ANTIOXIDANTS AND ENZYMES

Effect of Alpha-tocopherol, Propyl Gallate, and Nordihydroguaiaretic Acid on Enzymatic Reactions

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This survey of the effect of α-tocopherol, NDGA, and propyl gallate on fourteen enzyme systems was undertaken in order to determine if phenolic antioxidants will inhibit enzymic reactions typical of those which cause deterioration in refrigerated and frozen foods, determine inhibition of oxidative enzymes by α-tocopherol, and add to knowledge of enzyme inhibition by phenolic compounds. Peroxidase, catalase, and ethyl alcohol dehydrogenase were specifically inhibited by concentrations of $2 \times 10^{-4}M$ of the antioxidants. Ascorbic acid oxidase, D-amino acid oxidase, the cyclophorase system, and urease were inhibited at $2 \times 10^{-3}M$ because of the general denaturing properties of the phenolic antioxidants. This research shows that the commercial antioxidants, NDGA and propyl gallate widely used for the preservation of foods, can inhibit some enzymes at the concentrations allowed in foods. Most of the enzymes tested were not sensitive to inhibition by these antioxidants. The biologically important antioxidant and vitamin, α-tocopherol, at $3 \times 10^{-6}M$ inhibited alcohol dehydrogenase in the presence of metal ions.

PHENOLIC ANTIOXIDANTS are used extensively in the United States for the stabilization of edible unsaturated fats and of foods containing fats that readily undergo oxidative rancidity. Of the phenolic antioxidants approved for use in foods (*17, 18*) butylated hydroxyanisole, propyl gallate, and nordihydroguaiaretic acid are used in the largest quantities. Phenolic antioxidants have been advocated for protection of refrigerated and frozen foods, particularly meat (*14*), poultry (*13*), and fish (*4, 27*). The possibility that phenolic antioxidants can inhibit enzymatic deteriorative reactions in unblanched or uncooked foods has not been given much consideration.

The enzymes soybean lipoxidase, mushroom catecholase (*16*), and beef liver catalase (*8*) are inhibited by phenolic compounds. Several investigators (*5, 10, 15, 25*) have studied the inhibition of lipoxidase by phenolic antioxidants. Lipoxidase is strongly inhibited by propyl gallate and nordihydroguaiaretic acid. The inhibition of lipoxidase by nordihydroguaiaretic acid was due not only to interaction of nordihydroguaiaretic acid with the lipoxidase-linoleate complex in which nordihydroguaiaretic acid was preferentially oxidized, but also to the reversible inhibition of lipoxidase activity by a direct reaction of the antioxidant with the enzyme (*24*). Further knowledge of the effect of antioxidants on other enzymatic reactions should be useful in explaining the mechanism by which phenolic compounds inhibit enzymatic reactions.

One important biological function of vitamin E might be inhibition of enzymic oxidations. Except for studies of the inhibition of succinoxidase by α-tocopherol phosphate (*21*), the possibility of inhibition of enzymes by α-tocopherol remains largely unexplored.

This paper reports the inhibition of many enzyme-catalyzed reactions by the antioxidants α-tocopherol, propyl gallate, and nordihydroguaiaretic acid.

Materials and Methods

Antioxidants. α-Tocopherol and nordihydroguaiaretic acid dissolved in absolute ethyl alcohol and propyl gallate dissolved in 50% ethyl alcohol were added to the enzyme systems to give the desired concentrations. The maximum amount of ethyl alcohol added to any enzyme system was 3%. Identical amounts of ethyl alcohol were added to the controls.

Initial Reaction Rates. All estimates of initial reaction rates for enzyme catalysts are based on many determinations of the amount of reaction, as a function of time, except for ascorbic acid oxidase and pepsin. The reaction rates calculated for ascorbic acid oxidase and pepsin are from a single determination of the products formed after a short reaction time. All other reaction rates were calculated from the graphs—the amount of reaction plotted as a function of time. Reaction rates are given in Table I in terms of the measurement made and the time interval used.

Turnip Peroxidase. Fresh turnip tissue was disintegrated in a blender for 2 minutes with an equal weight of distilled water. The extract was separated from tissue by pressing through cheese cloth and centrifuging at 850 × gravity. The supernatant was held at 65° C. for 3 minutes to precipitate inactive protein and to denature catalase and polyphenolase, and was immediately cooled and centrifuged (*20*). The resulting preparation had high peroxidase activity and contained no measurable catalase or polyphenolase.

A direct spectrophotometric measurement of guaiacol oxidation, identical with that of Ponting and Joslyn (*20*), was used for measuring peroxidase. Absorbancies at 430 mμ were read at 15-second intervals at 22° C. The system contained $4 \times 10^{-3}M$ hydrogen peroxide and $1.7 \times 10^{-3}M$ guaiacol in $0.01M$ acetate buffer, pH 5.4.

Liver Catalase. Catalase (Armour & Co., Chicago, Ill.) was determined by manometric measurement of the oxygen

released in the decomposition of $0.75 \times 10^{-2}M$ hydrogen peroxide in $1 \times 10^{-3}M$ phosphate buffer at pH 7.0 and 0° C.

Squash Ascorbic Acid Oxidase. Crooked neck squash was disintegrated in a blender with 1.5 ml. per gram of cold distilled water and the supernatant liquid from centrifugation was used directly.

The method of Ponting and Joslyn (*20*), based upon the colorimetric determination of ascorbic acid using 2,6-dichlorobenzenone-indophenol, was used for measuring ascorbic acid oxidase activity at 20° C. The reaction system contained 1 mg. of ascorbic acid per 20 ml. of $0.1M$ oxalate-phosphate buffer, pH 6.0.

Potato Polyphenolase. White potato tissue was disintegrated in a blender with 5 ml. per gram of cold $0.02M$ acetate buffer, pH 5.4, and the supernatant liquid from centrifugation was used directly. A direct spectrophotometric measurement of pyrogallol oxidation as a function of time was used to determine polyphenolase activity in a system of $0.05M$ pyrogallol in $0.02M$ acetate buffer, pH 5.4, saturated with oxygen at one atmosphere. Absorbancies at 420 mμ were read at 10-second intervals at 22° C.

Yeast Ethyl Alcohol Dehydrogenase. Crystalline ethyl alcohol dehydrogenase was prepared from baker's yeast by the method of Racker (*22*). The rate of enzymic ethyl alcohol oxidation at 22° C. was determined by spectrophotometric measurement of reduced diphosphopyridine nucleotide at 340 mμ. The concentration of ethyl alcohol was $0.56M$ and the concentration of diphosphopyridine nucleotide was $4.0 \times 10^{-5}M$. Tris-(hydroxymethyl) aminomethane buffer, $0.1M$, at pH 7.50 was used.

Glyceraldehyde 3-Phosphate. Crystalline glyceraldehyde 3-phosphate dehydrogenase, prepared by the method of Cori *et al.* (*3*), was a gift from M. P. Stulberg and P. D. Boyer of the University of Minnesota. Enzymic oxidation of glyceraldehyde 3-phosphate at 22° C. was determined by a direct spectrophotometric measure at 340 mμ of reduced diphosphopyridine nucleotide.

DL-Glyceraldehyde 3-phosphate was prepared by hydrolysis of glyceraldehyde 1-bromide-3-phosphate (Concord Laboratories, Cambridge, Mass.). The reaction system contained $1 \times 10^{-3}M$ glyceraldehyde 3-phosphate and $1 \times 10^{-3}M$ diphosphopyridine nucleotide in $0.01M$ phosphate buffer, pH 7.0.

Glucose 6-Phosphate Dehydrogenase. The activity of glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.) was determined at 22° C. by direct spectrophotometric measurement at 340 mμ for reduced triphosphopyridine nucleotide. The concentration of the substrate, glucose-6-phosphate, was $1.67 \times 10^{-2}M$ and the concentration of coenzyme, triphosphopyridine nucleotide was $2.3 \times 10^{-5}M$. Tris-(hydroxymethyl) amino methane buffer, $0.1M$, at pH 7.26 was used.

D-Amino Acid Oxidase. D-Amino acid oxidase from acetone-dried pig kidney was purified by the method of Negelein and Brömel (*19*) as modified by Wood and Gunsalus (*31*). Amino acid oxidase activity was determined by manometric measurement of oxygen consumption with $0.0167M$ DL-alanine, tris-(hydroxymethyl) amino methane buffer, pH 8.0, at 30° C.

Pig Heart Succinoxidase. A crude preparation of particulate succinoxidase was obtained by blending minced-washed pig heart for 75 seconds with 4 volumes of cold $0.1M$ phosphate buffer, pH 7.3, centrifuging at 5000 × gravity for 15 minutes. and resuspending in 4 volumes of buffer. Succinoxidase was assayed by manometric measurement of the oxygen consumed in the oxidation of $0.03M$ succinate in $0.03M$ phosphate buffer, pH 7.3, at 30° C.

Hydrogenase. Hydrogenase was prepared from *Escherichia coli* by the method of Gest (*7*). Cells were ground with alumina and the nucleic acid was removed with manganese ions. The protein fraction precipitating between 0.37 and 0.40 saturation of ammonium sulfate was used in these studies. Hydrogenase was assayed by measuring hydrogen consumption manometrically at 30° C. in $0.05M$ phosphate buffer, pH 6.8, with $8 \times 10^{-3}M$ methylene blue as the electron acceptor.

Rat Liver "Cyclophorase." "Cyclophorase" was prepared from rat liver by the method of Green (*9*). The activity of the cyclophorase system at 30° C. was determined by manometric measurement of oxygen absorption. The Warburg flasks contained 1 ml. of "cyclophorase gel," $1.6 \times 10^{-3}M$ α-ketoglutarate as substrate, $1.3 \times 10^{-3}M$ magnesium sulfate, $3.3 \times 10^{-3}M$ phosphate buffer at pH 7.3, and 0.2 ml. of potassium hydroxide in the center well.

Bovine Intestinal Phosphatase. Alkaline phosphatase (Armour & Co., Chicago, Ill.) was assayed by direct spectrophotometric measurement of the formation of phenolphthalein by enzymic hydrolysis of sodium phenolphthalein phosphate (Sigma Chemical Co.) at 22° C. in ammonium chloride–ammonium hydroxide buffer at pH 9.0 (*11*).

Pepsin. The activity of pepsin (Nutritional Biochemicals Corp., Cleveland, Ohio) was measured by the method of Anson (*1*) except for tyrosine and other aromatic amino acids resulting from hydrolyzed hemoglobin, which were measured by spectral absorption at 280 mμ after precipitation of protein with trichloroacetic acid. The absorbance at 280 mμ of the added antioxidants was corrected for by using suitable controls.

Jack Bean Urease. A crude urease preparation was made by aqueous extraction of ground jack bean meal (*23*). Urease activity was determined by measuring manometrically the production of carbon dioxide released in the hydrolysis of $1.67 \times 10^{-2}M$ urea in $0.1M$ of citrate buffer at pH 6.0 and at 30° C. (*29*).

Results

Propyl gallate and nordihydroguaiaretic acid were chosen for this study because these two compounds are powerful antioxidants and are used widely for the stabilizing food containing unsaturated fats. α-Tocopherol, the most widely distributed biological antioxidant, was included because of its biological importance.

This research consists of a survey of the effect of these three antioxidants on enzyme systems. The majority of the enzyme systems used were chosen as representing broad classes of enzymes catalyzing oxidation-reduction reactions. The enzymes are listed in Table I according to their classifications. Peroxidase and catalase were chosen as the two examples of peroxidative enzymes which have a heme-prosthetic group. Ascorbic acid oxidase and polyphenolase, which are of great importance in food preservation, are oxidative enzymes with a copper-prosthetic group. Alcohol dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase require diphosphopyridine nucleotide as the coenzyme, and glucose-6-phosphate dehydrogenase specifically requires the coenzyme, triphosphopyridine nucleotide. Amino acid oxidase is a flavin-type enzyme. The succinoxidase system consists of succinic dehydrogenase linked to a cytochrome–cytochrome oxidase electron transport system. Hydrogenase is an enzyme capable of catalyzing the hydrogen electrode reaction and transferring the electrons to a variety of acceptors. The cyclophorase system of rat liver, mitochondria, contains all the enzymes of the citric acid cycle and their requisite cofactors.

One might assume that the three antioxidants under consideration, because of their ease of oxidation, would act primarily as inhibitors of oxidative-reductive enzyme reactions. However, early results suggested that these antioxidants might act as general enzyme inactivators. For this reason three hydrolytic enzymes, alkaline phosphatase, pepsin, and urease were included in this study.

The results expressed as initial reaction rates and per cent inhibition are given in Table I.

In some of the fourteen enzyme systems studied, propyl gallate and nordihydroguaiaretic acid inhibited at relatively high concentrations. Presumably this

Table I. Inhibition of Enzyme-Catalyzed Reactions by Antioxidants

Classification of Enzyme	Enzyme	Antioxidant	Concentration of Antioxidant, $\times 10^{-5}$M	Initial Reaction Rate		% Inhibition of Initial Reaction Rate
Peroxidases	Peroxidase	None		0.010		
		NDGA[a]	0.27	0.010	increase in absorbancy/	0
			2.7	0.0027	10 sec.	73
			27	0.0027		73
		Propyl gallate	27	0.000		100
		α-Tocopherol	2.7	0.000		100
	Catalase	None		115	cu. mm. O_2/2 min.	
		α-Tocopherol	2.0	115		0
			20	115		0
		NDGA	2.0	51		56
			20	51		56
		Propyl gallate	20	25		78
Oxidases with Cu prosthetic group	Ascorbic acid oxidase	None		0.024	absorbancy after 15 sec.	
		Propyl gallate	20	0.030		0
			200	0.022		0
		NDGA	2.0	0.016		0
			20	0.028		0
			200	0.000		100
	Polyphenolase	None		0.0125	increase in absorbancy/	
		α-Tocopherol	2.7	0.0125	10 sec.	0
		NDGA	27	0.0084		33
		Propyl gallate	27	0.0108		14
			270	0.0125		0
Dehydrogenases requiring DPN or TPN coenzymes	Alcohol dehydrogenase	None		0.0167	increase in absorbancy/	
		α-Tocopherol	2.7	0.0141	10 sec.	16
		Propyl gallate	2.7	0.0143		14
			27	0.0079		53
		NDGA	2.7	0.0141		16
			27	0.0049		71
	Glyceraldehyde-3-phosphate dehydrogenase	None		0.0064	increase in absorbancy/	
		α-Tocopherol	27	0.0097	10 sec.	0
		NDGA	27	0.0052		~20
		Propyl gallate	27	0.0080		0
			270	0.0067		0
	Glucose-6-phosphate dehydrogenase	None		0.0046	increase in absorbancy/	
		NDGA	27	0.0046	10 sec.	0
		Propyl gallate	27	0.0046		0
Flavin containing enzyme	Amino acid oxidase	None		28	cu. mm. O_2/10 min.	
		α-Tocopherol	250	28		0
		Propyl gallate	250	23		18
		NDGA	250	2		93
Dehydrogenase linked to cytochrome system	Succinoxidase	None		42, 52	cu. mm. O_2/5 min.	
		α-Tocopherol	27	56		0
			270	42		0
		NDGA	270	42		0
		Propyl gallate	270	52		0
Catalyzes hydrogen electrode reaction	Hydrogenase	None		21	cu. mm. H_2/5 min.	
		α-Tocopherol	2.7	21		0
			27	21		0
		Propyl gallate	27	21		0
		NDGA	2.7	19		10
			27	16		24
Cyclophorase system	Cyclophorase	None		3.1	cu. mm. O_2/3 min.	
		α-Tocopherol	27	5.0		0
			270	3.7		0
		Propyl gallate	27	2.7		0
			270	2.0		~35
		NDGA	27	3.0		0
			270	1.0		~70
Hydrolytic enzymes	Alkaline phosphatase	None		0.0184	increase in absorbancy/	
		α-Tocopherol	270	0.0184	10 sec.	0
		NDGA	27	0.0184		0
		Propyl gallate	270	0.0155		16
	Pepsin	None		1.0	$E^{1\text{ cm.}}_{280\text{ m}\mu} \times 10^{-2}$ after 10 min.	
		α-Tocopherol	20	0.7		~30
		NDGA	2.0	1.0		0
			20	1.2		0
		Propyl gallate	2.0	0.8		~20
			20	1.0		0
	Urease	None		34.4	cu. mm. CO_2/5 min.	
		α-Tocopherol	270	24.7		28
		Propyl gallate	270	22.0		36
		NDGA	2.7	22.5		35
			27	20.2		41
			270	0.67		99

[a] Nordihydroguaiaretic acid.

inhibition is nonspecific. These two antioxidants possibly are general protein denaturants which react chemically with the enzymes as do many other phenolic and polyphenolic compounds.

Three enzyme systems, peroxidase, catalase, and ethyl alcohol dehydrogenase of the fourteen studied were inhibited by relatively low concentrations of the antioxidants. Peroxidase was strongly inhibited by nordihydroguaiaretic acid, propyl gallate, and α-tocopherol. Concentrations of $2.7 \times 10^{-5}M$ and $27 \times 10^{-5}M$ nordihydroguaiaretic acid appear to give the same inhibition because at $27 \times 10^{-5}M$ and above, nordihydroguaiaretic acid was oxidized instead of guaiacol and its oxidation product caused increased absorbancy at 430 mμ. The brown oxidation product of nordihydroguaiaretic acid (*24*) is easily distinguishable from the red-brown oxidation product of guaiacol. Catalase, a specific peroxidase which readily catalyzes the oxidation of many phenols and amines (*26*), was strongly inhibited by nordihydroguaiaretic acid and propyl gallate. Although the initial reaction rate of catalase was not inhibited by α-tocopherol, after 5 minutes the rate of oxygen evolution in the systems containing α-tocopherol decreased below that of the control. All three of the antioxidants gave more powerful inhibition than did hydroxylamine which is a potent inhibitor of the ethyl alcohol dehydrogenase from peas, *Aerobacter aerogenes*, liver, and yeast (*12*).

During the studies of the inhibition of ethyl alcohol dehydrogenase a remarkable and, as yet, unexplained phenomenon was observed. Ethyl alcohol–ethyl alcohol dehydrogenase–diphosphopyridine nucleotide systems made up in $0.1M$ phosphate buffer, pH 7.5, with distilled water were extremely sensitive to inhibition by antioxidants in low concentrations. For example, under these conditions $2.7 \times 10^{-6}M$ α-tocopherol gave 50% inhibition and $2.7 \times 10^{-7}M$ nordihydroguaiaretic acid gave 25% inhibition. If the distilled water was passed through a mixed cation-anion exchanger, or if ethylenediaminetetraacetic acid (Versene) or tris-(hydroxymethyl) aminomethane was added, the inhibition was much less.

The effect of antioxidants on ethyl alcohol dehydrogenase was partially explained by a study of the inactivation of this enzyme in phosphate buffer prepared with distilled water. In the absence of ethyl alcohol and diphosphopyridine nucleotide, ethyl alcohol dehydrogenase lost less than 10% of its activity in 50 seconds and 80% of its activity in 500 seconds. In the same phosphate buffer with added $3 \times 10^{-6}M$ α-tocopherol the enzyme was completely inactivated in 50 seconds. The addition of diphosphopyridine nucleotide to the system largely prevented this rapid inactivation by antioxidants permitting estimation of activity. The inactivation by antioxidants required the presence of contaminants of the water (probably heavy metals) for deionization, the addition of tris-(hydroxymethyl) aminomethane, ethylenediaminetetraacetic acid, or cysteine prevented the inactivation. The enzyme was quite stable in the presence of tris-(hydroxymethyl) aminomethane either with or without antioxidant.

The inhibitions of ascorbic acid oxidase, D-amino acid oxidase, and the cyclophorase system by the relatively large concentrations of 2 to $2.7 \times 10^{-3}M$ nordihydroguaiaretic acid and propyl gallate can best be ascribed to a general inhibitory action of these powerful antioxidants. The same effect was shown by urease and to some extent by alkaline phosphatase.

Discussion

The antioxidants inhibited peroxidase, catalase, and ethyl alcohol dehydrogenase at low concentration suggesting a specific inhibition. Because peroxidase can react with many easily oxidizable compounds, it is probable that the three antioxidants compete with the substrate, guaiacol, in its reaction with peroxidase–hydrogen peroxide compounds I and II (*2*). A similar explanation is applicable to the inhibition of catalase by propyl gallate and nordihydroguaiaretic acid. These antioxidants may react with the catalase–hydrogen peroxide compound I yet be only slowly oxidized. This reaction would prevent the more rapid reaction of compound I to form oxygen. α-Tocopherol did not depress the initial rate of oxygen evolution from hydrogen peroxide but gradually depressed the rate of reaction below that of the control. This suggests that the antioxidants may react with compound I transforming it into catalytically inactive compound II. Certain phenols are known to inactivate catalase by this mechanism (*6, 8*). A detailed kinetic analysis is necessary to establish either of these suggested mechanisms.

The inhibition of ethyl alcohol dehydrogenase by antioxidants is apparently complex. Ionic impurities in distilled water cause inactivation which is greatly accelerated by antioxidants, yet antioxidants alone do not cause appreciable inactivation. The protection from inhibition by compounds which react with heavy metals suggests that heavy metals cause the inactivation *per se* and that antioxidants sensitize the enzyme to inactivation. Because diphosphopyridine nucleotide stabilizes the enzyme from inactivation and because it is probable that thiol groups are responsible for the binding of diphosphopyridine nucleotide in both ethyl alcohol dehydrogenase (*28*) and glyceraldehyde phosphate dehydrogenase (*30*), it appears that the antioxidants sensitize the enzyme by making the thiol groups available for inactivation by heavy metals. Possible biological significance of the strong inhibition properties of α-tocopherol, which were noted here, indicates that the mechanism of antioxidant inhibition of ethyl alcohol dehydrogenase should be studied further.

It is difficult to visualize any such specific mode of inhibition of the widely different oxidative-reductive enzyme systems, ascorbic acid oxidase, D-amino acid oxidase, and cyclophorase system. The possibility that α-tocopherol, propyl gallate nordihydroguaiaretic acid inhibits urease by some specific mode is even more remote. Rather, it is more probable that the antioxidants can inhibit these four enzymes in a similar fashion as do many other phenolic compounds. A large number of substituted phenols and polyphenols are bactericidal presumably because they react chemically with sensitive enzyme systems and render them catalytically inactive. Among the enzymes studied, polyphenolase and succinoxidase were not inhibited by nordihydroguaiaretic acid or propyl gallate at $2.7 \times 10^{-3}M$. It is not unusual to find such differences in sensitivity toward general inactivating agents. Urease, on the other hand, is an example of an enzyme which can be readily inactivated by heavy metals, fluorides, halogens, borates, quinones, formaldehyde, hydrogen peroxide, and other compounds.

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FOOD COATINGS

Permeability of Acetostearin Products to Water Vapor

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The acetostearins are modified fats capable of being formed into waxlike films which are potentially useful as protective coatings. For this use they should be relatively impermeable to moisture. In the present investigation the permeability of such films was investigated using a modification of the standard cup method. Measurements were made at various temperatures and relative humidities using films of different compositions and thicknesses. From these data permeability constants were calculated. The permeability constants for these fat films were not independent of the thickness of the film or the concentration of water vapor. The results obtained were compared with those found in the literature for various plastic films. The permeability of acetostearin films to water vapor is less than that of cellulose acetate and slightly greater than the permeability of nylon, ethylcellulose, and polystyrene. Cellophane and paraffin wax have significantly smaller permeabilities.

ACETOSTEARIN PRODUCTS consisting essentially of di- and triglycerides containing one and two acetyl groups, respectively, solidify to unique waxlike solids (*3*). This unusual physical property has been shown to be associated with the alpha polymorphic form of the fats (*7, 12*). Spontaneous transformation to the nonwaxy form does not occur readily unless the product is a single compound of high purity. At room temperature and below, technical grade products will remain in the waxy form for several years.

The physical properties of the acetostearin products made them potentially valuable as protective coating materials. They are being evaluated for use as protective coatings for processed meats like frankfurters and for dressed meats that are to be stored at low temperatures, and as coatings for cheese, fruits, nuts, candy, and other food products.

Commercial utilization involving food products must await proof of edibility, which should be furnished by tests now under way. Other types of information are desirable as an aid to commercial utilization—detailed chemical and physical data on the products.

In the present investigation the permeability of a number of acetostearin products to water vapor was investigated in order to obtain an indication of the performance of these compounds as protective films against moisture transfer.

A survey of the literature on the permeability of various types of films to water vapor reveals that measurements have been made on a wide variety of polymeric materials, including polystyrene, polyvinyl chloride, cellulose acetate, nylon, rubber, glue, gelatin, hardened shellac, and polymerized linseed oil. The permeability of paraffin wax has also been determined by a number of investigators. However, it appears that no measurements have been made on films of fat or fatlike materials.

Materials and Methods

Materials Two series of acetostearin products were prepared and examined. Series A was made from a technical grade monostearin derived from almost completely hydrogenated cottonseed oil (iodine value, 1). The method of preparation and purification was such that this technical grade monostearin contained about 60% of monoglycerides, 35% of diglycerides, 5% of triglycerides, and no free glycerol (*2*).

The other series of acetostearin products, series B, was made from a commercial, molecularly distilled monostearin (Myverol 18:05) derived from triple-pressed stearic acid having an average molecular weight of 270.9. The distilled monostearin had an actual monoester content of 91.5%, a hydroxyl value of 332.7, an iodine value of 1, and a free glycerol content of about 1%.

Both monostearins were converted