i>Micrococcus lysodeikticus</i>	Enzyme Dev. Corp.	Enzeco Catalase-L	
	<i>M. lysodeikticus</i>	Solvay Enzymes	Microcatalase	
	<i>A. niger</i>	Genencor	Oxygo 1500 <sup>b</sup>	
	Glucose Oxidase	...	Danisco Ingredients	TS-E 55
		<i>A. niger</i>	Novo Nordisk	Novozym 358 <sup>b</sup>
<i>A. niger</i>		Rhône-Poulenc	Glucos RF <sup>b</sup>	
<i>A. niger</i>		Rhône-Poulenc	Glucos PS <sup>b</sup>	
<i>A. niger</i>		Rhône-Poulenc	DP 1293	
<i>A. niger</i>		Sigma	...	
<i>A. niger</i>		Stern-Enzym	Gloxy 4080 <sup>b</sup>	
<i>A. niger</i>		Stern-Enzym	Gloxy 4090 <sup>b</sup>	
Laccase		<i>Pyricularia oryzae</i>	Sigma	...
Lipoxygenase		Soybean	Sigma	...
Peroxidase	Horseradish	Sigma	...	
	Milk	Sigma	Lactoperoxidase	
	Milk	Biopole	Lactoperoxidase	
	Soybean	Sigma	...	
	Horseradish	STC Laboratories	...	

<sup>a</sup> Contains glucose oxidase side activity.

<sup>b</sup> Contains catalase side activity.

TABLE II  
Degradation (%) of  $\beta$ -Carotene (5  $\mu$ g/mL) after Contact (30 min) with Oxido-Reductases (10,000 U/mL) in 0.1M Citrate Phosphate Buffer (pH 6.5)

Enzyme <sup>a</sup>	Control	+LA <sup>b</sup> (2 mM)	+H <sub>2</sub> O <sub>2</sub> (1.37 mM)	+LA and H <sub>2</sub> O <sub>2</sub>
Peroxidase (soybean, Sigma)	67	96	85	98
Peroxidase (horseradish, STC)	65	95	75	93
Peroxidase (horseradish, Sigma)	58	89	73	88
Lactoperoxidase (milk, Biopole)	51	91	60	83
Lactoperoxidase (milk, Sigma)	38	93	61	83
Lipoxygenase (soybean, Sigma)	1	65	3	11
Catalase (bovineliver, Sigma)	4	30	0	58
Catalase Hidelase (Amano)	3	14	0	40
Catalase NL (Amano)	0	7	0	26
Catalase Fermcolase 1000 (Genencor)	0	5	0	20
Catalase ( <i>A. niger</i> , Sigma)	0	5	0	17
Microcatalase (Solvay)	0	4	0	10
Enzeco Catalase-L (Enzyme Dev. Corp.)	0	2	0	11
Catalase Catazyme 50L (Novo Nordisk)	...	5	0	11
Catalase L43 (Rhône-Poulenc)	0	0	0	9

<sup>a</sup> Source, manufacturer in parentheses.

<sup>b</sup> Linoleic acid.

of 40 mM potassium phosphate buffer (pH 6.8, 25°C) containing 0.25% (w/v) bovine serum albumin (BSA) and 0.5% (v/v) Triton X-100. The increase in absorbance was recorded at 405 nm. One unit of peroxidase oxidized 1 μmol ABTS/min at pH 5.0 and 25°C.

### Determination of Lipase Activity

A commercial lipase preparation (Lipase AY 30, Amano) was used. Its protein content was determined by bicinchoninic acid assay (Pierce Chemical Co., Rockford, IL), using BSA as standard. Based on a procedure modified from Yang et al (1994), lipase activity was measured in 50-mL tubes by titration of fatty acids released in an emulsion containing 20% olive oil (Sigma) in 100 mM Tris buffer (pH 7.0) with 4% Triton X-100 (Sigma). The mixture was emulsified at 37°C for 30 sec with a homogenizer (T25-S1, Janke & Kunkel GmbH & Co., IKA-Labor Technik, Staufen, Germany) equipped with a S25N-106 generator. Lipase (10 mg) was added to 10 g of emulsion, and incubation proceeded at 37°C for 90 min at 300 rpm (Environ Shaker, Lab-Line Instruments, Melrose Park, IL). The reaction was stopped by adding 20 mL of ethanol, followed by freezing at -18°C. For control, no incubation was performed, and the reaction was immediately stopped by adding ethanol, followed by freezing. After thawing at 25°C, acidity formed by free fatty acids was titrated to pH 9.5 with 0.05N NaOH (Titrimo S719, Metrohm, Herissau, Switzerland); acidity of the control was subtracted from the results. One unit of lipase released 1 μmol fatty acid from olive oil in 90 min at pH 7.0 and 37°C.

### Screening Enzymes in Dough

Bread dough was prepared in duplicate, using enzymes (various types and concentrations), 100 g of unbleached flour (stored at -30°C; Keynote 80, Robin Hood Multifoods, Montreal, QC), 54 g of water, 3 g of sucrose, 3 g of compressed yeast, 3 g of canola oil (Crisco, Procter & Gamble, Toronto, ON), 2 g of salt, 0.38 g of sodium stearoyl-2-lactylate (Atlas SSL, ICI, Brantford, ON), and 10 mg of ascorbic acid. For some experiments, various quantities of linoleic acid were incorporated in the formulation (100 μL weighed 90 mg) and previously mixed with canola oil. Except where indicated, ingredients were mixed for 8 min (total contact period for enzymes) in a 100- to 200-g mixer (National Mfg. Co., Lincoln, NE). Dough was bench-rested for 2 min, and two dough samples (20 g each) were taken for β-carotene extraction.

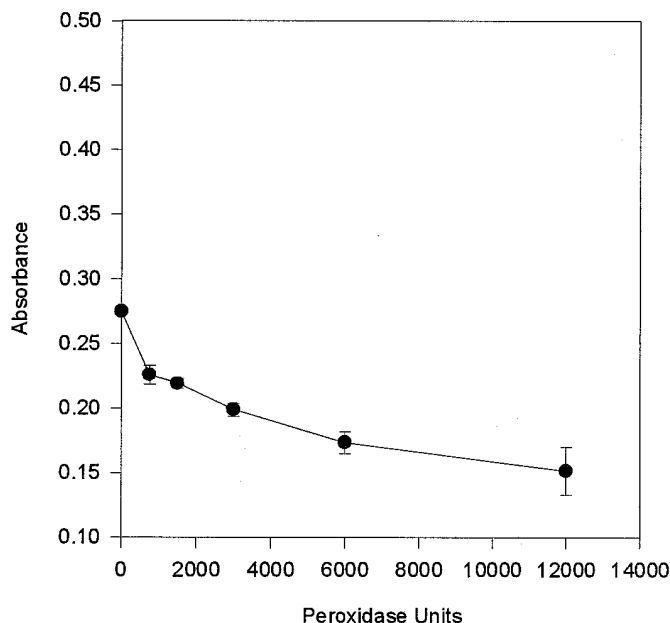


Fig. 2. Effect of soybean peroxidase on degradation of β-carotene after mixing dough with 200 mg of linoleic acid for 8 min. Complete bleaching obtained at 0.18 A.

For some experiments, the quantity of ingredients was doubled, and dough was baked. Dough was divided into two portions of 140 g each, sheeted-molded with a sheet-molder (L & M Co., Dowsview, ON), and placed in 55-mm-deep pans (145 mm × 80 mm). Fermentation was performed for 60 min at 37°C and 80% rh. Pigment extraction was performed on one dough; the other dough was baked at 210°C for 15 min in a revolving oven (Equipement de Boulangerie L. P., Victoriaville, QC). After cooling for 1 hr, extraction of β-carotene was performed in duplicate on 17.5 g of crumb taken from the center of the loaf.

### β-Carotene Determination by Total Lipids Extraction

The lipids extraction method used was adapted from Tsen et al (1962). Dough (20 g) was cut into small pieces and homogenized for 2 min in a micro-blender (Waring) with solvents (50 mL of methanol, 25 mL of chloroform, and 25 mL of water, the latter corrected for dough water content). Chloroform (25 mL) was added, and after blending for 30 sec, 25 mL of water was added and blended for 30 sec. The homogenate was centrifuged at 4°C at 16,000 × g for 15 min with a rotor (GSA) in a centrifuge (Sorvall RC-5B, Ingram and Bell Scientific, Weston, ON). Using a 500-mL funnel, the bottom layer (chloroform/carotenoid extract) was removed, and chloroform was evaporated under vacuum at 30°C (Rotavapor, Buchi, Brinkman Instruments, Rexdale, ON). Lipid extract was dissolved into 10 mL of cyclohexane and absorption was read on a spectrophotometer (DU-640, Beckman Instruments, Fullerton, CA) between 350 and 700 nm, and at 446 nm in 10-mm quartz-glass cells (Hellma Canada Ltd., Concord, ON).

For determination of β-carotene in unbleached and bleached flour, 12.1 g of flour was used (equivalent to the weight of flour in 20 g of dough). For bread, 17.5 g of crumb was taken to compensate for water loss during baking. Extractions were performed in duplicate.

## RESULTS AND DISCUSSION

### Selection of Oxido-Reductases in Liquid Media

Table II presents the effects of oxido-reductases (10,000 U/mL, pH 6.5) on percent β-carotene degradation in liquid media after contact for 30 min with and without linoleic acid, with and without hydrogen peroxide, and with linoleic acid and hydrogen peroxide. Whatever the source, peroxidases had the best bleaching

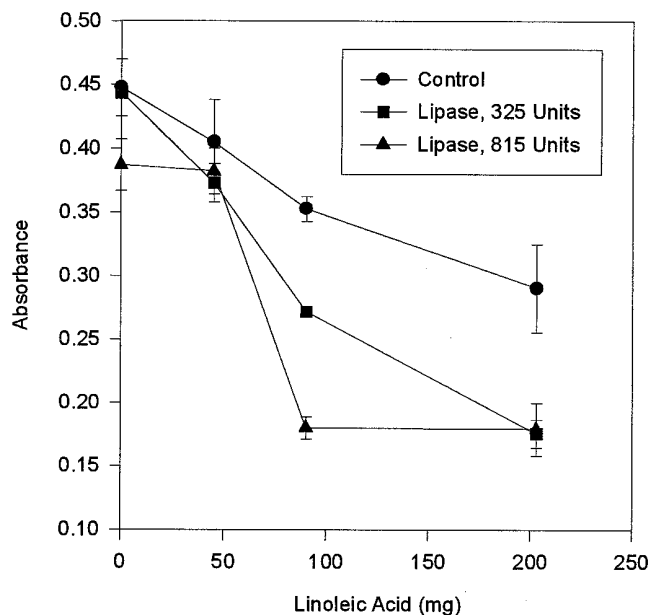


Fig. 3. Combined effects of lipase and linoleic acid on degradation of β-carotene after mixing dough for 8 min. Complete bleaching obtained at 0.18 A.

activity among enzymes tested, especially when linoleic acid was present, ≈90–95% degradation compared to 40–65% without linoleic acid. Supplementation with hydrogen peroxide increased β-carotene degradation by 10–20%. At 2,000 U/mL, instead of 10,000 U, the bleaching activity of peroxidases was generally reduced by 5–10% at pH 6.5; similar trends were observed at pH 5.5 (data not shown).

The presence of linoleic acid was essential to the activity of lipoxygenase (control). At 10,000 U of lipoxygenase/mL, bleaching was 65% complete, compared to 1% without linoleic acid (Table II); at 2,000 U, lipoxygenase activity dropped to only 11% (data not shown). The addition of hydrogen peroxide had no bleaching effect and almost suppressed the positive effect of linoleic acid on lipoxygenase activity. Catalases had low to intermediate bleaching activity (10–50%), depending on source and only when both hydrogen peroxide and linoleic acid were present (Table II). Catalase from bovine liver and hidelase from *Aspergillus niger* were the most active. When tested alone, linoleic acid had a positive effect on β-carotene degradation by catalases but not on hydrogen peroxide. Glucose oxidases and laccase had no bleaching activity, and combinations of various types of oxido-reductase did not accelerate β-carotene degradation (data not shown).

The literature has established that peroxidases have a bleaching action on β-carotene (Ben-Aziz et al 1971). Theoretically, peroxidase activity also may be linked to off-flavor development in plants, just as lipoxygenase is, especially when linoleic acid is present. However, intermediate bleaching activity was observed for peroxidases in the absence of linoleic acid, a cofactor in the reaction leading to off-flavor formation (Ben-Aziz et al 1971), suggesting that peroxidases may be preferable to lipoxygenase as bleaching agents. These enzymes also may have other positive effects on dough by forming cross-links between proteins in bread dough and improving consistency, crumb structure, and softness (Si 1994, Garti et al 1996).

### Selection of Oxido-Reductases in Dough

The best peroxidases and catalases screened in liquid media were tested in dough to check the potential of these enzymes in a complex system. During dough mixing, a great deal of air is incorporated, which was expected to affect the activity of enzymes compared to screening in liquid media. Extracts from unbleached flour and flour bleached with benzoyl peroxide had 0.48 and 0.18 A, respectively, so the target for bleaching dough with enzymes was fixed at 0.18 A.

After mixing bread dough prepared with 100 g of flour, no bleaching was observed with up to 15,000 U of soybean peroxidase (Sigma) (data not shown). However, 200–300 mg of linoleic acid (without enzyme) partially bleached dough, and complete bleaching was obtained by supplementing linoleic acid with 3,000 U of peroxidase (Fig. 1). With 200 mg of linoleic acid, the effect of peroxidase concentration (1,000–10,000 U) was slight (Fig. 2), which confirmed that linoleic acid plays a critical role in dough bleaching. Horseradish (STC Laboratories) and soybean (Sigma) peroxidase produced similar results (data not shown).

Contrary to trends observed in liquid media, catalases (10,000–50,000 U) from two sources did not bleach dough, either supplemented or not with linoleic acid (data not shown). These enzymes may have reacted nonspecifically with dough constituents without coming into close contact with flour pigments. According to these results, the use of liquid media for selecting bleaching enzymes would be of limited interest for bread dough applications, despite the fact that screening in liquid media is widespread in the literature.

### Effect of Lipase on Dough Bleaching

Despite the fact that lipases are not oxido-reductases and, therefore, were not screened for degradation of β-carotene in liquid media, it was expected that lipases might play some role in dough

bleaching (Si and Hansen 1994). When used alone, the commercial lipase tested (AY 30) had some dough-bleaching potential (Fig. 3). However, complete bleaching was obtained only when linoleic acid was present (90–200 mg) in combination with 325–815 U of lipase/100 g of flour. At the latter concentrations, it was estimated that lipases bleached ≈50% of β-carotene in flour. A combination of 815 U of lipase and only 90 mg of linoleic acid (Fig. 3) produced complete bleaching activity, which is similar to 3,000 U of peroxidase combined with 200 mg of linoleic acid (Figs. 1 and 2). Thus, under tested conditions in dough, lipase was an even more potent bleaching enzyme than peroxidase, because lower concentrations of linoleic acid were necessary for bleaching when lipase was present.

### Combining Peroxidases and Lipases for Dough Bleaching

In combining 815 U of lipase and 3,000 U of peroxidases, either from soybean or horseradish, we failed to reduce further the level (90 mg) of linoleic acid necessary to produce complete bleaching (data not shown). We then used another peroxidase not tested previously in liquid media (Novo Nordisk; source unknown). In dough, its activity was approximately equivalent to horseradish peroxidase; like the other peroxidases, the peroxidase from Novo Nordisk had only slight bleaching activity when tested alone at 3,000 U/100 g of flour and still required 90 mg of linoleic acid to bleach dough completely. However, contrary to results obtained with the other peroxidases, the peroxidase from Novo Nordisk (3,000 U) showed a synergistic effect with lipase (815 U) and enabled a further reduction of the linoleic acid concentration from 90 to 50 mg to produce complete bleaching. When 3,000 U of the peroxidase from Novo Nordisk were combined with 1,630 U of lipase (instead of 815 U), bleaching was complete without linoleic acid (data not shown). As for the other peroxidases tested in dough, the latter bleaching effect was not improved with higher concentrations (up to 12,000 U) of the peroxidase from Novo Nordisk (data not shown). These results stress the importance of the source of peroxidase as a dough-bleaching agent.

## CONCLUSIONS

As determined in both liquid media and dough, peroxidases and lipases did have bleaching activity. However, the use of liquid media for estimating enzymatic bleaching activity was markedly different for bread dough applications. Under the mixing conditions tested, linoleic acid was the most efficient dough-bleaching agent, but it could be replaced by specific combinations of peroxidase (3,000 U/100 g of flour) and lipase (815–1,630 U). Peroxidase source had a major impact on dough bleaching. Further investigations on the action of enzymes and fatty acids on dough bleaching are needed, such as their effects on dough rheology and bread flavor. Reasons for differences between liquid and dough systems for determination of enzyme activity also could be addressed.

## ACKNOWLEDGMENTS

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