

BIOCATALYTIC OXIDATION OF OILSEED-DERIVED ACYLGLYCEROLS

Castor oil is composed predominately of glycerol esters of ricinoleic acid, a unique hydroxy substituted fatty acid. Because of the presence of the hydroxyl group, castor oil and products derived from it are used in a number of industrial applications in which common seed oils cannot be substituted. Castor oil is not produced in the US; it is entirely imported. If ways could be found to hydroxylate glycerides derived from domestically produced vegetable oils, then the need to import castor oil would be eliminated. The enzyme lipoxygenase catalyzes a reaction between oxygen and polyunsaturated fatty acids to give fatty acid hydroperoxides. These may be easily reduced to give hydroxy fatty acid similar to ricinoleic acid. Previous work showed that soybean lipoxygenase can oxidize phosphoglycerides when surfactants are present in the reaction media. We have extended these studies by looking at the action of lipoxygenase upon neutral acylglycerols. It was found that under given reaction conditions neutral glycerides can be oxidized by soybean lipoxygenase.

Introduction

Soybean lipoxygenase (LOX; linoleate, oxygen oxidoreductase, EC 1.13.11.12) generates fatty acid hydroperoxides from polyunsaturated fatty acids and oxygen. In earlier work the high pH form of soybean LOX was shown to strongly prefer free fatty acid as a substrate, rather than esterified acid (1). However experimental evidence with structural analogues suggests that 15(S)-lipoxygenases, such as soybean LOX, recognize and bind their substrates through the methyl end, and therefore modification to the carboxylic end of the substrate should have a minimal impact upon substrate reactivity (2). In support of this contention, recently it was shown that LOX also acted upon esterified fatty acids in phosphoglycerides when a bile salt surfactant was present (3-5). Phosphoglyceride oxidation was highly positionally and stereochemically specific and therefore was not merely an enzyme promoted radical-mediated autoxidation (4,5). A link between fungal elicitor response in plants and the oxidation of phosphatidylcholine by LOX has been demonstrated (6). There are reports concerning the LOX catalyzed oxidation of phospholipids in liposomes and lipoprotein fractions (7,8). There is a single report demonstrating the action of LOX upon fatty amide (9).

That phosphoglyceride and fatty amide can be good substrates for LOX raises the question as to whether acylglycerols can also be oxidized by LOX. Therefore we have reexamined acylglycerol oxidation by LOX in the presence of the bile salt deoxycholate to determine whether LOX oxidizes acylglycerols as well as phosphoglycerides under these conditions. These studies are important prerequisites to possible industrial scale

processes in which lipoxygenase and other enzymes are used to introduce readily derivatizable oxygen functionality into fats and oils.

METHODS

Using selected acylglycerol substrates the influences of pH, deoxycholate concentration, and buffer composition on hydroperoxide formation by LOX were examined. Using optimized conditions, relative oxidation rates of several acylglycerols and phospholipids were measured, and reaction time courses were obtained. What follows is a description of a typical optimized assay for hydroperoxide formation.

Hydroperoxide formation. An aliquot of the substrate (6 μ mol linoleoyl residues) dissolved in chloroform was placed in a 10 ml Erlenmeyer flask, and the chloroform was evaporated under a stream of dry nitrogen. In addition to substrate, the reaction medium contained 0.2 mg LOX, 0.2 ml 100 mM deoxycholate, and 1.8 ml aqueous buffer (pH 8.0 or as indicated), consisting of an equal 0.1 M mixture (0.4 M total) of 2-amino-2-methyl-1-propanol hydrochloride (AMP), N-tris(hydroxymethyl)-methylglycine (TRICINE), N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), and 2-(N-morpholino)ethanesulfonic acid (MES). Oxidation was conducted at 15°C with agitation at 250 rpm for the indicated time. The reaction was quenched with 400 μ l 1 M citric acid, and fatty acid hydroperoxide was extracted within 1 h with two 1.5 ml aliquots of chloroform:methanol (2:1, v/v). After removing the solvent under a stream of nitrogen, the hydroperoxide was redissolved in 3.0 ml ethanol.

Hydroperoxide assay. The level of hydroperoxide was determined spectrophotometrically using the xylenol orange method as described before (10). Freshly diluted commercial cumene hydroperoxide was used for preparing a calibration curve each day. All results were corrected by subtracting readings from controls that were quenched with citric acid immediately after the addition of LOX. Confirmation of the readings from the xylenol orange method was accomplished with TLC on preadsorbent HPTLC-HL, 10 \times 10 cm silica gel plates that had been dipped 5% boric acid in methanol and allowed to dry before sample application. Development was achieved using a two stage system: toluene/ethyl acetate/diethyl ether/acetic acid (75:13:10:1.2, by volume); hexane/diethyl ether/formic acid (64:16:2, by volume). The solvent was allowed to rise to the top of the plate in each case, and the TLC plate was allowed to air dry between solvent systems. After development, the TLC plate was sprayed with 60% aqueous sulfuric acid and charred to visualize the hydroperoxide and starting material.

HPLC analyses of the oxidation of substrate mixtures. Time course of oxidation was monitored as described previously (11). The extent of reaction was followed by measuring the decrease in the area of the peak corresponding to the substrate. Reaction products were separated on an Alltech (Deerfield, IL) C18 hydroxyethylmethacrylate (HEMA) column (250 x 4.6 mm) installed on a Waters (Milford, MA) LCM1 HPLC instrument. The detector was a Varex evaporative light-scattering detector MK III (Alltech) operated at a temperature of 48°C, and with a nebulizing gas (N₂) flow rate of 1.3 mL/min. The mobile phase had the following composition and gradient: methanol:10 mM aqueous trifluoroacetic acid (TFA) (86:14), 0-4 min; methanol:10 mM TFA (90:20), 4-8 min; acetonitrile:methanol:10 mM TFA (30:65:5), 8-10 min; acetonitrile:methanol:10 mM TFA (51:48:1), 10-25 min. The flow rate was 1 mL/min.

Oxidation of trilinolein. Initial studies were performed with trilinolein in 10 mM deoxycholate (10). After oxidation with LOX, the products were analyzed by TLC on silica gel. The primary oxidation product ran immediately behind trilinolein and was well separated from linoleic acid and its oxidation product. That oxidized linoleic acid was not detected demonstrates that direct oxidation of esterified fatty acid is the primary route to hydroperoxide.

Figure 1 shows the influence of pH upon the amount of oxidized trilinolein using the deoxycholate-containing buffered system. The activity profile was the typical bell-shaped curve that is seen with many enzymes. The activity of LOX on trilinolein was optimal between pH values of 8 and 9 and decreased as the pH value of the medium was either reduced or raised from this range.

The influence of the bile salt surfactant deoxycholate upon trilinolein oxidation was examined at pH 8, and the results are shown in Figure 2. Trilinolein oxidation was observed even when no deoxycholate was present, but when 5 mM deoxycholate was added, the rate of oxidation nearly doubled. With further increases in deoxycholate, the oxidation rate decreased slightly. The rate of soybean LOX catalyzed oxidation of phosphatidylcholine was reported to be maximal in the presence of 10 mM deoxycholate (3).

The time course of trilinolein oxidation was determined and is shown in Figure 3. The oxidation rate was high during the first 20 min, but then rapidly decreased. The time course of trilinolein oxidation was examined with nonlinear regression analysis (curve, Figure 3) (10). Potentially 0.92 μmol of available linoleate (6 μmol) could be converted to hydroperoxide, equivalent to a 15% conversion.

One explanation for the limited production of hydroperoxide was that LOX became inactivated during the oxidation process through a mechanism that is observed even when linoleic acid is the substrate (1). To test this possibility a second addition of freshly prepared LOX was made 1 h after the first addition. Samples were withdrawn after an additional 1 h, analyzed for hydroperoxide content, and compared to controls that received only one addition of LOX. After 1 h, regardless of whether one or two additions of LOX were made, the levels of hydroperoxide in both treatments were the same within experimental error ($n = 6$). Thus it can be concluded that low conversions to oxidized product are not merely the result of substrate-independent LOX inactivation.

Oxidation of phospholipids, diacylglycerols, and triacylglycerols Table 1 shows the relative rate of LOX-catalyzed oxidation of linoleic acid and several phosphoglycerides and acylglycerols. All of the tested phosphoglycerides were substrates of LOX, although their rates of oxidation were considerably reduced compared to that of linoleic acid. The phosphoglyceride substrate that was hydrolyzed the fastest, phosphatidylserine (1,2-diL, 3-P-serine), reacted at 28% of the rate of linoleic acid. A comparison of the two tested phosphatidylcholine species (1-L, 2-S, 3-P-choline and 1-S, 2-L, 3-P-choline) suggested that a slight preference exists for the oxidation of a primary linoleate residue. However T-test analysis of the data demonstrated that the difference in the observed rates was not statistically significant. Trilinolein was oxidized at a very low rate compared to linoleic acid. Substitution of oleate and stearate for linoleate at positions 2 and 3 (1-L, 2-O, 3-S) resulted in a slightly better triacylglycerol substrate. However, the removal of

one fatty acid to form the diacylglycerol resulted in substrates (1-L, 3-L and 1-S, 2-L) that reacted more rapidly, approximately 40% as fast as linoleic acid.

Figure 4 shows the time course of 1,3-dilinolein oxidation by LOX. The extent of oxidation was determined by the xylenol orange method which measures hydroperoxide formation. The curve in Fig. 4 shows the results of nonlinear regression analysis. The maximum amount of product that can be formed was found to be 4.02 μmol or 67% conversion, a value considerably higher than that obtained with trilinolein.

Figure 5 shows the time course of 1,3-dilinolein oxidation by LOX, with the extent of oxidation determined by the peak area corresponding to 1,3-dilinolein after analysis using HPLC. The maximum degree of oxidation was found to be 5.39 μmol , equivalent to about 90% conversion to product. The reason for the discrepancy between the results obtained with the xylenol orange method and those obtained with HPLC is that slightly more than 20% of the 1,3-dilinolein was converted to products that are not hydroperoxide(s). Both HPLC and TLC revealed the formation of products that did not correspond to the mono and dihydroperoxides of 1,3-dilinolein. The structural nature of these other products is not known, but their formation cannot simply be the consequence of the anaerobic LOX cycle, since under the reaction conditions used, oxygen was not depleted. However, steric constraints may be causing 1,3-dilinolein to bind to LOX in a nonproductive or a less productive mode, allowing an increase in the rate of nonspecific radical reactions even when oxygen is available.

Optimizing 1,3-dilinolein oxidation. Since dilinolein was the most rapidly oxidized ester substrate, reaction parameters were rechecked in order to assure that they were optimal (12). Unlike the assays with trilinolein which were conducted for two hours, very short assay times (15 min) and suboptimal concentrations of LOX were used so that the assay results would approximate initial oxidation rates. The pH and deoxycholate concentration were varied to determine the optimal oxidation rate. Plotted in Figure 1 is the pH profile of 1,3-dilinolein oxidation in deoxycholate containing buffer. The dilinolein pH profile is similar to the profile of trilinolein, except that the dilinolein profile is slightly narrower at pH < 7. Figure 2 shows the effect of deoxycholate concentration on the amount of dilinolein oxidized in 15 min. The amount of hydroperoxide produced increased almost linearly up to 10 mM deoxycholate. At 10 mM deoxycholate oxidation was increased six-fold over that obtained in a medium containing no deoxycholate. Further increases in the amount of deoxycholate caused a small decrease in the amount of oxidation.

The influence of LOX concentration upon dilinolein oxidation in 15 min was also investigated. Reaction conditions were identical to those used in Figure 1 at pH 8. The degree of oxidation increased as the level of LOX increased up to 1.25 mg (~0.6 mg protein). Further increases in LOX caused no increase in the rate of oxidation, probably because oxygen became limiting. The amount of oxidized linoleate decreased somewhat at the highest levels of LOX tested, probably because of the anaerobic action of LOX upon the initially produced hydroperoxide (13).

The experiments discussed above were conducted in a multi-component buffer described in Materials and Methods. When oxidations were conducted in 0.1 M AMP, HEPES, MES, or TRICINE, the extent of dilinolein oxidation in 15 min was reduced approximately 2 to 3-fold. The possibility that this decrease is due in part to diminished ionic strength is shown by the data in Figure 6. Here different amounts of NaCl were added to 0.1 M TRICINE buffer. The extent of oxidation remained nearly constant to

10 mM NaCl, then increased about two-fold at 200 mM NaCl. Further increases in the NaCl concentration caused the extent of oxidation to diminish. The stimulatory effect of lower NaCl concentrations may be due to the enhancement of complex formation between dilinolein and deoxycholate in a more polar environment. Apparently this stimulatory effect is overcome by inhibition of LOX at very high concentrations of NaCl. In 0.1 M TRICINE containing 200 mM NaCl, the rate of dilinolein oxidation was approximately 90% of that in the multi-component buffer. Thus high ionic strength is the main reason why oxidations occur more rapidly in the multi-component buffer.

Oxidation rates of linoleate acyl glycerols and methyl linoleate Having established optimal reaction conditions for dilinolein, other esters of linoleate were tested, and their extent of oxidation in 15 min was compared to that of free linoleic acid (Table 2). Monolinolein was oxidized more than two times faster than dilinolein and slightly faster than linoleic acid. An entry from Table 1 on the oxidation of trilinolein in deoxycholate is included in Table 2 to emphasize that the addition of a third linoleoyl residue on the glycerol backbone causes a large decrease in reactivity. The methyl ester of linoleic acid was oxidized at approximately the same rate as linoleic acid. In contrast, earlier work that was performed with Tween 20 (polyoxyethylene-sorbitan monolaurate), but without deoxycholate showed that the methyl ester of linoleic acid was a poor substrate for LOX (1). Since the results obtained in deoxycholate were at such variance with these earlier results, assays were repeated in the presence of no added surfactant and in Tween 20. Table 2 shows that without surfactant or in the presence of Tween 20 the oxidation of linoleic acid was accelerated, but monolinolein and methyl linoleate were oxidized at rates that were significantly reduced compared to those obtained in deoxycholate. No oxidation of dilinolein could be detected in the presence of Tween 20 or without surfactant.

Relative oxidations rates of linoleate acyl glycerols and methyl linoleate. The data in Table 3 show that when the oxidation of dilinolein, methyl linoleate, and monolinolein was followed in the presence of linoleic acid, their rates of oxidation were reduced relative to that of linoleic acid (Compare Table 2 and Table 3, entries 1, 2, and 3). However, even in the presence of linoleic acid, monolinolein was still the most rapidly oxidized ester substrate. When 1,3-dilinolein and methyl linoleate were oxidized together in the presence of linoleic acid, their relative rates of oxidation were reduced further, although the amount of LOX was increased proportionately (Table 3, entry 4). The combination of 1,3-dilinolein and monolinolein was also oxidized in the presence of linoleic acid (Table 3, entry 5). Each substrate was oxidized at approximately the same relative rate as before when assayed separately against linoleic acid.

Work by several research groups has demonstrated the importance of the methyl terminus of the fatty acid chain in directing the site of hydrogen removal by L[+2] type LOX, such as the high pH form of soybean LOX (14,15,16). However, the rate and specificity of hydroperoxide formation is also influenced by the presence of the free carboxylic residue in the substrate. For example, at high pH values (13S)-(9Z,11E)-13-hydroperoxy-9,11-octadecadienoic acid is the major product of the action of soybean LOX on linoleic acid. As the pH is lowered, and the carboxylic acid moiety becomes increasingly protonated, increasing amounts of the 9-hydroperoxide isomer are formed (17). Additionally, the rate at which structural analogues of naturally occurring fatty acids are oxidized by LOX is profoundly diminished as the system of methylene-

interrupted *cis* double bonds is displaced from its natural distance to the carboxyl group (18).

The data found in Tables 2 and 3 show that the presence of a free carboxyl group on the substrate influences the magnitude of the interaction between the substrate and LOX. Linoleic acid is oxidized most rapidly in the competition experiments (Table 3), but monolinolein is oxidized most rapidly when the substrates are assayed individually (Table 2). If the order of reactivity of the substrates were determined solely by the ability of deoxycholate to solubilize the substrates, then a change in the reaction order would not be expected depending on whether the substrates were assayed separately or together. That linoleic acid is always oxidized most rapidly in the competition experiments must be because of preferential binding of linoleic acid by LOX.

In conclusion the results demonstrate that methyl linoleate as well as mono- and diacylglycerol that contain linoleoyl residues are rapidly oxidized by LOX in buffers that contain deoxycholate surfactant. The oxidized products are useful chemical intermediates for further transformation to emulsifiers and lubricants with enhanced hydrophilic properties.

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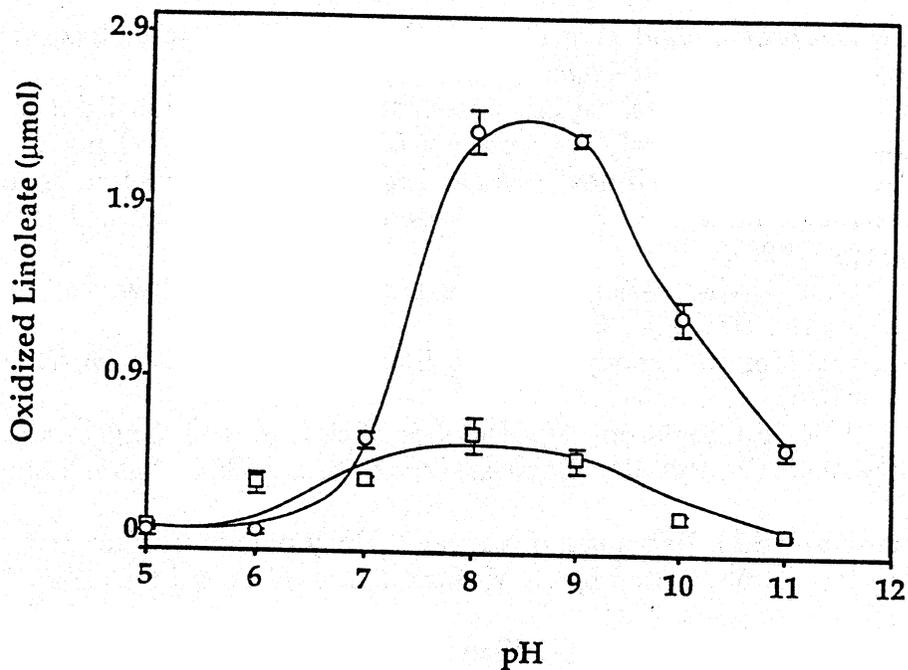


FIG 1. Influence of pH on the oxidation of 2 μM trilinolein (\square) and 3 μM 1,3-dilinolein (\circ) by LOX in the presence of 10 mM deoxycholate. Hydroperoxide was determined by the xylenol orange assay (11). Data are means \pm SEM of three to five repetitions.

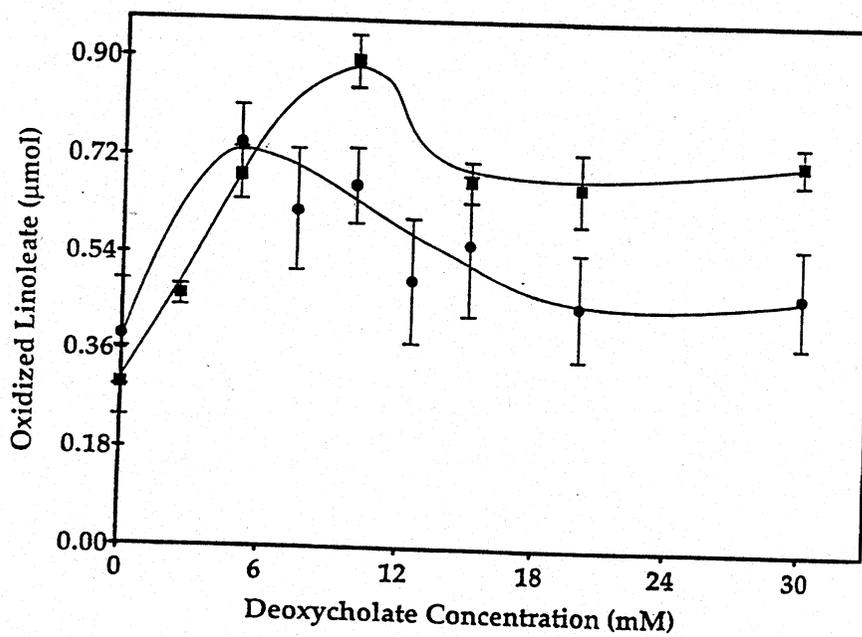


FIG. 2. Influence of deoxycholate concentration on the oxidation of 2 μM trilinolein (\bullet) and 3 μM 1,3-dilinolein (\blacksquare) by LOX. Hydroperoxide was determined by the xylenol orange assay. The data are means \pm SEM for seven determinations.

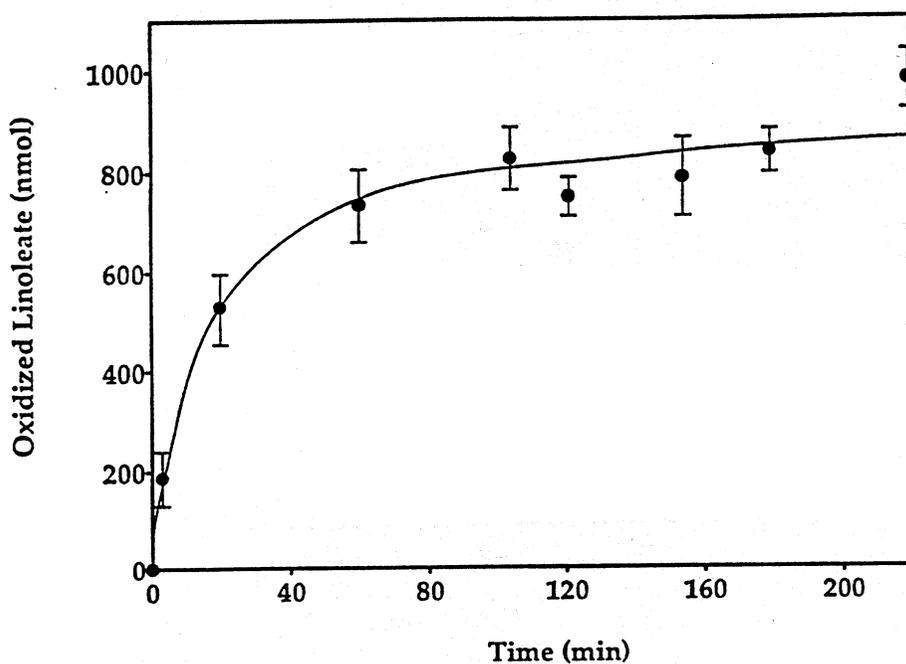


FIG. 3. Time course of 2 μM trilinolein oxidation by LOX in the presence of 10 mM deoxycholate. Hydroperoxide was determined by the xylenol orange assay. The data are the mean \pm S.E.M. for seven determinations.

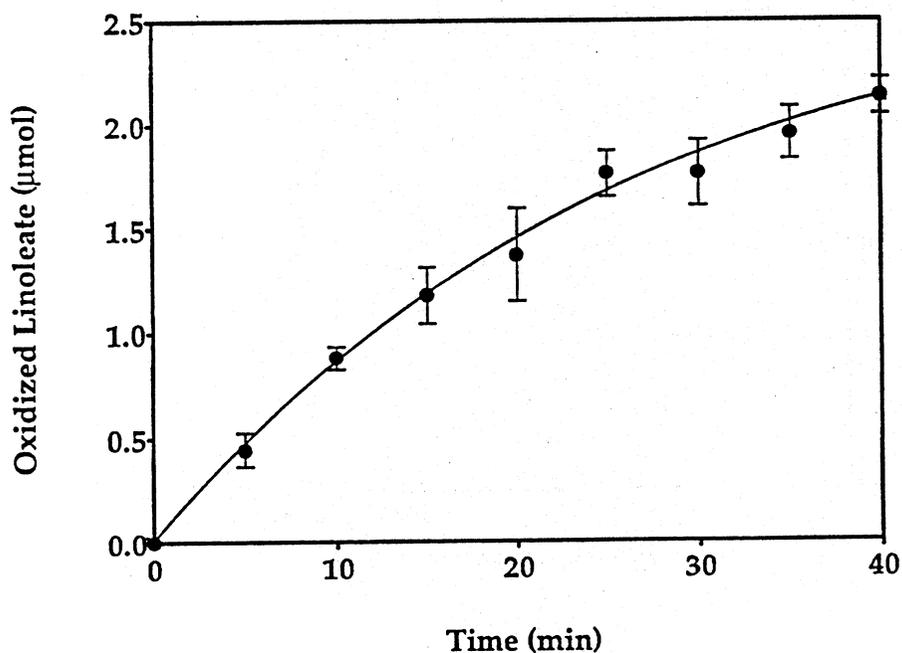


FIG. 4. Time course of 3 μM 1,3-dilinolein oxidation by LOX in the presence of 10 mM deoxycholate. Hydroperoxide was determined by the xylenol orange assay. The data are the mean \pm S.E.M. for four determinations.

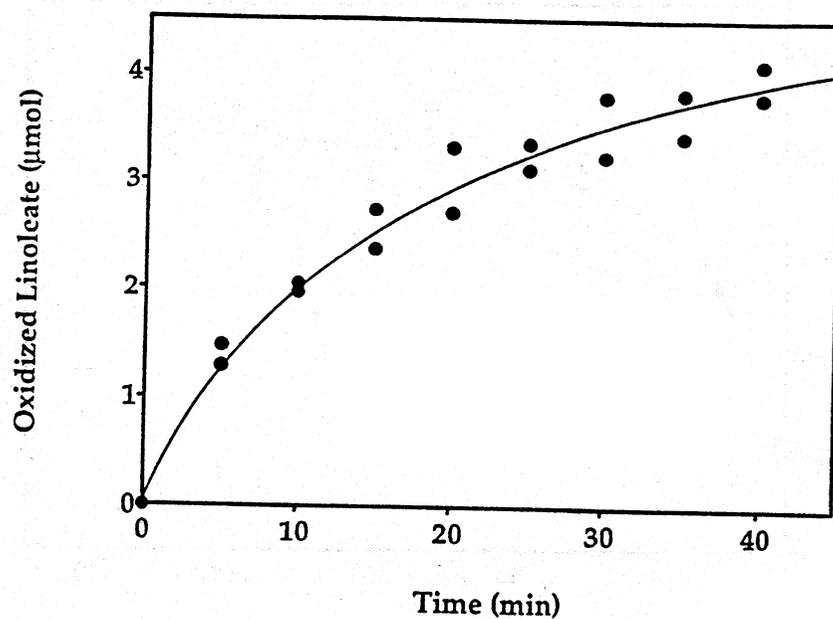


FIG. 5. Time course of 3 μM 1,3-dilinolein oxidation by LOX in the presence of 10 mM deoxycholate as determined by using HPLC to follow the rate of disappearance of the substrate. Analyses were conducted as described in Ref. 10.

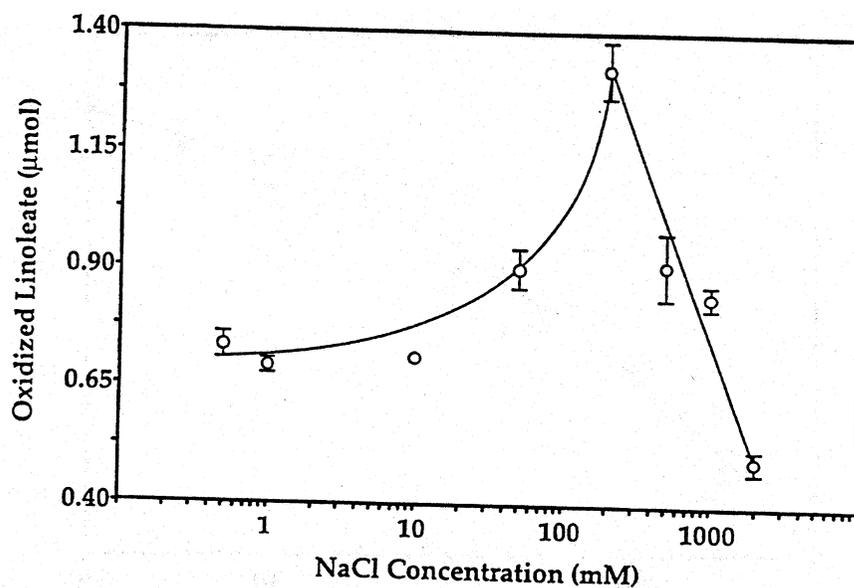


FIG. 6. The effect of NaCl on 3 μM 1,3-dilinolein oxidation by LOX in the presence of 10 mM deoxycholate. Hydroperoxide was determined by the xylenol orange assay. The data are means \pm SEM for four determinations.

TABLE 1. Relative Rate of Oxidation of Linoleic Acid and Several Phosphoglycerides and Acylglycerols Catalyzed by LOX

Substrate	Oxidation Rate (%) ^a
Linoleic acid	100
1,2-diL, 3- <i>P</i> -serine ^b	28 ± 8
1-L, 2-S, 3- <i>P</i> -choline	12 ± 4
1-S, 2-L, 3- <i>P</i> -choline	7 ± 3
1-P-, 2-L, 3- <i>P</i> -ethanolamine	13 ± 2
1-P, 2-L, 3- <i>P</i>	15 ± 5
Trilinolein	3 ± 1
1-L, 2-O, 3-S	11 ± 4
1-L, 3-L	40 ± 5
1-S, 2-L	43 ± 4

^a Assays were conducted as described in Ref. 11. The data are means ± S.E.M. for five to ten determinations.

^b Substrate abbreviations: 1,2-diL, 3-*P*-serine, 1,2-dilinoleoyl-*sn*-glycero-3-phospho-L-serine; 1-L, 2-S, 3-*P*-choline, 1-linoleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine; 1-S, 2-L, 3-*P*-choline, 1-stearoyl, 2-linoleoyl-*sn*-glycero-3-phosphocholine; 1-P, 2-L, 3-*P*-ethanolamine, 1-palmitoyl-2-linoleoyl-*sn*-glycerol-3-phosphoethanolamine; 1-P, 2-L, 3-*P*, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphate; 1-L, 2-O, 3-S, 1-linoleoyl-2-oleoyl-3-stearoyl-*rac*-glycerol; 1-L, 3-L, 1,3-dilinolein; 1-S, 2-L, 1-stearoyl-2-linoleoyl-*sn*-glycerol.

TABLE 2. Relative Amounts of Oxidized Linoleate Formed in 15 Min by lipoxygenase (LOX)

Substrate	Relative Amount of Oxidation ^a		
	Without		
	Deoxycholate ^b	Surfactant	Tween 20 ^c
Linoleic Acid	100 ± 6	186 ± 1	195 ± 7
Methyl Linoleate	90 ± 8	28 ± 1	12 ± 1
1-Monolinolein	126 ± 6	11 ± 1	72 ± 3
1,3-Dilinolein	55 ± 4	NS ^d	NS
Trilinolein	3 ± 1 ^e		

^a Assays were conducted as described in Ref. 12. Data are the mean ± SEM for three determinations.

^b The deoxycholate concentration was 10 mM.

^c The Tween 20 concentration was 0.25% (v/v) (18).

^d Results were not significantly different than zero.

^e Data from Ref. 9. Reaction conditions were identical except 200 µg LOX was added.

TABLE 3. Relative Rate of Oxidation of Mixtures of Substrates by LOX in Deoxycholate

	Substrate Mixtures	Relative Oxidation Rates ^a
1.	Linoleic acid	100 ^a
	1,3-Dilinolein	36 ± 2
2.	Linoleic acid	100 ^b
	Methyl linoleate	39 ± 3
3.	Linoleic acid	100 ^b
	1-Monolinolein	75 ± 2
4.	Linoleic acid	100 ^b
	1,3-Dilinolein	10 ± 2
	Methyl Linoleate	18 ± 2
5.	Linoleic acid	100 ^c
	1,3-Dilinolein	36 ± 9
	1-Monolinolein	65 ± 4

QUICK METHODS FOR THE DETERMINATION OF FREE FATTY ACID CONTENT IN COTTONSEED

Abstract

The official method (AOCS Aa6-38) for the determination of free fatty acid content in cottonseed requires 40 - 50 gram samples and 4 hour extractions before final titration. This research is to study the feasibility of using a hydraulic press to remove oil from seed instead of extraction and to compare several commercially available free fatty acid determination methods as opposed to titration. The hydraulic press has been practiced by individual labs. Our test results indicated that to be able to press oil out at room temperature from fuzzy seed, the moisture content can not be lower than 8%, and from dehulled kernels, its moisture can not be higher than 7.5%. Titration is still the most accurate method for the determination of free fatty acid content in oil. Other simple methods, such as indicator paper, fatty acid kit, and conductivity meters, were compared. Both indicator paper and fatty acid kit gave acceptable estimations of free fatty acid content in oil. The conductivity meter only works with purified oils.

Quick Methods for the Determination of Free Fatty Acid Content in Cottonseed

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BACKGROUND

Free fatty acid (FFA) content is one of the major quality factors for cottonseed. There are trading rules that define its limit set up by the National Cottonseed Products Association (1).

For cottonseed crusher: seed with FFA < 1.8% is defined as Prime, and > 12.5% as off grade (Rule #116)

For feed industry: seed with FFA < 3% is defined as Prime (Rule F-4).

Shipment of cottonseed is valued by its amount of FFA and other properties. The amount of FFA in cottonseed is presently determined by the Official Method Aa 6-38 of American Oil Chemists' Society (2).

Official Method for Free Fatty Acids Determination (AOCS Official Method Aa 6-38)

Grind seed with Bauer mill

Remove meats from hulls
on a 4 - 6 mesh screen

Grind the meats in a Universal Food Chopper

Place 40 - 50 g in the extractor and
percolate with 50 mL petroleum ether or hexane

Repeat with two 25 mL portions of solvent

Official Method for Free Fatty Acids Determination

(AOCS Official Method Aa 6-38)
Continued

Desolventize solvent from oil

Weight 7.05 g of extracted oil

Add 50 - 75 mL alcohol

Add 1 mL indicator

Titrate with 0.25 N NaOH

Concerns About the Official Method

Experimental Condition	Concerns
Operates at room temperature and pre-heating of seed is not a required step	Could underestimate FFA% in the seed oil
Organic solvent is used to extract oil from ground meats	Flammable solvent Disposal of solvent Add more than 40 minutes to the test

To alleviate the concerns of official FFA method, we propose to include a pre-heating step for the seed and to use a hydraulic press instead of solvent to extract oil. This report presents the criteria for a good hydraulic press operation in a laboratory environment.

Pre-heating of Seed

- Microwave heating of seed four minutes or longer will
- melt solidified fat or FFA, and
 - minimize lipase activity.

Effect of Hydraulic Pressure on Yield of Oil

Yield of oil from cottonseed meats reached a steady state as the hydraulic pressure increased above 1000 psi.

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Effect of Moisture in Cottonseed Meats on Yield of Oil

Whole cottonseed which has a moisture of $< 8\%$ yields no oil under 5000 psi hydraulic pressure.

Oil yield drops dramatically when the moisture in meats is greater than 8%.

Oil yield decreases slowly when meats moisture is less than 6%.

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SUMMARY

Hydraulically pressed cottonseed meats eliminate the use of solvent and reduce the test time from approximately 1 hour to 15 minutes for the determination of free fatty acid content in cottonseed. The proposed procedure is outlined as follows:

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Effect of Cottonseed Meat Size on Yield of Oil

When 57.6% of meat particles are smaller than 2.8 mm (14mesh), no oil was pressed out by hydraulic press at 5000 psi.

When 99.8% of meat particles are larger than 2.8 mm (14mesh), 48.4% available oil was obtained by hydraulic press.

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Dehull the seed in a Waring Blender

Pre-heat meats in a microwave oven for four minutes

Place 80 g of meats in the hydraulic cell

Press meats to 5000 psi to produce oil sample

Determine FFA in the oil by titration or 3M Shortening Strips or Fatty Acid Kit

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Free fatty acid content in oil is officially determined by titration method. It requires 7.05 g of oil sample, NaOH solution, indicator liquid, and burette. The titration procedure is easy and swift. If titration is not available, other simpler FFA determination kits might be useful. Several commercial FFA kits have been considered for this purpose.

Commercially Available Kits for FFA Determination

Shortening Monitor Strip - 3M

FFA Kit - Test Kit Technologies, Inc.

Foodoil Sensor (Model NI-21B) -

Northern Technologies International Corp

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Shortening Monitor Strip - 3M

Regular Shortening Monitor Strip -
2, 3.5, 5.5, 7% FFA
(+/- 0.5%)

Low Range Shortening Monitor Strip -
1, 1.5, 2, 2.5% FFA
(+/- 0.25%)

Ultra Low Shortening Monitor Strip -
0.5, 1, 1.5, 2% FFA
(Only available to Japanese users)

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FFA Kit - Test Kit Technologies, Inc.

2 Kits available - 0.7 & 3% FFA
3.5 mL oil required per test
Spectrophotometer for end point
\$820 initial kit then \$2 per test

The kit has not been tested by this lab yet.

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Foodoil Sensor (Model NI-21B)
Lubri Sensor (Model NI-2C) -
Northern Technologies International Corp.

Simple
Economic
Sensitive to polar impurities
therefore, less specific to FFA in oil

COST

Shortening Monitor Strip - 3M
< 40 cents / test

FFA Kit - Test Kit Technologies, Inc.
\$820 start up kit + \$2 /test

Foodoil Sensor (Model NI-21B) -
Northern Technologies International Corp.
\$500 for a unit

REFERENCES

1. Trading Rules, National Cottonseed Products Association, 1995, p. 51-52 and p.186.
2. Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th edn., AOCS Press, Champaign, 1993.