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Comparison of Some Lipoxidases and Their Mechanism of Action

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GENERALIZATIONS about the lipoxidase-catalyzed oxidation of the essential fatty acids require comparative studies of these plant enzymes. Soybean 8, 17, 26), pea (20), and alfalfa (21) lipoxidases have been studied in some detail, and these studies have indicated the absence of prosthetic groups and coenzymes. Further studies relative to the active group of lipoxidases are necessary before the mechanism of its catalysis can be determined.

This paper reports comparative studies on the catalytic properties of urd bean, mung bean, soybean, wheat, and peanut lipoxidases. A mechanism for lipoxidase action is proposed.

Materials and Methods

Plant Extracts Containing Lipoxidases. The urd bean *(Phaseolus mungo)* and mung bean *(Phaseolus aureus)* powders were extracted with 10 vol. of distilled water for 30 min., squeezed through cheesecloth, and centrifuged at 500 x g. for 10 min. The supernatant was diluted 10 times with 0.1 M phosphate buffer, pH 7.0, before use. Ten grams of freshly powdered whole wheat of the Ovos 41 Variety were ground with 40 ml. of distilled water and 3 g. of sand in a mortar, squeezed through cheesecloth, and centrifuged at 500 x g. for 10 min. The supernatant was used as a source of lipoxidase. After a preliminary analysis of 16 varieties of peanuts obtained fom the Southern Utilization Research Branch, New Orleans, La., the variety Var-199-22-A-2 was chosen for study since it contained the most lipoxidase. Peanuts were shelled, ground, and defatted. Ten grams of the defatted powder were ground with 30 ml. of distilled water and 3 g. of sand in a mortar, squeezed through cheesecloth, and centrifuged at 500 x g. for 10 min. The supernatant was used as the source of the enzyme. The preparation of soybean and pea extracts containing lipoxidase has been previously described (20, 26).

Partial Purification of Urd Bean Lipoxidase. Ten grams of 30-mesh urd bean powder were extracted with 100 ml. of 0.15 M sodium chloride for 15 min. and centrifuged at 500 x g. for 10 min. Fifty ml. of the supernatant were adjusted to pH 5.1 with 1 M acetic acid, the inactive precipitate was removed, and the supernatant was stirred with 2.25 g. (dry weight) of calcium phosphate gel (11) for 15 min. The suspension was centrifuged at 500 x g. for 10 min., and the supernatant was discarded. The packed gel was washed first with 50 ml. of distilled water, then with 50 ml. of 0.05 M phosphate buffer, pH 5.7. The lipoxidase was then eluted with four 25-ml. portions of 0.08 M citrate-phosphate buffer, pH 7.0. In each elution, as in each washing, the gel was stirred mechanically with the solvent for 15 min., then centrifuged. The above treatment yielded an urd-bean lipoxidase preparation with an enrichment of approximately eight times as much lipoxidase activity on the basis of total protein and a yield of 67% lipoxidase activity retained over the original extract.

Preparation of Substrates. Preparation of linoleate substrate has been previously described (26). Linolenate and arachidonate were used as ammonium salts and prepared similarly.

Measurement of Lipoxidase-Catalyzed Oxidations. For measurement of relative activity and the effect of antioxidants, three ml. of ammonium linoleate (4 x 10^{-3}M), pH 7.0, were placed in the main compartment, and 1 ml. of enzyme preparation was placed in the side-arm of the Warburg flask. The bath temperature was 20°C. The gas phase was air. Antioxidants dissolved in 0.1 ml. alcohol were added to the linoleate. Oxygen-absorption studies were performed by conventional techniques.

For measurement of nordihydroguaiaretic acid inhibition, the final reaction mixture contained 0.01 M linoleate, pH 7.0, and 1.4 Holman units of soybean lipoxidase per flask. The gas phase was air, and the temperature was 0°C.

For measurement of the effect of fatty-acid composition and molecular extinction coefficients, three ml. of ammonium salts of the fatty acids at 1 x 10^{-2}M and pH 7.0 were placed in the main compartment, and 1 ml. of the lipoxidase preparation was put in the side-arm of the Warburg flask. After oxidation at 20°C. the reaction was stopped with 4 ml. of absolute alcohol, an aliquot was diluted with 60% alcohol, and its spectral absorption was determined at 235 mμ.

In the inhibition studies three ml. of ammonium linoleate (2 x 10^{-2}M) substrate at pH 7.0 and 1% egg albumen were placed in the main compartment. After incubation of the enzyme, preparation for 15 min. with the inhibitor, 1 ml. of aliquot was placed in the side-arm. For reactivation experiments the enzyme-inhibitor mixture was incubated with the reactivator for 15 min. before putting it in the side-arm. Proper controls were maintained. The bath temperature was 20°C., and the gas phase was oxygen.

Results and Discussion

Relative Activity of Lipoxidases in Some Plant Seeds. The relative lipoxidase activity of the most active plant materials studied is listed in Table I.

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TABLE I

Relative Activity of Lipoxidases in Various Plants

Source	x Dilution	Initial velocity μl. O_2/ min.	Percentage of activity relative to soybeans
Soybeans	100	32.0	100
Urd beans	100	19.2	60
Mung beans	100	15.2	47
Peas	100	11.2	35
Wheat	4	15.0	2
Peanut	3	8.4	1

For comparative purposes, results have been calculated on the basis of 100 for soybeans, the most potent known source for lipoxidase.

The data in Table I indicate that urd beans and mung beans are a very good source of the enzyme, ranking next to soybeans. While urd and mung beans have never been assayed before for lipoxidase activity, Reiser and Fraps (18) determined the lipoxidase content of 13 different kinds of beans and found them to possess 3–24% as much lipoxidase activity as soybeans. The Thomas Laxton Variety of peas used in this study has about as much lipoxidase activity as any pea assayed.

The reports of lipoxidase activity of wheat show marked differences. Thus Miller and Kummerow (16) report soybeans to be 200 times as active as wheat germ while Sumner (23), using the iron thiocyanate method of assay, found soybeans to be only 40 times as active as wheat germ. Blain and Todd (5), utilizing the carotene destruction method of assay, reported soybeans to be 20–30 times as active as wheat germ. These differences could be explained by the observations of Irvine and Anderson (10), who report large variations in the lipoxidase content of wheat with variety.

As is evident from Table I, peanuts are not a very good source of the enzyme containing less than 1% activity as compared to soybeans.

Effect of Antioxidants. Table II summarizes the results of antioxidant-inhibition studies. It is evident from these data that the antioxidants vary considerably in their inhibitory effect on various lipoxidases. Nordihydroguaiaretic acid is the most potent agent, inhibiting the oxygen absorption of all four lipoxidases completely in all cases at 1 x 10^{-3}M. Propyl gallate ranks next to nordihydroguaiaretic acid while results with α-tocopherol and hydroquinone are more variable. It may well be that nordihydroguaiaretic acid and propyl gallate are more efficient hydrogen donors to the linoleate free radical as compared with α-tocopherol and hydroquinone.

Since nordihydroguaiaretic acid is the most potent lipoxidase inhibitor known (20, 21, 26, 27), its mode of inhibition of soybean lipoxidase was studied further. The data in Table III show a 50% inhibition at about 2 x 10^{-4}M. The data in Figure 1 plotted as 1/v *vs.* 1/s show that nordihydroguaiaretic acid is a competitive inhibitor. The only chemical similarity between linoleate and nordihydroguaiaretic acid is that they both possess hydrogens which are easily abstracted. The importance of hydrogen abstraction in the reaction mechanism of the lipoxidases will be discussed later. It was again noted that nordihydroguaiaretic acid was readily cooxidized in the lipoxidase-catalyzed linoleate oxidation. This is apparently caused by the abstraction of hydrogens by the intermediate linoleate peroxide free radicals.

TABLE II

Effect of Antioxidants on Linoleate Oxidation-Catalyzed by Lipoxidases

Antioxidant	Molarity	Percentage inhibition of initial velocity			
		Urd beans	Mung beans	Wheat	Peanut
Nordihydroguaiaretic acid	1 x 10^{-4}	0	0	0	0
		100	100	100	100
Propyl gallate	1 x 10^{-3}	24	69	53	80
	2 x 10^{-3}	100	100	100	100
α-Tocopherol	3 x 10^{-3}	0	35	69	0
Hydroquinone	1 x 10^{-2}	0	17	53	100

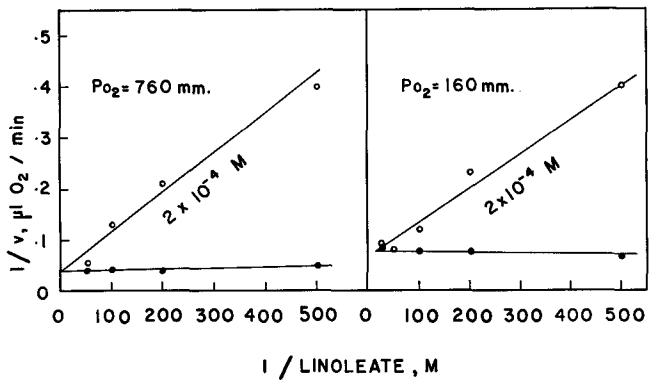

FIG. 1. Competitive inhibition of lipoxidase-catalyzed, linoleate oxidation by nordihydroguaiaretic acid.

TABLE III

Inhibition of Soybean Lipoxidase by Nordihydroguaiaretic Acid

Nordihydroguaiaretic acid M x 10^{-4}	Percentage inhibition of initial velocity
1	13
2	45
3	57
4	63
5	70
6	75
10	88

Molecular Extinction-Coefficients. The ϵ_M of the products of linoleate oxidation catalyzed by urd bean, mung bean, and wheat lipoxidases were 15,000, 11,000, and 17,000, respectively. For the sake of comparison the ϵ_M values of soybean and pea enzymes are 27,000 and 8,000, respectively. On the basis of their observation that lipoxidase produced conjugated diene hydroperoxides of *cis-trans* configuration, Privett *et al.* (17) suggested that the ϵ_M cannot be theoretically greater than 28,000. Furthermore the quantitative characterization of the products of soybean lipoxidase-catalyzed, linoleate oxidation showed the presence of products other than conjugated hydroperoxides. Their amount depended upon enzyme concentration, method of enzyme addition, and the rate and level of oxidation (17). It is also possible that the crude extracts used in the above study contain compounds which partially break down the conjugated peroxides formed by lipoxidase, thus resulting in low values of ϵ_M. For example, various hematin compounds catalyze this breakdown (25).

Absence of Known Prosthetic Groups by Inhibition Analysis and Dialysis. Cyanide, azide, diethyldithiocarbamate, fluoride, pyrophosphate, ethylene diamine tetraacetate, and quinacrine at a final concentration of 1 x 10^{-3}M did not inhibit the linoleate oxidation catalyzed by urd bean, mung bean, wheat, and peanut lipoxidases. These results are in agreement with the

previous work on pure soybean lipoxidase (8), pea lipoxidase (20), and alfalfa lipoxidase (21). The noninhibition by this wide variety of efficient metal inhibitors demonstrates that the plant lipoxidases give no direct evidence of possessing essential metals. Since quinacrine is also noninhibitory, there is no evidence for essential flavin groups.

Dialysis of the above enzyme preparations in the cold against buffers of pH values ranging from 5.1 to 8.0 did not result in any loss of activity, suggesting the absence of a freely dissociable, essential group in the enzyme preparations.

Effect of Thiol Reagents. It has been previously reported from this laboratory that linoleate-oxidation catalysis by pea-lipoxidase preparations (20) and alfalfa-lipoxidase preparations (21) is not inhibited by 1×10^{-3}M p-chloromercuribenzoate. Likewise it was observed in this study that the catalysis of linoleate oxidation by wheat and peanut lipoxidase preparations was not inhibited. Similar negative results were obtained when soybean, pea, peanut, and wheat enzyme preparations were subjected to the action of 1×10^{-3}M ferricyanide, 1×10^{-2}M iodoacetate, and 1×10^{-2}M maleate. These results suggest the nonessentiality of thiol groups in these lipoxidases.

However results with lipoxidase preparations from mung beans and urd beans were significantly different. Preliminary experiments with 1×10^{-3}M p-chloromercuribenzoate gave 35% inhibition of mung bean lipoxidase and 100% inhibition of urd bean lipoxidase. Since urd beans proved to be a good source of the enzyme, purification was undertaken, and further thiol inhibitor experiments were performed on this partly purified enzyme preparation. Table IV lists the results obtained. It will be observed from Table IV that all the thiol inhibitors tested except sodium arsenite inhibit the reaction. Since sodium arsenite is the least satisfactory among the arsenicals (3) as a thiol inhibitor, the negative results observed cannot be used to exclude the possibility of urd-bean lipoxidase having essential thiol groups. The results with p-chloromercuribenzoate show the high sensitivity of urd-bean lipoxidase to this reagent. The low concentrations of the reagent required to produce inhibitions make any denaturation of the protein unlikely, a fact supported by partial reactivation of the enzyme by cyanide and glutathione. The low degree of reactivation of cyanide may be due to its weak reducing-action. In contrast, glutathione reactivation was good. This is significant in view of the great variations observed earlier on the ability of glutathione to reactivate thiol-inhibited systems (3) and strongly suggests essential thiol groups in urd-bean lipoxidase. Considering the lack of absolute thiol oxidation specificity by ferricyanide and the weak reducing power of cyanide, 42% inhibition by ferricyanide and 25% reactivation by cyanide also indicate essential thiol groups. The complete inhibitions with Cu^{++}, Hg^{++}, and Ag^{+} ions show results similar to those obtained in the case of L-amino acid dehydrogenase by Stumpf and Green (22). However the interpretation of inhibition by heavy metals is complicated by the fact that in addition to the ability of metals to form mercaptides, they are also known to combine with the amino groups of the protein (1). The necessity of large concentrations of iodoacetate and maleate required to produce inhibition is not uncommon (1). These reagents, though not very specific, have been extensively used in the past for the testing of essential thiol groups in enzymes.

TABLE IV
Inhibition and Reactivation of Urd-Bean Lipoxidase

Inhibitor	Molarity of inhibitor x 10^4	Reactivator	Molarity of reactivator x 10^4	% Inhibition	% Reactivation
p-Chloromercuribenzoate	.025	.....	.....	0	.....
p-Chloromercuribenzoate	.25	.....	.....	63	.....
p-Chloromercuribenzoate	.25	Cyanide	2.5	34	29
p-Chloromercuribenzoate	2.5	.....	.....	100	.....
p-Chloromercuribenzoate	2.5	Glutathione	25	16	84
Cu^{++}	2.5	.....	.....	100	.....
Ag^{+}	2.5	.....	.....	100	.....
Hg^{++}	2.5	.....	.....	100	.....
Ferricyanide	2.5	.....	.....	42	.....
Ferricyanide	2.5	Cyanide	25	17	25
Maleate	30	.....	.....	0	.....
Maleate	300	.....	.....	100	.....
Iodoacetate	30	.....	.....	0	.....
Iodoacetate	300	.....	.....	100	.....
Arsenite	100	.....	.....	0	.....

This collective evidence presented in Table IV gives ample support for the participation of thiol groups in urd-bean lipoxidase-catalysis. Questions regarding the number of thiol groups, their relation to enzymatic activity, and the identity of the compound possessing these groups in the enzyme protein cannot be answered until extensive purification of urd-bean lipoxidase is accomplished. To see if partial purification would affect the inhibition characteristics, soybean and pea extracts were absorbed and desorbed from calcium phosphate gel. The gel eluates thus obtained were treated with p-chloromercuribenzoate (1×10^{-3}M), ferricyanide (1×10^{-3}M), iodoacetate (1×10^{-2}M), and maleate (1×10^{-2}M). None of these reagents produced any inhibition. The simplest explanation of these observations would be the existence of two types of lipoxidase in nature.

Further Characterization of Urd-Bean Lipoxidase. The use of the inhibitors: cyanide, azide, diethylthiocarbamate, fluoride, pyrophosphate, ethylenediaminetetraacetate, and quinacrine and the dialysis experiments reported elsewhere in this study were repeated, using the partly purified, urd-bean-lipoxidase preparation. No inhibition was observed, confirming the results obtained earlier.

TABLE V
Effect of Fatty-Acid Composition on the Initial Velocity of Urd-Bean Lipoxidase and the Molecular Extinction-Coefficients

Substrate	Initial velocity μl. O_2/min.	Molecular extinction-coefficient at 235 mμ
Linoleate	15.2	15,000
Linolenate	18.0	7,700
Arachidonate	14.8	8,100

In order further to test whether urd-bean lipoxidase is a typical lipoxidase, the rates of oxidation of linoleate, linolenate, and arachidonate by the enzyme preparation were compared. Table V lists the results obtained. It will be noticed that the rates are practically the same. This is in agreement with the results obtained by Holman and Elmer (9), using soybean-lipoxidase preparations, and is a distinguishing feature of lipoxidase catalysis. Table V also lists the ϵ_M of the products of oxidation of the three substrates. It is seen that the ϵ_M values for linolenate and arachidonate are the same order of magnitude

but are considerably lower than that of linoleate oxidation. Similar results were observed in soybean-lipoxidase catalysis (8) and can be ascribed to the known unstability of the peroxides of these more highly unsaturated acids.

This study indicates that, except for the evidence of essential thiol groups, urd-bean lipoxidase is similar to the other plant lipoxidase.

Proposed Mechanism of Lipoxidase Catalysis. This present study and the work of others (8, 17, 20, 21, 26) allows the formulation of a more definite hypothesis for the mechanism of lipoxidase catalysis. For more complete understanding of the mechanism, certain fundamental questions of electron transfer need consideration. If the function of lipoxidase is to abstract an electron or a hydrogen from the methylene group of the fatty acid, how does the enzyme bring about such an abstraction without possessing any metallic prosthetic group or a coenzyme? Our present knowledge suggests that the lipoxidase protein itself might act as an electron sink whereby it could momentarily hold an electron from the α-methylene group of linoleate, thus allowing oxidation. The validity of considering the protein molecule as an electron sink is based on the original suggestions of Szent-Györgyi (24) and others (6, 19, 29) and has been recently reviewed by Leach (12).

From these considerations the following mechanism of lipoxidase catalysis of linoleate oxidation may be visualized. Although any diagram presented at this time will be deficient in representing some phases and factors in the actual mechanism, Figure 2 has been drawn.

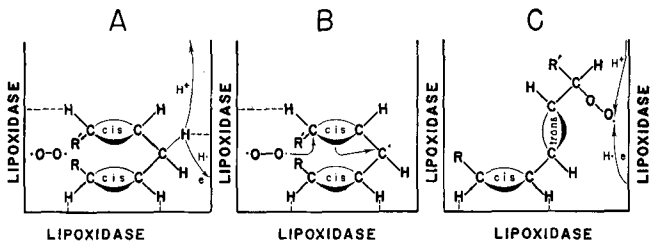

FIG. 2. Proposed mechanism of lipoxidase-catalyzed, linoleate oxidation. R′ represents the $CH_3(CH_2)_4$ portion of linoleate molecule while R represents the $(CH_2)_7COOH$ portion.

The first step is the formation of an enzyme-substrate complex. The fact that only *cis-cis* linoleate is acted upon and that the enzymatic oxidation products are optically active, whereas the products obtained by autoxidation are optically inactive (17), show the high degree of specificity resulting from a definite spatial arrangement of the linoleate on the lipoxidase. This necessitates the following:

a) the proper attachment of the α-methylene group of the linoleate to the enzyme protein;

b) the proper attachment of oxygen to the enzyme protein. (The enzyme absorbs oxygen and holds it in the proximity of the linoleate molecule until the linoleate accepts it. In a stereospecific manner the enzyme transfers the oxygen to the linoleate radical, thus bringing about an optically active product.)

The second step in the reaction proper is the creation of a free radical at the methylene group by the removal of a hydrogen atom. Figure 2-A indicates two possibilities of such removal. Working with atomic models, it was observed that the formation of the required *cis-trans* product could occur only if the methylene hydrogen pointing upwards in Figure 2-A is removed. If this treatment is valid, the necessity of a highly specific attachment of the methylene hydrogen to the enzyme protein is obvious. A free radical would be produced if the enzyme protein abstracted an electron *via* the methylenic hydrogen bridge. The hydrogen ion thus formed would go to the medium. Alternatively the hydrogen atom might be directly transferred to the protein.

The third step is the isomerization of double bonds leading to conjugation and the production of a *cis-trans* peroxyl radical (Figure 2-B). The thus-formed radical is capable of resonance, resulting in the isomerization of double bonds producing conjugation and creating a free radical on carbon atom No. 9 in proximity to the oxygen absorbed on the lipoxidase. At the instant the electron moves to conjugate the double bonds, the diradical oxygen is ready to attack the newly created, free radical in a precise manner so that an asymmetric center is created. This migration of an electron from the *cis* double bonds to the methylenic carbon radical produces the *trans* isomerization. The reasons why such an isomerization occurs may be as follows:

a) the mode of attachment of the methylene hydrogen and its subsequent removal might lead to a steric situation favoring the formation of a *trans* configuration;

b) if the groups attached to the newly created, double bonds possessed a *cis* configuration, it can be seen that the bulky groups attached to the two double bonds would be on the same side, thus capable of producing steric hindrance (the molecule is probably less strained in the *cis-trans* configuration);

c) the *cis-trans* compound is probably more thermodynamically stable than the *cis-cis*.

The formation of hydroperoxide from this radical could be visualized (Figure 2-C) in the following manner. After the above series of reactions the oxygen of the peroxyl radical is brought into proximity with the enzyme protein holding the electron or hydrogen radical. Lipoxidase transfers this electron to oxygen, and the hydrogen ion is picked up from the medium or the lipoxidase transfers the hydrogen radical in total. This would lead to the formation of the linoleate hydroperoxide. Alternatively the peroxyl radical may remove a hydrogen atom from another methylene group of an adjacent linoleate molecule. This would lead to a lipoxidase-modulated-and-controlled chain reaction.

The competitive inhibition by nordihydroguaiaretic acid can be explained as an abstraction of an electron or a hydrogen radical from this inhibitor by lipoxidase, resulting in a decrease in the capacity of lipoxidase for initiating linoleate oxidation. Cooxidation of polyphenolic antioxidants, carotenoids, and other easily oxidized molecules is probably caused by abstraction of hydrogens by the linoleate, peroxide-free, radical intermediates.

The mechanism as depicted in Figures 2-A, 2-B, 2-C incorporates and successfully explains all the known features of lipoxidase catalysis. It is in harmony with free radical mechanisms in biological systems (12) and with the well established theory of the oxidation of 1–4 diolefins.

Proposed Mechanism of Catalysis by Lipoxidases Possessing Essential Thiol Groups. The results reported in Table IV give preliminary indications of the participation of thiol groups in urd-bean-lipoxidase catalysis. The oxidoreduction enzymes, possessing thiol groups, have been reviewed by Barron (1), and invariably they also possess either a nucleotide

or flavin-type of coenzyme. All these other oxidoreduction systems are two electron-transfer systems. Lipoxidase catalysis involves only the momentary transfer of one electron. This clearly emphasizes the essential difference between the two types of catalysis.

According to Leach (12), the role of thiol groups in the protein moiety may be two-fold; as one electron system mediating between pairs of bivalent redox systems and as the initiator of reaction chains. In lipoxidase catalysis however there is no need for mediation since the nature of oxidation is univalent itself. Hence the purpose of thiol groups in urd-bean lipoxidase may be the initiation of reaction chains. The theoretical basis for this surmise is afforded by the papers of Waters and associates (7, 15, 28), Leach (12), and Barron and associates (1, 2). Bickel and Kooyman (4) have pointed out that these reactions should have low-activation energy and may be reversible at room temperature. The existence of thiol radicals has been repeatedly demonstrated (1, 13, 14).

In the light of the above the first step in the catalysis of linoleate oxidation could be represented as follows:

$$-CH=CH-CH_2-CH=CH-+\cdot SR-Lipox \rightleftarrows$$
$$-CH=CH-\dot{C}H-CH=CH-+HSR-Lipox$$

This reaction depicts the essential thiol radical initiating the lipoxidase reaction. The remaining sequence of steps in the oxidation could be exactly the same as depicted in Figures 2-B and 2-C, which is fully discussed in the previous section.

Summary

The comparative properties of lipoxidases from urd beans, mung beans, wheat, and peanuts have been determined and discussed. Evidence has been presented to suggest the participation of essential thiol groups in urd-bean and mung-bean lipoxidase catalysis but not in soybean, wheat, pea, and peanut lipoxidase catalysis. This suggests the possible occurrence of two types of lipoxidases in nature.

On the basis of these and other available experimental results, mechanisms for lipoxidase catalysis are proposed.

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The Composition of Bloom Fat in Chocolate

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THE FORMATION of a white film on the surface of chocolate, whether in the form of molded bars or coatings for centers, has plagued the industry since the use of chocolate began. Since this discoloration is supposed to resemble the bloom on grapes, it is known as "bloom." There are two distinct blooms which occur on chocolate, "fat bloom," so called because it is composed largely of fat, and "sugar bloom," composed mainly of sugar crystals. It is with "fat bloom" that we will deal here, and we shall call it simply bloom.

There has not been a great deal published on the actual composition of bloom. Whymper was the pioneer in the field of studying fat bloom on chocolate (16, 17). Sachsse (14, 15) described the different types of fat bloom. He found that the fat located in streaks below the surface of the chocolate had a melting point of 30°C. The fat which had appeared as bloom on the surface of well-tempered chocolate after six months had a melting point of 34°C. and, on chocolates which had been stored for several years, had the appearance of feathers and melted at 36°C. These melting points confirm unpublished findings of one of the authors (5). Neville *et al.* (12) found the iodine number of the surface layer of fat on a slab of cocoa butter to be lower than that of cocoa butter. Several investigators studied the effect of various centers upon the fat blooming of chocolate coatings (2, 5, 7, 14). Whymper (17) and Fincke (6) studied the effect of oil from covered nutmeats.

Harris and Shillaber (8) as well as Hettich (9) studied fat bloom by observing the surface of blooming chocolate under a microscope and noticed tiny beads of oil coming to the surface and then crystallizing, after which the typical white discoloration formed on the surface. From this and other observations Fincke (6) and others (9, 10, 11, 14) state that fat bloom is a physical phenomenon, that is, when an unstable, polymorphic modification of the fat

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