constant m calculated from the data in Table II was found to be 2.724. Using this constant, viscosities can be calculated for any temperature with an accuracy of about 5 percent or better from the viscosity of the oil at a given temperature.

A comparison of the density and viscosity characteristics for cottonseed oils (15) and peanut oils shows striking similarities between oils of the same iodine value (Table IV). This agreement is not surprising in view of the known likeness in the composition of these two oils. It emphasizes the fact that certain physical properties of a vegetable oil may be correctly inferred from the same properties of another vegetable oil of similar chemical characteristics, a fact which may be of considerable help in designing processing equipment for an oil which has not been thoroughly studied.

TABLE IV

Comparison of Density and Viscosity Characteristics of Cottonseed Oil and Peanut Oil.

Characteristic	For cottonseed oil	For peanut oil
Density at 100° C.		
For oils having iodine value of 100	0.8675	0.8622
For oils having iodine value of 50	0.8570	0.8550
Temperature density coefficient $d_2=d_1-k(t_2-t_1)$ k	0.000638	0.000646
Mean coefficient of cubical expansion, 30°-200° C.	0.000764	0.000764
Viscosities (centipoises)		
For oils having iodine value of 100		
Viscosity at 50° C.	23.5	22.7
Viscosity at 100° C.	7.10	7.18
Viscosity at 200° C.	1.96	1.96
For oils having iodine value of 60		
Viscosity at 50° C.	29.5	27.8
Viscosity at 100° C.	8.05	8.26
Viscosity at 200° C.	2.03	2.07
Walther constant m (Relating temperature and viscosity)	2.805	2.724

Acknowledgment

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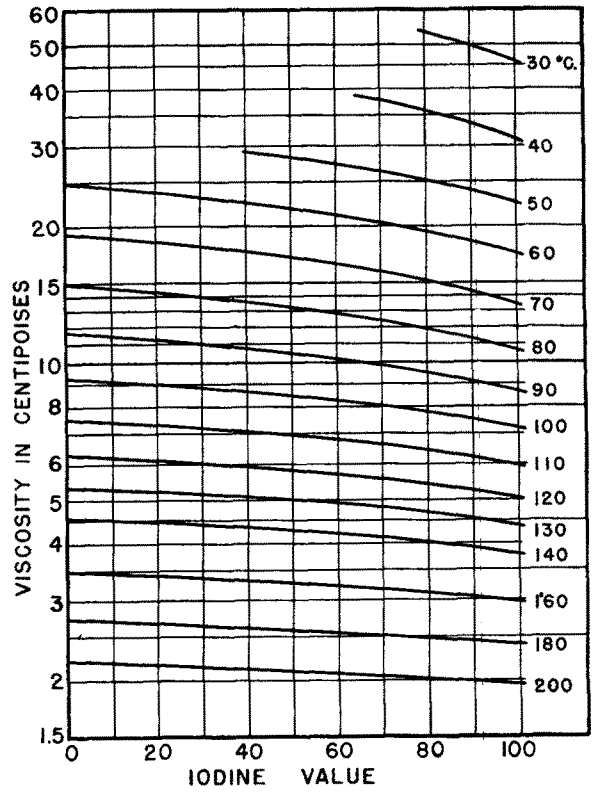

FIG. 2. Viscosities of hydrogenated peanut oils.

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An Application of the Barcroft-Warburg Apparatus to the Study of Antioxidants in Fats*

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FOR some years the Research Laboratory of the American Meat Institute has been investigating the keeping quality of lards with and without the addition of various antioxidants that have been submitted for examination. The active oxygen method (AOM) (1) was used extensively. More recently tests have been made also with the Barcroft-Warburg constant volume manometric apparatus as described by Johnston and Frey (3).

This paper gives the results of studies by the two methods on the effect of d-isoascorbyl esters of fatty acids** alone and in combination with soybean lecithin on the keeping time of lard. When 0.01 to 0.10 per cent of ascorbyl esters were added to lard. the keeping times as determined by the Barcroft-

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Warburg apparatus were in much better agreement with storage tests at room temperature than those obtained by the AOM.

Riemenschneider, Turner, Wells and Ault (5) have recently studied the effect of fatty acid monoesters of l-ascorbic and d-isoascorbic acids as antioxidants for fats and oils, using the AOM and the oven test at 100° C.

Methods

1. The active oxygen method (AOM), as described by King, Roschen and Irwin (1) was used.

2. Accelerated oxidation with the Barcroft-Warburg constant volume manometric apparatus. This apparatus was described by Dixon (2), Johnston and Frey (3), Perkins (4) and others. In this study the method as outlined by Johnston and Frey was followed with minor modifications.

To minimize the effect of temperature it was intended to carry out the experiments at 50° C. with air as the source of oxygen. This plan was abandoned as too time-consuming and a temperature of 70° C. was used throughout with tank oxygen. A sample of 850-900 mg. of lard was used and the shaking rate was adjusted to 60 oscillations per minute to avoid splashing.

To observe the rate of oxygen absorption, readings of the manometer were made at intervals of 30 to 60 minutes from the start of absorption as indicated by the manometer. The oxygen absorption was allowed to proceed only far enough to form a peroxide content of 20 to 30 milliequivalents in 1000 g. of lard. With a 850 to 900 mg. sample a 25 to 35 mm. negative pressure, depending on the condition of the lard at the start of the experiment, was usually sufficient. After oxidation the sample was transferred to a 250 ml. Erlenmeyer flask with the help of 30 ml. of a mixture of glacial acetic acid and chloroform in 5 or 6 portions. The peroxide content was determined in the usual manner with 0.01 N. sodium thiosulphate.

The Barcroft-Warburg technique at 70° C. with oxygen requires, on the average, a period five times as long for a lard to reach a peroxide content of approximately 20 milliequivalents per 1000 g. as for the active oxygen method. With this latter method, organoleptic detection of rancidity in lard begins at approximately 20 milliequivalents peroxide per 1000 g. Organoleptic observation is not possible with the Barcroft-Warburg apparatus without disrupting the experiment and on account of disguising odor developed in the stopcock lubricants during the oxidation.

3. The storage tests were carried out by placing 10 g. samples of the lard in 4 oz. mayonnaise jars with screw cap and storing them at room temperature with the exclusion of light. At intervals, samples were removed and tested for peroxide content and organoleptically for rancidity.

Results

Effect of D-Isoascorbyl Fatty Acid Esters When Used Alone in Lard. When the d-isoascorbyl fatty acid esters were used in a concentration of 0.01% in a lard of eight hours keeping quality, (A.O.M.), only slight antioxidative effect was observed with the Barcroft-Warburg technique. An increase of the d-isoascorbyl ester content to 0.1% sharply decreased the keeping quality far below that of the original lard (Table 1), but with the active oxygen method 0.01% of added ester increased the keeping quality.

TABLE 1

The effect of d-isoascorbyl palmitate on the keeping quality of lard.

Per cent d-iso-ascorbyl palmitate added	Barcroft-Warburg method using oxygen at 70° C.		Active oxygen method using air at 98° C.	
	Keeping quality in hours	Difference in hours	Keeping quality in hours	Difference in hours
0.00	35	....	8	....
0.01	42	+7	....	....
0.05	7	−28	....	....
0.10	6	−29	40	+32

To study the effect of the concentration of the d-isoascorbyl fatty esters in the lard, ten samples of the same lard, containing 0.01 to 0.10% d-isoascorbyl monostearate, were made up and examined in the Barcroft-Warburg apparatus. A very marked lowering of the keeping quality resulted with increasing concentration of the d-isoascorbyl ester (Fig. 1).

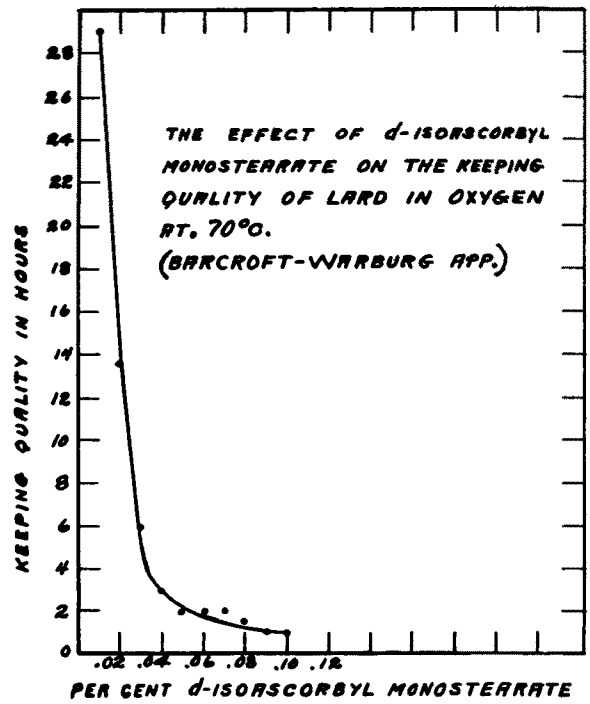

FIG. 1.

By plotting the keeping quality in hours against the concentration of the d-isoascorbyl monostearate, a steeply dropping curve was obtained, the upper part of which could be plotted roughly by multiplying the keeping quality of the lard by two, for each subsequent 0.01% decrease in the d-isoascorbyl ester content.

A number of determinations were made on lard containing 0.01, 0.005, and 0.001% d-isoascorbyl esters. In most cases an increase in the keeping quality was noted with the decrease in d-isoascorbyl ester content below 0.01%, when determinations were made in the Barcroft-Warburg apparatus, but little change or decrease with the active oxygen method (Tables 2, 3).

Combined Effect of d-Isoascorbyl Fatty Acid Esters and Lecithin in Lard. Lecithin in the amount of 0.05% in every case improved the keeping quality, both with the Barcroft-Warburg apparatus and the active oxygen method. The addition of 0.01% or 0.005% of d-isoascorbyl palmitate to the lard already containing 0.05% lecithin increased the keeping qual-

TABLE 2

The effect of d-isoascorbyl palmitate on the keeping quality of lard.

Per cent d-iso-ascorbyl palmitate added	Barcroft-Warburg method using oxygen at 70° C.		Active oxygen method using air at 98° C.	
	Keeping quality in hours	Difference in hours	Keeping quality in hours	Difference in hours
0.000	84	....	14	....
0.001	92	+ 8	17	+ 3
0.005	79	—5	21	+ 7
0.010	68	—16	22	+ 8
0.050	10	—74	24	+10
0.100	4	—80	28	+14

TABLE 3

The effect of d-isoascorbyl monostearate on the keeping quality of lard.

Per cent d-iso-ascorbyl added	Barcroft-Warburg method using oxygen at 70° C.		Active oxygen method using air at 98° C.	
	Keeping quality in hours	Difference in hours	Keeping quality in hours	Difference in hours
0.000	86	....	14	....
0.001	95	+ 9	16	+ 2
0.005	91	+ 5	20	+ 6
0.010	76	—10	20	+ 6
0.050	10	—76	23	+ 9
0.100	3	—83	32	+18

ity still further when the determination was made by both the Barcroft-Warburg apparatus and the active oxygen method (Table 4), but with the active oxygen method the combined effect of lecithin and d-isoascorbyl ester is more pronounced. In some cases pronounced synergistic effect was observed in the Barcroft-Warburg apparatus despite the fact that the d-isoascorbyl ester when used alone decreased the keeping quality of the same lard.

In order to compare the data obtained by the Barcroft-Warburg apparatus and the active oxygen method with storage tests at room temperature, a number of samples containing d-isoascorbyl esters and combinations of them with lecithin were stored in 4 oz. mayonnaise jars in the dark (Tables 5, 6, 7).

When used alone 0.01 per cent of either of the d-isoascorbyl esters retarded the development of rancidity slightly in storage while 0.10 per cent promoted it. The use of 0.05 per cent of either d-isoascorbyl ester with 0.05 per cent lecithin decreased the keeping time of the lard compared with the samples to which only lecithin was added. Under these conditions the data obtained with the Barcroft-Warburg apparatus are in much better agreement with the storage tests than are those obtained with the active oxygen method (Table 8). When no antioxidant is added to the lard or when lecithin alone is added the

TABLE 4

The combined effect of lecithin (L) and d-isoascorbyl palmitate (P) on the keeping quality of lard.

Samples	Barcroft-Warburg method using oxygen at 70° C.		Active oxygen method using air at 98° C.	
	Keeping quality in hours	Difference in hours	Keeping quality in hours	Difference in hours
Control	84	....	14	....
Control + 0.05% L	99	+15	27	+13
Control + 0.001% P	92	+ 8	17	+ 3
Control + 0.05% L. +0.001% P	91	+ 7	27	+13
Control + 0.005% P	79	— 5	21	+ 7
Control + 0.05% L. + 0.005% P	106	+22	33	+19
Control + 0.01% P	68	—16	22	+ 8
Control + 0.05% L. + 0.01% P	133	+49	45	+31

results of both the Barcroft-Warburg and active oxygen methods are in good agreement with the storage tests.

The fact that results obtained by the active oxygen method on lard to which d-isoascorbyl fatty acid esters have been added differ widely from the storage

TABLE 5

Effect of lecithin, d-isoascorbyl palmitate, and d-isoascorbyl monostearate on the keeping quality of lard stored at room temperature.

Sample	Accelerated stability, hours		Peroxide values in milliequivalents after aging at room temperature					
	Active oxygen method	Barcroft-Warburg method	40 days	61 days	82 days	103 days	138 days	159 days
Control	3	12	5	7	8	rancid	rancid	rancid
Control + 0.05% L	5	20	4	5	6	8	17	rancid
Control + 0.10% P	2	1	rancid	rancid	rancid	rancid	rancid	rancid
Control + 0.01% P	2	14	7	9	12	17	rancid	rancid
Control + 0.05% L. + 0.05% P	20	3	13	rancid	rancid	rancid	rancid	rancid
Control + 0.025% L + 0.01% P	8	12	5	6	8	10	rancid	rancid
Control + 0.10% S	2	1	rancid	rancid	rancid	rancid	rancid	rancid
Control + 0.01% S	3	14	7	10	13	20	rancid	rancid
Control + 0.05% L. + 0.05% S	20	4	8	12	23	rancid	rancid	rancid
Control + 0.025% L. + 0.01% S	8	15	5	6	8	8	rancid	rancid

L = lecithin. P = palmitate. S = stearate.

TABLE 6

Effect of lecithin, d-isoascorbyl palmitate, and d-isoascorbyl monostearate on the keeping quality of lard stored at room temperature

Sample	Accelerated stability, hours		Peroxide values in milliequivalents after aging at room temperature						
	Active oxygen method	Barcroft-Warburg method	50 days	71 days	92 days	113 days	148 days	168 days	212 days
Control	4	20	4	6	9	18	rancid	rancid	rancid
Control + 0.05% L	9	40	3	4	4	6	9	11	rancid
Control + 0.10% P	9	1	rancid	rancid	rancid	rancid	rancid	rancid	rancid
Control + 0.05% L. + 0.05% P	47	19	3	4	7	10	rancid	rancid	rancid
Control + 0.10% S	9	1	rancid	rancid	rancid	rancid	rancid	rancid	rancid
Control + 0.05% L. + 0.05% S	38	22	3	4	6	10	rancid	rancid	rancid

L = lecithin. P = palmitate. S = stearate.

TABLE 7

Effect of lecithin, d-isoascorbyl palmitate, and d-isoascorbyl monostearate on the keeping quality of lard stored at room temperature.

Sample	Accelerated stability, hours		Peroxide values in milliequivalents per 1000 g. after aging at room temperature							
	Active oxygen method	Barcroft-Warburg apparatus	46 days	88 days	109 days	144 days	165 days	208 days	244 days	280 days
Control	9	44	2	4	6	5	11	rancid	rancid	rancid
Control + 0.05% L.	11	55	1	3	3	6	....	....	13	rancid
Control + 0.10% P.	13	2	1	4	6	rancid	rancid	rancid	rancid	rancid
Control + 0.10% S.	13	1	1	4	5	rancid	rancid	rancid	rancid	rancid
Control + 0.05% L. + 0.05% P.	104	44	1	4	8	23	rancid	rancid	rancid	rancid
Control + 0.05% L. + 0.05% S.	88	45	1	3	9	8	11	rancid	rancid	rancid

L = lecithin. P = palmitate. S = stearate.

data emphasizes the need for more work to correlate the existing accelerated methods with actual storage tests.

Summary

THE results of the active oxygen method and those obtained with the Barcroft-Warburg apparatus at 70° C. are in good agreement with storage tests at room temperature on lard to which no antioxidant is added and on lard to which lecithin alone is added. With lard to which d-isoascorbyl palmitate or d-isoascorbyl monostearate, in concentrations between 0.01 and 0.10 per cent, is added the results with the Barcroft-Warburg apparatus are in much better agreement with storage tests than are those of the active oxygen method.

The d-isoascorbyl esters in 0.01 to 0.10 per cent in lard usually behave as antioxidants with the active oxygen method and as pro- or antioxidants, depending on their concentration, with the Barcroft-Warburg apparatus and during storage at room temperature.

This study demonstrates that in testing new compounds for antioxidant properties conclusions should not be drawn from results obtained when the experimental conditions of the test are very different from the conditions under which the antioxidant is to be used. It emphasizes the importance of correlating the results of accelerated tests with storage tests.

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TABLE 8

Comparison of protection factors calculated from data in Tables 5 to 7.

Sample	Keeping quality			Protection factors		
	Storage test	Active oxygen method	B-W apparatus	Storage test	Active oxygen method	B-W apparatus
	days	*hours*	*hours*			
Control	103	3	12	1.00	1.00	1.00
Control + 0.1% + d-isoascorbyl P	40	2	1	0.39	0.67	0.08
Control + 0.01% d-isoascorbyl P	138	2	14	1.34	0.67	1.14
Control + 0.05% lecithin + 0.05% d-isoascorbyl P	61	20	3	0.59	6.67	0.25
Control + 0.025 lecithin + 0.01% d-isoascorbyl P	138	8	12	1.34	2.67	1.00
Control + 0.1% d-isoascorbyl S	40	2	1	0.39	0.67	0.08
Control + 0.01% d-isoascorbyl S	138	3	14	1.34	1.00	1.17
Control + 0.05% lecithin + 0.05% d-isoascorbyl S	103	20	4	1.00	6.67	0.33
Control + 0.025% lecithin + 0.01% d-isoascorbyl S	138	8	15	1.34	2.67	1.25
Control	148	4	20	1.00	1.00	1.00
Control + 0.1% d-isoascorbyl P	50	9	1	0.34	2.25	0.05
Control + 0.05% lecithin + 0.05% d-isoascorbyl P	148	47	19	1.00	11.75	0.95
Control + 0.1% d-isoascorbyl S	50	9	1	0.34	2.25	0.05
Control + 0.05% lecithin + 0.05% d-isoascorbyl S	148	38	22	1.00	9.50	1.10
Control	208	9	44	1.00	1.00	1.00
Control + 0.1% d-isoascorbyl P	144	13	2	0.69	1.45	0.05
Control + 0.1% d-isoascorbyl S	144	13	1	0.69	1.45	0.03
Control + 0.05% lecithin + 0.05% d-isoascorbyl P	165	104	44	0.79	11.55	1.00
Control + 0.05% lecithin + 0.05% d-isoascorbyl S	208	88	45	1.00	9.78	1.02
Control	103	3	12	1.00	1.00	1.00
Control + 0.05% lecithin	159	5	20	1.54	1.67	1.67
Control	148	4	20	1.00	1.00	1.00
Control + 0.05% lecithin	212	9	40	1.43	2.25	2.00
Control	208	9	44	1.00	1.00	1.00
Control + 0.05% lecithin	280	11	55	1.34	1.22	1.25

P = palmitate. S = stearate.