Effect of Lipid Oxidation on Dough Bleaching

M. Mercier[1] and P. Gélinas[2]

ABSTRACT

Cereal Chem. 78(1):36–38

The effect of lipid composition and oxidation on dough bleaching has been determined. At >2.25% (flour basis), pure linoleic acid was very efficient in degrading β-carotene in dough, unlike colza, corn, peanut, soy, or sunflower oil, which were mainly characterized by different polyunsaturated fatty acids content. In a very oxidized state, as determined by a peroxide index of >15 meq/kg of oil, sunflower oil (rich in polyunsaturated fatty acids) had a major bleaching activity on β-carotene when compared with colza oil (less polyunsaturated), especially in combination with long mixing times. A combination of lipase (815 U), slightly oxidized oil (peroxide index of 2–5 meq/kg of oil), and linoleic acid (90 mg/100 g of flour) significantly degraded flour pigments ($P < 0.05$).

Wheat flour is naturally yellowish due to the presence of carotenoids. During flour storage, pigments are slowly bleached within two to three weeks. However, in most Canadian and U.S. milling plants, benzoyl peroxide is normally used to bleach flour and the process is complete within 24 hr. As an alternative to this additive in breadmaking, lipoxygenases present in soy or broad bean flour are effective but would have a negative effect on bread flavor (Wolf 1975). At the dough stage, peroxidases and lipases also showed some bleaching potential (Gélinas et al 1998).

The most studied bleaching enzymes, lipoxygenases, are very specific toward polyunsaturated fatty acids with nonconjugated double links (*cis-cis*-1,4 pentadiene). These enzymes preferably attack linoleic and linolenic acids (Hugues et al 1994), especially as free fatty acids or monoglycerides (Drapron and Beaux 1969; Mann and Morrison 1974; Tait and Galliard 1988).

During mixing, oxygen uptake by dough would increase due to the presence of polyunsaturated fatty acids (linoleic and linolenic acids) but not oleic acid in dough (Smith and Andrews 1957). Through oxidation of polyunsaturated fatty acids, free hydroxyls are formed and react with flour pigments; they would be the true bleaching agents considering their very unstable nature (Drapron 1973; Nicolas and Potus 1994). Hawthorn and Todd (1955) showed that supplementation of dough with pure linoleic acid accelerated pigment discoloration. These authors also suggested that the degree of unsaturation of fat used in bread formulation might affect dough color. Dough mixing conditions are likely to affect these reactions because longer or more intense mixing is likely to increase reaction rates, which improves pigment discoloration (Hawthorn and Todd 1955; Nicolas et al 1978).

Because dough lipid composition might affect its bleaching during mixing, the objective of this study was to determine the effect of fat composition and oxidation on dough bleaching. It was hypothesized that the use of slightly oxidized fats and long dough mixing periods might replace benzoyl peroxide.

MATERIALS AND METHODS

Dough Preparation

Bread dough was prepared with 100 g of unbleached flour (hard red spring wheat; 12.8% protein and 14.1% moisture) (Robin Hood Multifoods Inc., Montreal, QC), 57 g of water, 3 g of sucrose, 3 g of colza oil (Crisco, Procter & Gamble Inc., Toronto, ON), 2 g of salt, 1 g of instant dry yeast (Fermipan, Lallemand, Inc., Montreal, QC), and 100 ppm of ascorbic acid (American Chemicals Ltd., Montreal, QC). Control was the same flour but previously bleached with 26 ppm of benzoyl peroxide. For some experiments, linoleic acid (Sigma-Aldrich, St. Louis, MO) was used as replacement for colza oil. Other oils also tested in another set of experiments were corn (Best Foods, Inc., Montreal, QC), peanut (Best Foods), soy (Bioforce Canada, Inc., St. Laurent, QC) and sunflower (Bioforce Canada). Oils were stored at 4°C in jars covered with aluminum foil.

Except where indicated, dough was mixed in triplicate for 4 min in a 100- to 200-g mixer (National Mfg. Co., Lincoln, NE). Dough was bench-rested for 20 min, and two dough samples (20 g each) were taken for β-carotene extraction.

β-Carotene Determination

Lipids extraction and determination were performed in duplicate according to Gélinas et al (1998). A standard curve was used to transform absorbance units into concentration of carotene.

Fatty Acids Analyses

In triplicate, 0.02 g of oil was transmethylated with a 3:1:2 mixture of BF_3-methanol, diethylic ether, and hexane (Sigma Aldrich). After vortexing, the preparation was heated at 65°C for 30 min, cooled, and supplemented with water (0.6:1). Head fraction was separated by gas chromatography (model 5890; Hewlett Packard, Avondale, PA) using a DB-225 column (30 m × 0.25 mm, i.d.) (J & W Scientific, Folsom, CA). Temperature of the column was fixed at 160°C for 2 min, then raised to 180°C at 20°C/min and to 210°C at 5°C/min with a 3-min hold at the end.

Fat Oxidation Assays and Analyses

The method was adapted from von Gadow et al (1997) and Gordon and Kouřimská (1994). In triplicate, 200 g of oil was placed in a jar and put in a water bath at 90°C. Under direct lighting, oxygen was injected into the oil through two outlets at 300 mL/min. After treatment for ≤12 hr, oil was cooled at 4°C under nitrogen (500 mL/min) and frozen at –28°C. Peroxide index was determined in triplicate according to Cocks and Van Rede (1966).

Dough Rheology Analyses

Farinograms were performed in triplicate with the 300-g bowl according to ICC (1998).

Lipase Assays

Lipase (815 U, AY30, from *Candida rugosa*; Amano Enzymes U.S.A., Ltd., Lombard, IL) was added to dough in combination with fresh or slightly oxidized sunflower oil (peroxide index of 2, 3.5, or 5.25 meq/kg of oil) and linoleic acid (90 mg/100 g of flour). Enzyme concentrate was previously diluted with water. One unit of lipase released 1 μmol of fatty acids from olive oil in 90 min at pH 7.0 and 37°C.

[1] Département des Sciences des aliments et de nutrition, Pavillon Paul-Comtois, Université Laval, Ste. Foy, Quebec, Canada G1K 7P4.
[2] Corresponding author: Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Blvd. West, St. Hyacinthe, Quebec, Canada J2S 8E3. E-Mail: gelinasp@em.agr.ca

Publication no. C-2000-1201-07R.

Statistical Methods

Results from linoleic acid assays were analyzed with the general linear models (GLM) procedure (SAS Institute, Inc., Cary, NC) using a Dunnett test at a confidence level of 95%. The effect of oil source was determined with the GLM procedure and a least significance difference test ($P < 0.05$). Combined effects of linoleic acid, lipase, and fat oxidation ($2 \times 2 \times 3$ levels) were analyzed with the GLM procedure using least significant difference (LSD).

RESULTS AND DISCUSSION

Effect of Linoleic Acid and Oil Type

Residual β-carotene content in dough according to replacement of colza oil by linoleic acid is shown in Table I. Linoleic acid had a significant dough-bleaching effect when used at >75% in colza oil (3%, flour basis), which corresponded to >2.25% of pure linoleic acid in dough. These results confirm the dough-bleaching potential of linoleic acid (Hawthorn and Todd 1955; Mann and Morrison 1975). However, such a large quantity of linoleic acid is impossible to use in practice, considering its prohibitive cost. Tocopherol, an oil antioxidant, also has been used as replacement for colza oil to check potential bleaching due to oxidation of colza oil during dough mixing. Colza oil and tocopherol had a similar degradation effect of β-carotene in dough. Therefore, oxidation of colza oil during dough mixing did not occur or was minor, probably due to limited contact under aerobic conditions (4 min).

Commercial oils with different polyunsaturation levels had no dough-bleaching potential whatever their linoleic acid (25–65%) and linolenic acid (0–9%) (Table II). These results stress the importance of the state of the added fats; the free form of polyunsaturated acids has more bleaching potential than the glyceride form. Considering that hydroperoxides are involved in bleaching (Frankel 1991), a series of assays involved artificial oxidation of specific oils to determine their importance in dough bleaching according to mixing time.

TABLE I
Degradation of β-Carotene in Dough After Replacement of Colza Oil (3%, flour basis) by Linoleic Acid

Linoleic Acid in Colza Oil (%)[a]	Residual β-Carotene (mg/kg of dough)
0	1.09a (0.15)[b]
10	1.07a (0.08)
33	1.03a (0.33)
50	0.98a (0.18)
75	0.83a (0.17)
100	0.65b (0.20)
Standard (bleached flour)	0.45b (0.05)
Tocopherol[c]	1.08a (0.08)

[a] Peroxide index of fresh colza oil was 2 meq/kg of oil.
[b] Values followed by the same letter are not significantly different ($P < 0.05$) (Dunnett). Standard errors in parentheses.
[c] Antioxidant used instead of colza oil.

TABLE II
Degradation of β-Carotene in Dough by Oil Type (3%, flour basis)

Oil[a]	Fatty Acids Profile (C_{18}) (%) Oleic	Linoleic	Linolenic	Residual β-Carotene (mg/kg of dough)
Colza	60.9	26.5	9.3	1.13a (0.12)[b]
Corn	30.9	58.8	0.1	1.06a (0.10)
Peanut	50.3	33.8	0	1.05a (0.05)
Soy	23.2	56.4	9.1	1.20a (0.28)
Sunflower	22.6	65.3	0	1.08a (0.10)
Standard[c]	…	…	…	0.44b (0.03)

[a] Peroxide index of fresh colza oil was 2 meq/kg of oil.
[b] Values followed by the same letter are not significantly different ($P < 0.05$) (Dunnett). Standard errors in parentheses.
[c] Bleached flour.

Effect of Oil Oxidation and Mixing Time

Using oils with a high (sunflower) or low (colza) linoleic acid content, the combined effects of fat oxidation and mixing time were determined on dough bleaching. After ≈6–10 hr of forced oxidation, the most unsaturated oil (sunflower) was a more effective bleaching agent than colza oil (Table III). With sunflower oil oxidized for 6 hr, but not colza oil, mixing under intense conditions for >9 min and ≤16 min, had a significant dough bleaching action. However, mixing for such a long period was much too long for standard dough development and had a lower bleaching effect compared with oil oxidation. Whatever the oil type and oxidation level, dough mixing stability and water absorption were constant (data not shown).

Sunflower oil was mainly transformed after 6 hr of forced oxidation, in contrast to colza oil, which was much more resistant to this treatment (Fig. 1). A high peroxide index (15–135 meq/kg of oil) of sunflower oil corresponded to major dough-bleaching activity (Table III). This means that marked bleaching was only observed when sunflower oil became very oxidized (rancid). Because a peroxide index of ≈5 meq/kg of oil is generally considered as the limit for oil rancidity and human acceptance (Warner 1994), this limit value was used in the assays.

Effect of Lipase

A series of assays involved a combination of slightly oxidized sunflower oil (peroxide index of 2, 3.5, or 5.25 meq/kg of oil), lipase, and linoleic acid. This enzyme (815 U) in combination with linoleic acid (90 mg/100 g of flour) has shown some potential for dough bleaching using a longer mixing time (8 min instead of 4 min) (Gélinas et al 1998). Fresh or slightly oxidized sunflower oil (peroxide index of ≤5.25 meq/kg of oil) had no dough-bleaching activity, even when supplemented with linoleic acid ($P < 0.05$) (Table IV). The use of lipase with fresh oil (peroxide index of 2 meq/kg of oil) had some degradation effect on dough pigments. A combination of lipase with either fresh or slightly oxidized oil slightly improved dough bleaching, but its effect was increased with 90 mg of linoleic acid/100 g of flour. These results revealed the potential of using slightly oxidized oil in combination with lipase and pure linoleic acid. Among the three factors tested, lipase was the most effective for degradation of β-carotene, followed by slight oil oxidation or linoleic acid.

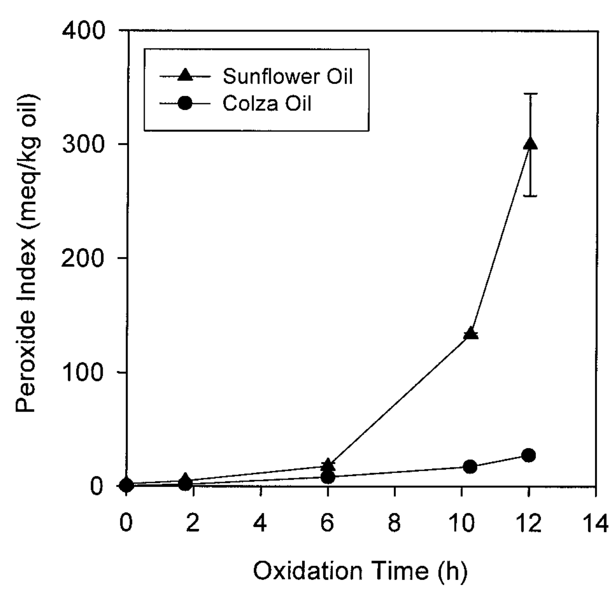

Fig. 1. Effect of oxidation time on peroxide index of sunflower or colza oil. Oxidation obtained through twin injection of oxygen (300 mL/min) into oil sample.

TABLE III
Degradation of β-Carotene in Dough by Oxidation State of Sunflower or Colza Oil[a] (3%, flour basis) and Mixing Time

Oil Oxidation Time (hr)[b]	Mixing Time (min)	Residual β-Carotene (mg/kg of dough)	
		Sunflower Oil	Colza Oil
0	9	1.31a (0.06)[c]	1.13a (0.10)
1.75	4	1.21a (0.10)	1.01a (0.04)
1.75	14	1.10a (0.05)	1.08a (0.07)
6	2	1.04a (0.09)	1.07a (0.04)
6	9	1.06a (0.05)	0.98a (0.06)
6	16	0.88b (0.06)	0.88a (0.04)
10.25	4	0.37c (0.06)	0.99a (0.04)
10.25	14	0.31c (0.07)	0.80a (0.05)
12	9	0.29c (0.07)	0.77a (0.08)
Standard[d]	4	0.44c (0.03)	0.44b (0.03)

[a] Peroxide index of fresh colza oil was 2 meq/kg of oil.
[b] Fat oxidation obtained through twin injection of oxygen (300 mL/min) into oil sample.
[c] Values followed by the same letter are not significantly different ($P < 0.05$) (Dunnett). Standard errors in parentheses.
[d] Bleached flour.

TABLE IV
Degradation of β-Carotene in Dough by Sunflower Oil Oxidation Time (3%, flour basis) in Combination with Lipase and Linoleic Acid

Oil Oxidation Time (hr)[a]	Peroxide (meq/kg of oil)	Lipase (U)	Linoleic Acid (mg/100 g of flour)	Residual β-Carotene (mg/kg of dough)
0	2	0	0	1.49a (0.05)[b]
1	3.5	0	0	1.45a (0.13)
2	5.25	0	0	1.38a (0.13)
0	2	0	90	1.37a (0.21)
0	2	815	0	1.22b (0.09)
1	3.5	815	0	1.13bc (0.11)
2	5.25	815	0	1.12bc (0.02)
0	2	815	90	1.18b (0.05)
1	3.5	815	90	1.07bc (0.11)
2	5.25	815	90	1.00c (0.06)
Standard[c]	2	0	0	0.45d (0.08)

[a] Oxidation of sunflower oil obtained through twin injection of oxygen (300 mL/min).
[b] Values followed by the same letter are not significantly different ($P < 0.05$) (Dunnett). Standard errors in parentheses.
[c] Bleached flour.

CONCLUSIONS

Free linoleic acid (≈2.25%, flour basis), as well as highly oxidized sunflower oil (peroxide index of 15–135), had major dough-bleaching potential, especially in combination with long mixing times. This effect was not observed with colza oil, which was characterized by a lower polyunsaturation level. In comparison, lipase (815 U) could degrade less pigment, but this effect increased in combination with slightly oxidized oil (peroxide index of ≤5.25 meq/kg of oil) and 90 mg of linoleic acid/100 g flour.

Considering the prohibitive cost of linoleic acid, the use of slightly oxidized oil (5.25 meq/kg of oil) and lipase (815 U) would be an acceptable compromise for bakery applications requiring partial degradation of flour β-carotene. Results presented need to be confirmed in breadbaking experiments where high temperatures might affect β-carotene degradation. Bread sensory analyses would also be useful to optimize acceptable oil oxidation level. Further research is also needed to determine the precise effects of lipids, lipases, and dough mixing conditions (speed and time) on dough bleaching.

ACKNOWLEDGMENTS

We thank Elyse Poitras (FRDC) and Jean Amiot (Département des Sciences des aliments et de nutrition, Université Laval, Ste. Foy, Quebec) for advice during the planning of the experiments; Keith Bruce (Robin Hood Multifoods Inc., Montreal, QC) for providing flour samples; Claude Gosselin (Département des Sciences des aliments et de nutrition, Université Laval) for help during lipids analyses; and Natalie Rodrigue (FRDC) for help in statistical design and analyses.

LITERATURE CITED

Cocks, L. V., and Van Rede, C. 1966. Laboratory Handbook for Oil and Fats Analysis. Academic Press: New York.

Drapron, R. 1973. Intervention de la lipoxygénase en panification: Oxydation des acides gras essentiels à l'état libre, des pigments caroténoïdes et des tocophérols, formation d'hexanal. Rev. Fr. Corps Gras. 20:83-87.

Drapron, R., and Beaux, Y. 1969. Sur l'oxydation des acides gras essentiels par la lipoxygénase en panification et la formation de composés volatils intervenant dans l'arôme du pain. CR Acad. Sci. 268:2598-2601.

Frankel, E. N. 1991. Recent advances in lipid oxidation. J. Sci. Food Agric. 54:495-511.

Gélinas, P., Poitras, E., McKinnon, C. M., and Morin, A. 1998. Oxido-reductases and lipases as dough-bleaching agents. Cereal Chem. 75:810-814.

Gordon, M. H., and Kouřimská, L. 1995. The effects of antioxidants on changes in oils during heating and deep frying. J. Sci. Food Agric. 68:347-353.

Hawthorn, J., and Todd, J. P. 1955. Some effects of oxygen on the mixing of bread doughs. J. Sci. Food Agric. 6:501-511.

Hugues, M., Boivin, P., Gauillard, F., Nicolas, J., Thiry, J.-M., and Richard-Forget, F. 1994. Two lipoxygenases from germinated barley—Heat and kilning stability. J. Food Sci. 59:885-889.

ICC. 1998. Standard Methods of the International Association for Cereal Science and Technology. Method 115. ICC: Vienna, Austria.

Mann, D. L., and Morrison, W. R. 1974. Changes in the lipids during mixing and resting of flour water doughs. J. Sci. Food Agric. 25:1109-1119.

Mann, D. L., and Morrison, W. R. 1975. Effects of ingredients on the oxidation of linoleic acid by lipoxygenase in the bread doughs. J. Sci. Food Agric. 26:493-505.

Nicolas, J., Launay, B., Roussel, P., and Drapron, R. 1978. Influence de la composition, du pétrissage et du repos sur la destruction des pigments caroténoïdes et les propriétés viscoélastiques des pâtes de farine de blé tendre. Bull. Ec. Fr. Meun. 287:247-282.

Nicolas, J., and Potus, J. 1994. Phénomènes d'oxydation enzymatique et cooxydations. Exemples du rôle de la lipoxygénase en panification et de la polyphénoloxydase en technologie des fruits. Sci. Aliments 14:627-642.

Smith, D. E., and Andrews, J. S. 1957. The uptake of oxygen by flour dough. Cereal Chem. 34:323-336.

Tait, S. P. C., and Galliard, T. 1988. Oxidation of linoleic acid in doughs and aqueous suspensions of wholemeal flours: Effect of storage. J. Cereal Sci. 8:55-67.

von Gadow, A., Joubert, E., and Hansmann, C. F. 1997. Effect of extraction time and additional heating on the antioxidant activity of rooibos tea (*Aspalathus linearis*) extracts. J. Agric. Food Chem. 45:1370-1374.

Warner, K. 1994. Sensory evaluation of oils and fat-containing foods. Pages 49-65 in: Methods to Assess Quality and Stability of Oils and Fat-Containing Food. K. Warner and N. A. M. Eskin, eds. AOCS Press: Champaign, IL.

Wolf, W. J. 1975. Lipoxygenase and flavor of soybean protein products. J. Agric. Food Chem. 23:136-141.

[Received February 15, 2000. Accepted October 2, 2000.]