

Effect of Exogenous Lipase on Dough Lipids During Mixing of Wheat Flours

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ABSTRACT

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In control dough, endogenous wheat lipase was inactive, because the triacylglycerol (TAG), 1,2-diacylglycerol (DAG_{1,2}), and 1,3-diacylglycerol (DAG_{1,3}) fractions of nonpolar lipids were not affected by mixing. Conversely, the free fatty acid (FFA) and monoacylglycerol (MAG) fractions decreased, mainly due to the oxidation of polyunsaturated fatty acids (PUFA) catalyzed by wheat lipoxygenase. Addition of exogenous lipase to flour (15 lipase units [LU] per gram of dry matter) resulted in substantial modification of nonpolar lipids during dough mixing. Due to the 1,3 specificity of the lipase used in this experiment, the TAG and DAG_{1,3} fractions decreased, whereas the MAG and FFA fractions increased. The DAG_{1,2} fraction increased at the beginning of mixing and decreased after

40 min of mixing. Moreover, part of the PUFA released by lipase activity was oxidized by wheat lipoxygenase, resulting in major losses of PUFA. Conversely, the net content of the saturated and monounsaturated fatty acids (SMUFA) remained constant, because the free SMUFA content increased primarily at the expense of the esterified forms. For a constant mixing time of 20 min, increasing the amount of lipase added to dough (from 2.5 to 25 LU/g of dry matter) resulted in a linear decrease in the TAG fraction and a linear increase in the SMUFA content in the FFA fraction. At the same time, the PUFA content of the FFA fraction increased only for additions of lipase to flour of >5 LU/g of dry matter, due to partial oxidation by wheat lipoxygenase.

Lipids form a relatively minor compound in wheat, after extraction and purification procedures they constitute between 2 and 2.8% of dry matter in wheat flour (Morrison 1983, Genot et al 1984, Chung 1991). Approximately half of the lipids are polar, and the ratio of polar to nonpolar lipids is of great importance in breadmaking because of its strong correlation with bread volume (McRitchie 1984). Wheat lipids currently are classified as free or bound, according to extractability by different solvents (Morrison 1983, Genot et al 1984). Among bound lipids, starch lipids, which account for ≈1%, require extreme conditions, such as hot aqueous-alcohol mixtures, for extraction (Chung 1991). Bound lipids, which are primarily phospholipids (Acker 1974), are mainly present as inclusion compounds in starch granules and, therefore, essentially are unavailable to affect dough processing before starch gelatinization (Pomeranz and Chung 1983). The role of nonstarch lipids in flour has been studied extensively, and although they are a minor component of flour, nonstarch lipids are important determinants of breadmaking quality (Pomeranz 1967, McRitchie 1984, Panozzo et al 1993). During dough mixing, the increase of nonstarch lipid binding, which is related to dough development (Chiu et al 1968, Daniels et al 1971, Chung et al 1978), is mainly due to specific interactions between lipids and proteins in the gluten network (Frazier et al 1981, Marion et al 1987). According to Mann and Morrison (1974), polar lipids and triglycerides in nonpolar lipids are the main fractions bound during mixing.

In addition to lipid binding, dough mixing under air induces lipid oxidation (Tsen and Hlynka 1962). This reaction is catalyzed by lipoxygenase that is present naturally in wheat, mainly in germ (Nicolas et al 1982; Shiiba et al 1991), as well as in soy flour, which is often added to wheat flour (Mann and Morrison 1975, Kieffer and Grosch 1980). Lipoxygenase is able to act on polyunsaturated fatty acids (PUFA) in the presence of oxygen to form hydroperoxides (Gardner 1988). Hydroperoxides, as well as intermediary free radicals formed during mixing, can cooxidize several compounds, including carotenoids, tocopherols, and thiol groups of peptides and proteins (Nicolas and Drapron 1983). These reactions explain the bleaching effect of lipoxygenase on crumb color

and its improving effect on the rheological properties of dough, such as increasing relaxation time and mixing tolerance (Frazier et al 1977, Nicolas 1978, Hoseney et al 1980, Faubion and Hoseney 1981). Wheat lipoxygenase catalyzes the oxidation of PUFA (either free or in monoglycerides) in dough (Graveland 1970, Mann and Morrison 1974, Morrison and Panaprai 1975), whereas soy flour contains lipoxygenase isozymes that act on almost all esterified forms of linoleic and linolenic acids (Morrison and Panaprai 1975), although less rapidly than with free forms (Whitaker 1991). According to Morrison (1976), most of the oxygen consumed during dough mixing is due to lipid oxidation catalyzed by lipoxygenase. However, the extent of lipid oxidation, such as losses of free PUFA and carotenoid pigments, are affected by dough ingredients as well as by mixing conditions (Mann and Morrison 1975, Nicolas 1978, Tait and Galliard 1988).

For a long time, flour lipase activity has been associated primarily with its deleterious effects on breadmaking properties (Drapron and Uzzan 1968). Thus, both wheat and fungal lipases have been implicated in the increase of free fatty acids (FFA) during long-term storage of flour (Gracza 1965). Wheat lipase is concentrated mainly in the germ and bran fractions (Drapron 1983; Galliard 1983, 1986). A few works have been devoted to the study of wheat lipase properties (Stauffer and Glass 1966, Caillat and Drapron 1974, O'Connor and Harwood 1992, Fadiloglu and Söylemez 1996). However, the lipase activity of white flour is very weak (Drapron and Sclafani 1969, Galliard 1986).

With the exception of a patent in 1968 (Johnson et al 1968), only during the last decade has the use of exogenous lipases (EC 3.1.1.3) in wheat doughs been proposed to retard bread staling and improve dough rheological properties (Mohsen et al 1986; Olesen et al 1994; Si 1995, 1997; Mutsaers 1996). Because only limited data are available on the lipid fractions of added-lipase doughs (Si and Hansen 1994, Borne 1996), the aim of this work was to elucidate the effect of exogenous lipase on nonpolar lipids in dough during mixing.

MATERIALS AND METHODS

The flour used for these experiments was an untreated, improver-free, straight-grade flour commercially milled by Les Moulins Soufflet (Nogent-sur-Seine, France). Moisture content was 15.3%, and protein (N × 5.7) and ash contents were 9.7 and 0.61%, respectively (dry basis).

Lipase Novozym 677 BG was purchased from Novo Nordisk (Bagsvaerd, Denmark) and had an activity of 59,000 lipase units (LU) per gram. This lipase, which originated from *Humicola lanuginosa*, was produced in a recombinant strain of *Aspergillus oryzae*.

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According to the Novo data sheet, 1 LU corresponds to the amount of enzyme that releases 1 μmol of titratable butyric acid per minute from tributyrin under the reaction conditions described by Greenough et al (1996). All chemicals were of analytical reagent grade from Prolabo (Paris, France) and Sigma Chemical Co. (St. Louis, MO).

Mixing Conditions

Dough was prepared by adding 108 mL of distilled water to 180 g of flour in a micromixer of a Chopin (Villeneuve-la-Garenne, France) alveograph equipped with a heat-transfer system and speed regulator. The mixing parameters were set to 75 rpm and 23°C. Exogenous lipase was dissolved in water added to the dough.

Lipid Extraction

The extraction procedure was adapted from methods described by Folch et al (1957) and Tsen et al (1962). Fresh dough samples (10–12 g) were collected directly in centrifuged tubes, and enzyme activities were stopped by adding 100 μL of glacial acetic acid and 20 mL of methanol. Extraction were performed by successively adding 10 mL of chloroform containing butyl-hydroxy-toluene (1%), 10 mL of chloroform, and 16 mL of NaCl (1.25%). After each new addition, the mixture was homogenized by a 20-sec blending period (Ultra-turrax, IKA, Staufen, Germany). The mixture was centrifuged for 10 min at $6,000 \times g$. Lipids were obtained by collecting an aliquot of the chloroform phase. During the extraction procedure, tubes were kept at 0–4°C.

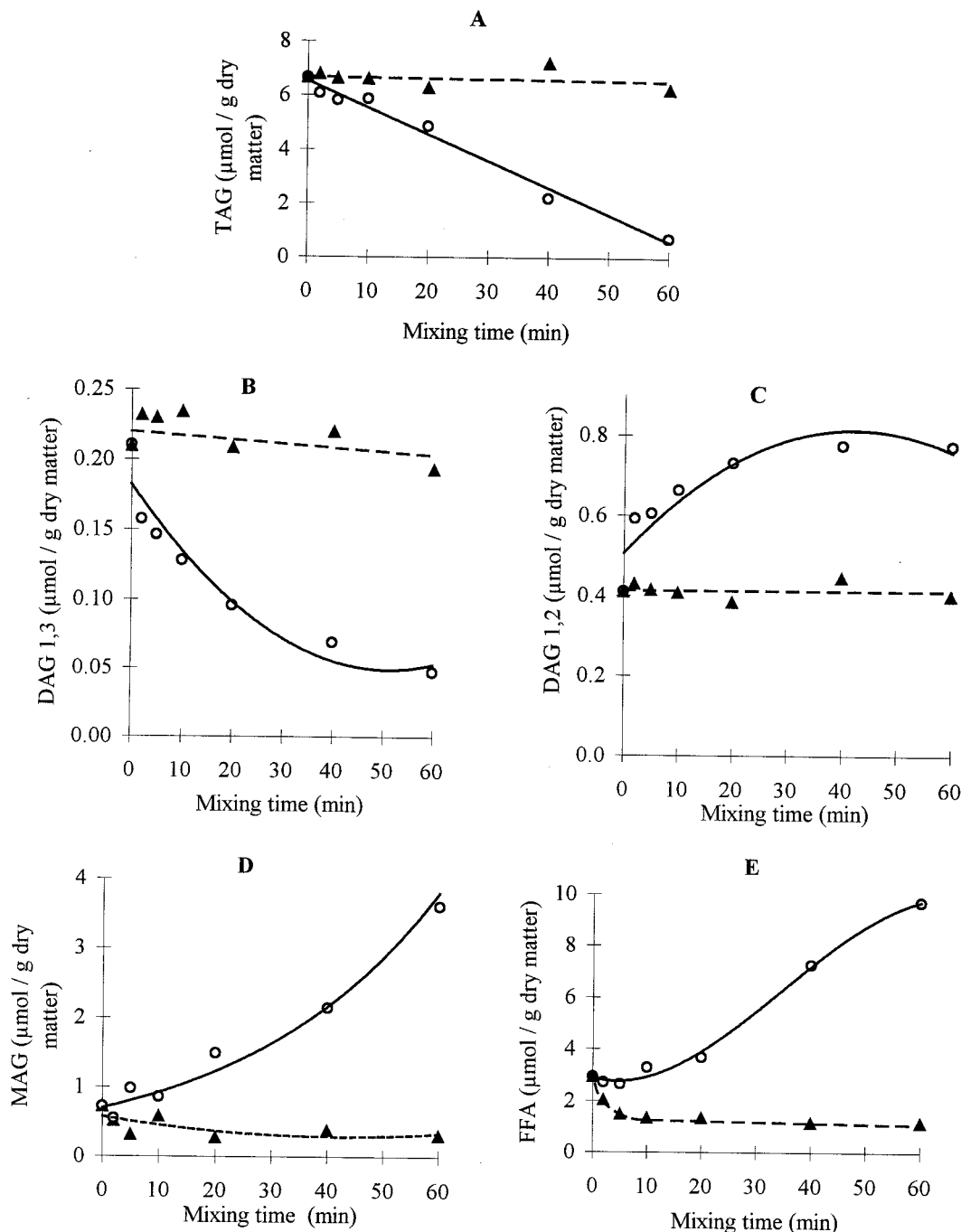


Fig. 1. Evolution of different fractions of nonpolar lipids during dough mixing with and without lipase (15 lipase units/g of dry matter). **A**, triacylglycerol (TAG) fraction; **B**, 1,3-diacylglycerol (DAG_{1,3}) fraction; **C**, 1,2-diacylglycerol (DAG_{1,2}) fraction; **D**, monoacylglycerol (MAG) fraction; and **E**, free fatty acid (FFA) fraction. ▲ = control dough; ○ = dough with lipase.

Lipid Fractionation

TAG, FFA, DAG_{1,2}, DAG_{1,3}, and MAG were fractionated by thin-layer chromatography with silica-gel plates (Macherey-Nagel, Hoerd, France) and the development system proposed by Mangold (1961). A 0.01% primulin solution was sprayed on the plates, and the lipid fractions were identified under UV light by comparing the R_f values with those of pure standard compounds. After scraping, 20 µg of heptadecanoic acid (internal standard) was added to each of the FFA, DAG, and MAG fractions, and 80 µg was added to the TAG fraction. Fatty acids of each fraction were hydrolyzed and methylated with 3 mL of boron trifluoride-methanol (1:5) by incubation at 65°C for 15 min. Fatty acid methyl esters (FAME) were extracted from methanol by adding 3 mL of pentane and 2 mL of water. After collecting the pentane fraction, the solvent was evaporated under a nitrogen flow, and FAME was solubilized in 20–100 µL of heptane (depending on the lipid fraction).

Lipid Quantification

FAME was analyzed with a gas chromatograph (Varian 3400 CX, Les Ulis, France) equipped with a split injector, a 25 m × 0.32 mm CPwax 52 CB (Macherey-Nagel) capillary column, and a flame-ionization detector. The injector, oven, and detector were set at 250, 200, and 270°C, respectively. The flow rate of the carrier gas (helium) was set at 0.7 mL/min. Analyses of the chromatogram were performed with Star (Varian) chromatography software 4.0.

Data Analysis

A mixture of pure palmitic (C₁₆), stearic (C₁₈), oleic (C_{18:1}), linoleic (C_{18:2}), and linolenic (C_{18:3}) acid methyl esters was injected together with heptadecanoic (C₁₇) acid methyl ester to calibrate the detector and calculate its relative response for each FAME (k_i).

For each fraction, the FAME content (C_i) was calculated by the relationship:

$$C_i \text{ (}\mu\text{mol/g of dry matter)} = (k_i \times S_{i \text{ peak area}} \times m_{C17} \times D) / (S_{C17 \text{ peak area}} \times MW_{C_i} \times DM)$$

where C_i, k_i, S_{i peak area}, and MW_{C_i} are the molar concentration, relative response factor of the detector, peak area, and molecular weight, respectively, for each FAME (i = 16, 18, 18:1, 18:2, and 18:3). S_{C17 peak area} and m_{C17} are the peak area and amount of internal standard. DM and D are the dry matter of the sample and dilution factor.

The molar concentration of each lipid fraction is calculated by the relationship:

$$\text{Molar concentration (}\mu\text{mol/g of dry matter)} = (\sum_i C_i) / n$$

where n = 3 for the TAG fraction, n = 2 for the DAG_{1,2} and DAG_{1,3} fractions, and n = 1 for the MAG and FFA fractions.

All the data resulted from the analyses of two samples per mixing time, and each mixing condition was duplicated. Between the two samples per mixing time, the maximum deviation was 5% for each lipid fraction. Between two similar mixing conditions, a maximum deviation of 8% was tolerated for each lipid fraction (15% for the MAG fraction).

Carotenoid Assay

The loss of carotenoid pigments during mixing was estimated based on the method described by Nicolas (1978).

RESULTS AND DISCUSSION

Lipase Hydrolysis in Dough and Its Effect on Lipid Content

Model doughs composed of water and wheat flour were mixed with and without exogenous lipase. Aliquots of dough were collected after 2, 5, 10, 20, 40, and 60 min of mixing, and their lipid content was analyzed. The amount of polar lipids extracted by the

method of Tsen et al (1962) remained constant during mixing with or without added lipase (data not shown), indicating these lipids were not affected by lipase hydrolysis. Therefore, most of our work in this article will concentrate on describing and analyzing the evolution of nonpolar lipids.

The evolution of TAG, which was the most abundant lipid fraction (Mann and Morrison 1974, Tait and Galliard 1988), is shown in Fig. 1A. The TAG content remained constant throughout the mixing time in control dough, whereas in lipase-added dough a TAG content decreased linearly, indicating TAG was unaffected by wheat lipase. When 15 LU/g of flour was added, ≈90% of TAG was hydrolyzed after 60 min of mixing, and the hydrolysis rate estimated by the slope was close to 1.5%/min. Moreover under our mixing conditions and at 15 LU/g of flour, the added lipase did not appear to be substrate limited because the rate of hydrolysis remained constant even when the residual amount of TAG dropped as low as 0.7 µmol/g of flour. Therefore, it can be assumed that this small amount of TAG is sufficient to saturate the lipase added to the dough, and that the mixing conditions allowed enough contact between the enzyme and its substrate to ensure a constant rate of hydrolysis.

Fig. 1B and C show that both the DAG_{1,2} and DAG_{1,3} fractions were unaffected during mixing in control dough. When exogenous lipase was added to the dough, the amount of DAG_{1,3} decreased during the first 40 min of mixing and remained constant during the next 20 min (Fig. 1B). Conversely, the DAG_{1,2} fraction increased at the beginning of mixing but did not change during the last 20 min (Fig. 1C). This result can be explained by the behavior of the lipase Novozym 677 BG, which specifically hydrolyzed the outer ester bonds of TAG (positions Sn1 and Sn3), which agreed with the specificity of lipase from *H. lanuginosa* described by Godfrey (1995). Thus, the increase in the DAG_{1,2} forms was due to the hydrolysis of TAG. DAG_{1,3} cannot originate from TAG hydrolysis by exogenous and wheat lipases, which is ineffective during mixing. Therefore, the only variations in these forms were due to hydrolysis by the exogenous enzyme, which explains their continuous decrease during mixing.

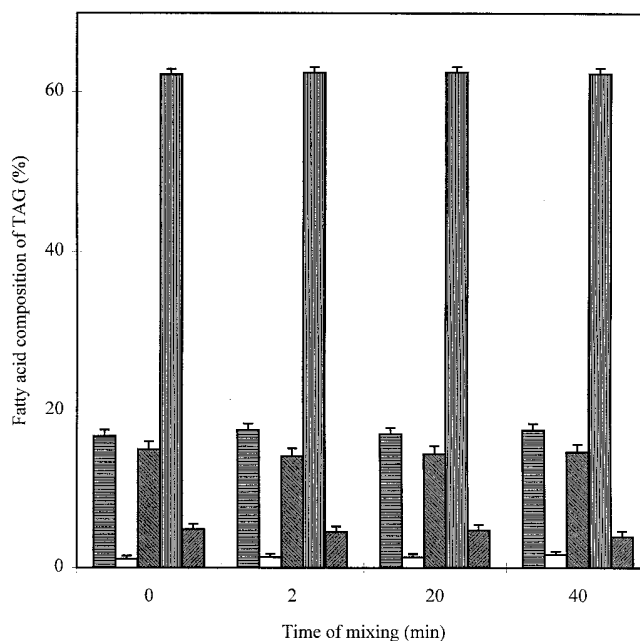


Fig. 2. Evolution of fatty acid composition in the triacylglycerol (TAG) fraction during dough mixing in the presence of lipase (15 lipase units/g of dry matter). Mixing times: 2 min of mixing = 14% of TAG hydrolysis; 20 min of mixing = 32% of TAG hydrolysis; and 40 min of mixing = 71% of TAG hydrolysis. Histogram order for each mixing time: C_{16:0}, C_{18:0}, C_{18:1}, C_{18:2}, and C_{18:3}. Vertical bars indicate standard error.

In addition, the amount of DAG_{1,2} began to decrease only when the level of TAG dropped too low (i.e., after 40 min of mixing). Moreover, the rate of DAG_{1,2} increase was much lower than the hydrolysis rate of TAG (Fig. 1A and C). These two observations confirm that the lipase added to the dough was able to hydrolyze DAG_{1,2}.

Figure 2 shows lipase specificity. Fatty acid composition of TAG remained constant during mixing, independent of hydrolysis level. Therefore, if the distribution of fatty acids that esterified the glycerol is random, it can be assumed that lipase Novozym 677 BG is not specific for the nature of the fatty acid present in wheat lipid TAG. This result agrees with the properties of *H. lanuginosa* lipase (Godfrey 1995).

The evolution of MAG and FFA is presented in Fig. 1D and E. Both fractions decreased in control dough, whereas they increased in lipase-added dough. However, in lipase-added dough, the increase seemed to be preceded by a plateau in the MAG fraction (Fig. 1D) and a small decrease in the FFA fraction (Fig. 1E). Further examination of the fatty acid distribution in these fractions explains their behavior during mixing.

Simultaneous Action of an Exogenous Lipase and Endogenous Lipoxygenase

To discriminate between the effects of the activities of exogenous lipase and endogenous lipoxygenase, we separately determined the evolution of PUFA and that of saturated and monounsaturated fatty acids (SMUFA) in each fraction of nonpolar lipids. SMUFA, which is not sensitive to lipoxygenase oxidation (Whitaker 1991), allows specific analysis of the effect of lipase. The results obtained for control dough are presented in Fig. 3A and B. SMUFA

content in MAG and FFA fractions remained constant during mixing, whereas the PUFA content strongly decreased. After 10 min of mixing, the decreases in PUFA content were close to 90% for the FFA fraction (Fig. 3A) and 75% for the MAG fraction (Fig. 3B). Conversely, the SMUFA and PUFA contents of TAG, DAG_{1,2}, and DAG_{1,3} fractions were not affected (results not shown). These results are consistent with lipid oxidation catalyzed by wheat lipoxygenase because this enzyme is able to catalyze the oxidation of PUFA only in the free and monoglyceride forms (Mann and Morrison 1974). Moreover, the bulk of the oxidation occurred at the beginning of mixing, when both the oxygen content in dough and, according to Delcros et al (1998), lipoxygenase activity were maximum.

When lipase was added to dough, the SMUFA content of the FFA fraction increased linearly with mixing time (Fig. 3D). SMUFA content also increased in the MAG fraction but only after a small delay and much less rapidly than in the FFA fraction (Fig. 3C).

In the FFA fraction, PUFA content decreased during the first 10 min of mixing and began to increase after 20 min but less rapidly than for SMUFA (Fig. 3D). For the MAG fraction, PUFA content increased only after 10 min of mixing but more rapidly than for SMUFA (Fig. 3C). Moreover, during the last 40 min of mixing, the increases in PUFA content in MAG and FFA fractions were almost similar. The rate of SMUFA increase in the FFA fraction was more than six times higher than that observed in the MAG fraction. Therefore, it can be assumed that the fatty acid release by lipase was due mainly to TAG hydrolysis.

The evolution of PUFA confirms that lipids were oxidized and shows that the oxidation rate is a function of mixing time. Until 10 min of mixing, the oxidation rate of FFA appeared faster than

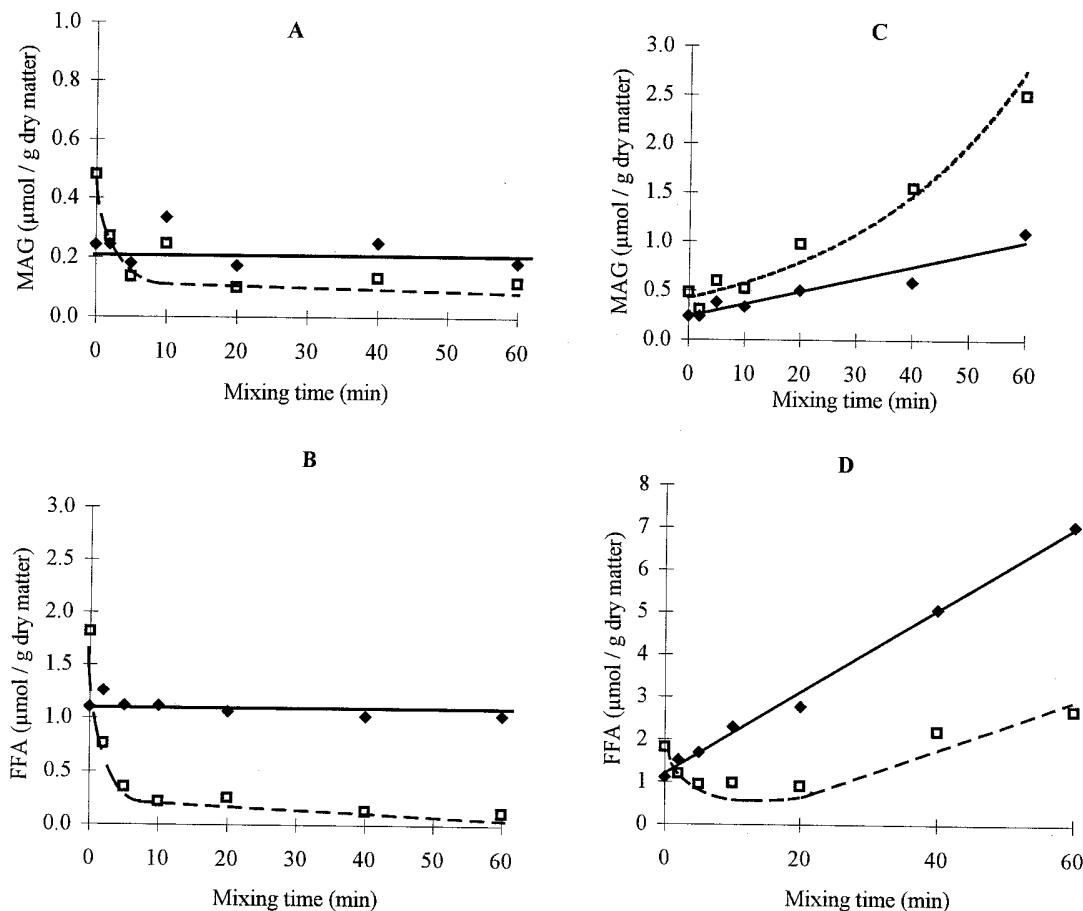


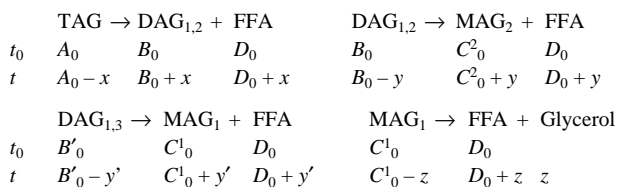
Fig. 3. Evolution of saturated and monounsaturated fatty acids (SMUFA) and polyunsaturated fatty acids (PUFA) contents in the monoacylglycerol (MAG) and free fatty acid (FFA) fractions during dough mixing with and without lipase (15 lipase units/g of dry matter). **A**, MAG in control dough; **B**, FFA in control dough; **C**, MAG in dough with lipase; and **D**, FFA in dough with lipase. \blacklozenge = SMUFA: C₁₆ + C₁₈ + C_{18:1}; \square = PUFA: C_{18:2} + C_{18:3}.

the rate of release, leading to decreasing content. After 20 min of mixing, the increase of PUFA was probably due to a decrease in the oxidation rate because the release of SMUFA was unaffected by mixing time. The decrease of PUFA oxidation could be due to a decrease in oxygen availability or lipoxygenase activity in dough (Delcros et al 1998). Lastly, when the behavior of PUFA and SMUFA are compared in the FFA and MAG fractions, it appears that the increase of PUFA was comparatively higher in the MAG fraction than in the FFA fraction, probably indicating wheat lipoxygenase is more active on PUFA in the free form than in its monoglyceride form.

These data clearly demonstrate that in the presence of lipase, dough lipids are simultaneously hydrolyzed and oxidized (in the absence of salt and yeast). Nevertheless, the importance of each reaction varies according to mixing time. A mass balance of each molecular species should help independently quantify the rate of these reactions.

Molar Balance of Lipid Fractions and Evaluation of Oxidation-to-Hydrolysis Ratio

The hydrolysis of triglycerides by a 1,3-specific lipase may be described by the following successive reactions:



where $A_0, B_0, B'_0, C^1_0, C^2_0,$ and D_0 represent the initial molar amounts of TAG, DAG_{1,2}, DAG_{1,3}, MAG₁, and MAG₂, respectively, and x, y, y' and z represent the fractions of TAG, DAG_{1,2}, DAG_{1,3} and MAG, respectively, that are hydrolyzed after time t .

From this model, the relationships among the initial concentrations ($A_0, B_0, B'_0, C_0,$ and D_0), concentrations at time t ($A_t, B_t, B'_t, C_t,$ and D_t), and hydrolyzed fractions ($x, y, y',$ and z) can be determined for each molecular species:

$$\text{TAG} \quad A_t = A_0 - x \quad (1)$$

$$\text{DAG}_{1,2} \quad B_t = B_0 + x - y \quad (2)$$

$$\text{DAG}_{1,3} \quad B'_t = B'_0 - y' \quad (3)$$

$$\text{MAG}_1 \quad C^1_t = C^1_0 + y' - z \quad (4)$$

$$\text{MAG}_2 \quad C^2_t = C^2_0 + y \quad (5)$$

The accumulation of FFA is given by:

$$\text{FFA} \quad D_t = D_0 + x + y + y' + z \quad (6)$$

Because $C_t = C^1_t + C^2_t$ and $C_0 = C^1_0 + C^2_0$:

$$C_t = C_0 + y + y' + z \quad (7)$$

By eliminating $x, y, y',$ and z with combinations of Eqs. 1–3 and 7, Eq. 6 becomes:

$$D_t - D_0 = 3(A_0 - A_t) + 2(B_0 - B_t) + 2(B'_0 - B'_t) + (C_0 - C_t) \quad (8)$$

The validity of the relationship (Eq. 8) was tested first on the SMUFA fraction. Excellent agreement was observed between the loss of SMUFA in glyceride fractions and its accumulation in the FFA fraction (Fig. 4), indicating that in the absence of oxidation, no lipid in the nonpolar fractions was lost.

If the same relationship is applied to PUFA, an increasing difference is observed between PUFA loss (right side of Eq. 8) in glyceride fractions, and the amount of PUFA accumulated in the FFA fraction (left side of Eq. 8) (Fig. 5). Because the SMUFA content resulting from lipase hydrolysis was found in the FFA fraction, a similar behavior for PUFA can be assumed. Therefore, the difference between losses in esterified fractions and accumu-

lation in the FFA fraction represents the amount of lipids oxidized during mixing. A percentage of oxidation in the nonpolar lipid fraction (amount of oxidized PUFA/initial PUFA content) can be calculated as a function of mixing time.

Figure 6 shows that the percentage of oxidized PUFA in control dough increased quickly during the first minute of mixing to reach a limited level ($\approx 12\%$) after 20 min, which roughly corresponds to the PUFA content of the FFA and MAG fractions in flour. When lipase (15 LU/g) was added to the dough after a similarly rapid increase during the first 10 min of mixing, the level of oxidized lipid increased steadily during mixing.

This result confirms that lipid oxidation occurs mainly during the first minutes of mixing and shows that oxidative reactions are quickly limited in control dough. The addition of lipase appears to

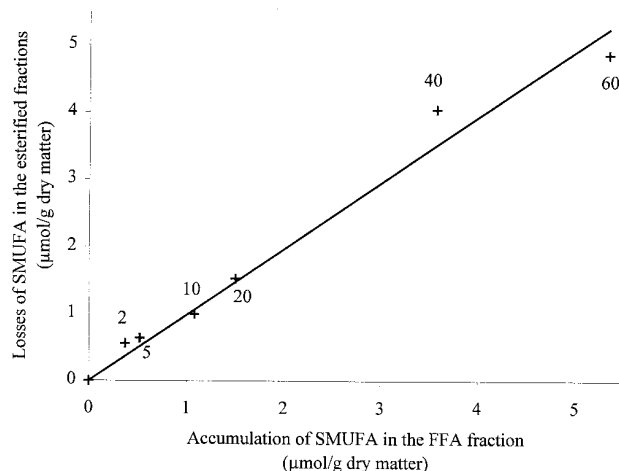


Fig. 4. Comparison of accumulation of saturated and monounsaturated fatty acids (SMUFA) in the free fatty acid (FFA) fraction and their losses in esterified fractions during dough mixing in the presence of lipase (15 lipase units/g of dry matter). Losses in esterified fractions: $3(A_0 - A_t) + 2(B_0 - B_t) + 2(B'_0 - B'_t) + (C_0 - C_t)$. Accumulation in the FFA fraction: $D_t - D_0$ for $C_{16}, C_{18},$ and $C_{18:1}$ (Eq. 8). Values at each experimental point indicate mixing time (min).

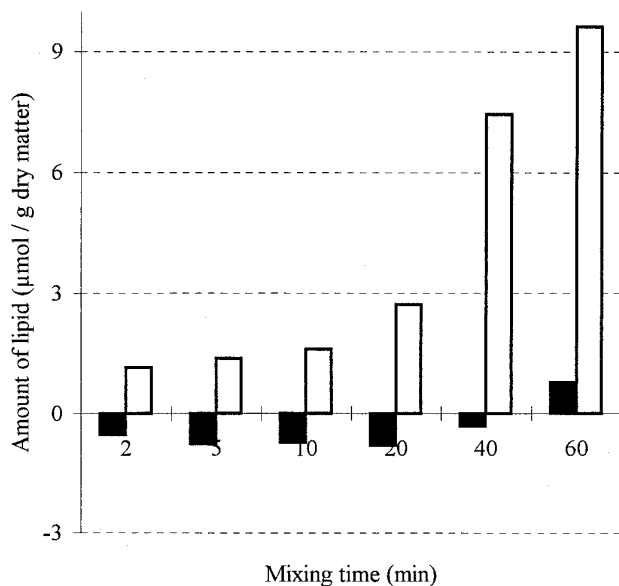


Fig. 5. Comparison of accumulation of polyunsaturated fatty acids in the free fatty acid (FFA) fraction and their losses in the glyceride fractions during dough mixing in the presence of lipase (15 lipase units/g of dry matter). Losses in esterified fractions (open columns): $3(A_0 - A_t) + 2(B_0 - B_t) + 2(B'_0 - B'_t) + (C_0 - C_t)$. Accumulation in the FFA fraction (solid columns): $D_t - D_0$ for $C_{18:2}$ and $C_{18:3}$ (Eq. 8).

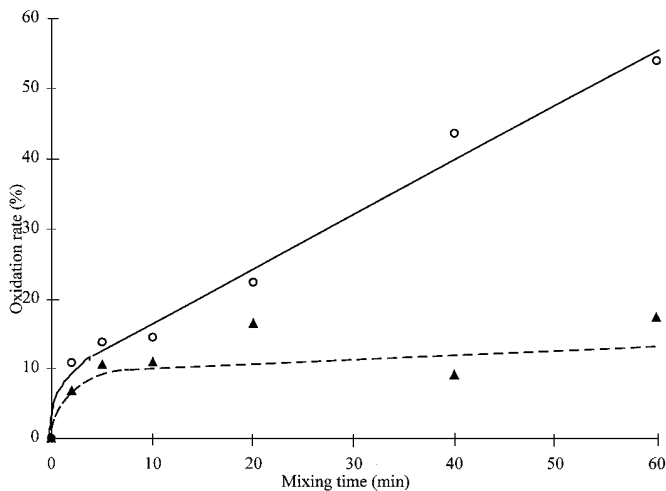


Fig. 6. Evolution of polyunsaturated fatty acid oxidation during dough mixing with and without lipase (15 lipase units/g of dry matter). ▲ = control dough; ○ = dough with lipase.

TABLE I
Effect of Mixing Time on Loss (%) of Carotenoid Pigments During Dough Mixing With and Without Lipase (15 lipase units/g of dry matter)

	Mixing Time (min)			
	0	2	20	40
Control dough	0	10.3	31	38
Dough with lipase	0	12	38	93

suppress this limitation, suggesting that the main limiting factor in control dough (in the absence of sodium chloride and yeast) is the amount of free PUFA. Therefore, the exogenous lipase could be considered an activator of oxidative reactions by furnishing substrate to endogenous lipoxygenase during mixing.

This hypothesis can be confirmed by following the loss of carotenoid pigments during mixing because lipoxygenase is able to bleach these pigments by cooxidation (Weber et al 1973, Nicolas 1978). Table I shows that losses in carotenoid pigments were higher in the dough containing lipase.

In another experiment, we measured the effect of different amounts of lipase, ranging from 2.5 to 25 LU/g of flour, on loss of the TAG fraction and accumulation of SMUFA and PUFA in the FFA fraction after 20 min of mixing (Fig. 7). The loss of TAG was roughly proportional to the amount of added enzyme (Fig. 7A) as well as the accumulation of SMUFA in the FFA fraction (Fig. 7B). The PUFA content of the FFA fraction after 20 min of mixing remained very low until lipase was added at 5 LU/g of flour, after which it increased rapidly to reach a value similar to that of SMUFA obtained with lipase at 25 LU/g of flour (Fig. 7B).

During 20 min of mixing, the amount of hydrolyzed TAG remained proportional to the amount of lipase until at least 25 LU/g of flour. This indicates that for these levels of enzyme the rate of TAG hydrolysis was not limited by TAG amount during this mixing time. For lipase levels higher than 5 LU/g of flour, the percentage of oxidation of PUFA released by lipase decreased as the amount of added enzyme increased. This phenomenon could be due to a limited oxygen supply during mixing or progressive loss of lipoxygenase activity in dough (Delcros et al 1998).

In conclusion, we observed that lipase from *H. lanuginosa* was able to act specifically on the nonpolar fractions of dough lipids by decreasing the amounts of TAG and DAG_{1,3}, whereas it increased the amounts of MAG and FFA fractions. The amount of the DAG_{1,2} fraction increased at the beginning of mixing and then decreased. Thus, the variation of this fraction was dependent on both the

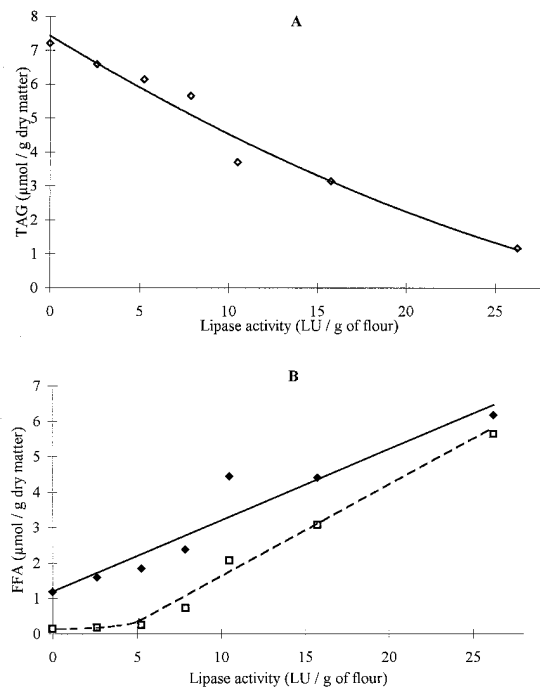


Fig. 7. Effect of amount of lipase added to dough on triacylglycerol (TAG) and free fatty acid (FFA) fractions after 20 min of mixing. **A**, Evolution of TAG fraction; **B**, Evolution of saturated and monounsaturated fatty acids (◆) and polyunsaturated fatty acids (□) contents in the FFA fraction.

amount of added enzyme and mixing time. Second, lipase induced large increases in the amounts of PUFA in the MAG and FFA fractions. Therefore, lipase allowed a much higher percentage of PUFA oxidation by wheat lipoxygenase, leading to an enhanced enzyme effect during dough mixing.

These mechanisms could provide an explanation for the beneficial effects of lipase in breadmaking (Mohsen et al 1986; Olesen et al 1994; Si 1995, 1997; Mutsaers 1996). However, further work is needed to determine the optimal amount of lipase and dough mixing conditions to obtain a good balance between the levels of hydrolysis and oxidation of lipids during dough mixing. Optimization also could depend largely on the lipid content of wheat flour and specificity of lipase.

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