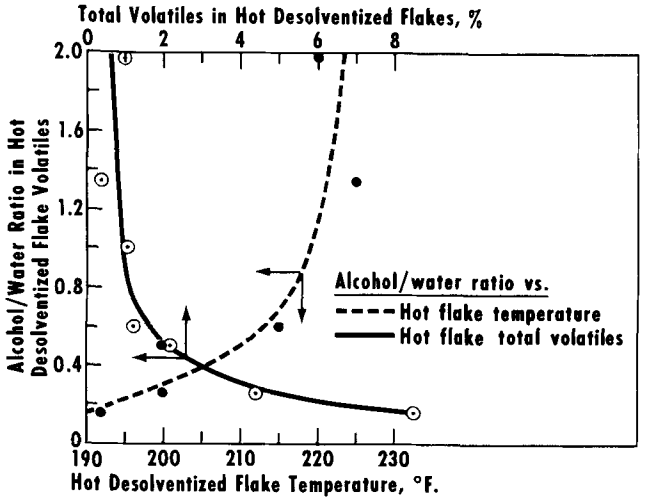

FIG. 5. Alcohol/water ratio in hot desolventized flake volatiles; 50% methanol system.

desolventizing, the protein was further denatured to an NSI range of 7–16.

The products obtained with the methanol-wash had a better flavor than those washed with either ethanol or isopropyl alcohol. The lower level of taste acceptance for these two alcohols was due partly to some residual taste of solvent. Organoleptic evaluation detected the alcohol even though the chemical analysis for it was relatively low.

Water absorption values of 328 to 410 were obtained for the desolventized products. Absorption values were significantly highest with the 50% methanol-wash. A wide variation in data was obtained for the remaining wash systems. Table III shows the average absorption value obtained for each system with the 95% confidence limits calculated for all the samples analyzed.

TABLE III

Water Absorption of Desolventized Flakes for Alcoholic Wash Systems

Alcohol	Alcohol conc., %	Water absorption	
		Average value [a]	95% Confidence limit
Methanol	50	379	10.3
Ethanol	50	352	15.7
Isopropyl alcohol	50	341	10.3
Methanol	70	358	35.3
Ethanol	70	341	6.7
Isopropyl alcohol	70	342	10.2

[a] Basis—grams water absorbed per 100 g solids.

Conclusions

The flash vapor-type desolventizer can handle soybean flakes washed with aqueous alcohols of rather high water content. Two-stage operation, as well as a less concentrated alcohol, is effective in minimizing residual alcohol in the end product. Steam injection during the second stage gives further reduction in residual alcohol. A small amount of alcohol is strongly bound to the solids and is difficult to remove even under conditions of high-temperature desolventizing.

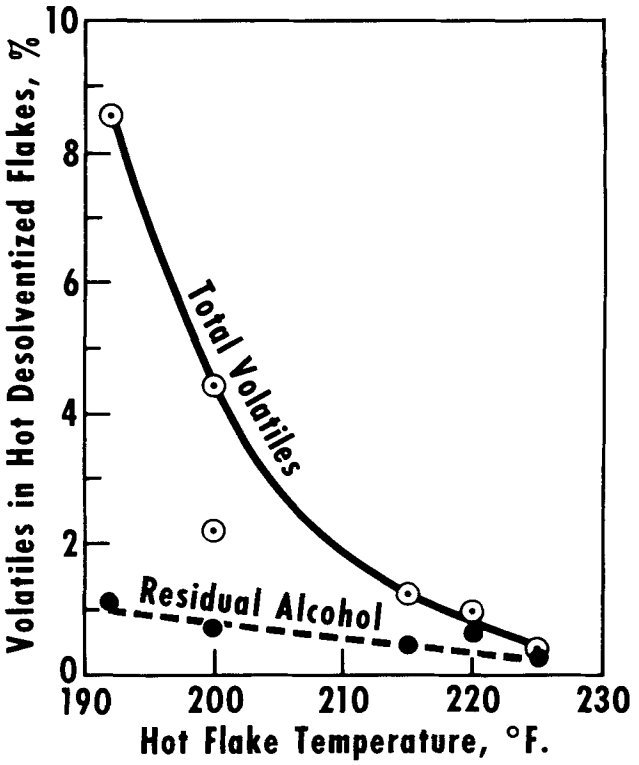

FIG. 6. Variation of hot desolventized flake total volatiles and alcohol content with temperature; 50% methanol system.

Important advantages of the flash desolventizer are: 1) Low product holdup, 2) high temperature with short retention time. 3) a system capable of rapid changes, and 4) a granular, free flowing product with minimum color degradation and maximum water absorption.

Acknowledgment

The authors express their appreciation to J. R. Dilley and F. C. Wohlrabe for mechanical design and installation of the new superheater and U-tube extension leg units on the original flash desolventizer; L. T. Black, A. Clark, and D. E. Uhl for analytical determinations; and J. N. Boyd, Biometrical Services, USDA, for statistical advice.

REFERENCES

1. Beckel, A. C., P. A. Belter, and A. K. Smith, Soybean Dig., *10*, 17–18 (1949).
2. Belter, P. A., O. L. Brekke, G. F. Walther, and A. K. Smith, JAOCS, *31*, 401–403 (1954).
3. Brekke, O. L., G. C. Mustakas, M. C. Raether, and E. L. Griffin, *Ibid.*, *36*, 256–260 (1959).
4. Mustakas, G. C., L. D. Kirk, and E. L. Griffin, *Ibid.*, *38*, 473–479 (1961).
5. Black, L. T., L. D. Kirk, and G. C. Mustakas, *Ibid.*, *38*, 484–485 (1961).
6. Smith, A. K., and S. J. Circle, Ind. Eng. Chem., *30*, 1414–1418 (1938).

[Received November 28, 1961]

A Comparison of Several Analytical Techniques for Prediction of Relative Stability of Fats and Oils to Oxidation

W. D. POHLE, R. L. GREGORY, and J. R. TAYLOR, Research Laboratories, Swift & Company, Chicago, Illinois

Abstract

Three analytical methods proposed by various workers for predicting the relative stability of fats and oils to oxidation have been compared on a series of samples. The Eckey Oxygen Absorption, the modification of the A.S.T.M. Oxy-

gen Bomb (4), and the Active Oxygen Method (AOM) were applied to combinations of animal and vegetable fats and oils, with and without added monoglycerides and antioxidants. The results indicate that the Eckey Oxygen Absorption and modified ASTM Oxygen Bomb methods are more precise than the AOM method, and more in keeping with the experience in the fat and oil field in relation to actual performance stability than the AOM. Experimental relationships of these factors and methods to shelf life storage studies measured organoleptically will be the subject of a later paper.

Introduction

THE ESTIMATION of the stability of fats with respect to oxidation has been investigated by many workers. The currently accepted method for this purpose is the Active Oxygen Method (1) which was developed by King, Roschen, and Irwin (2) in 1933. This method is now considered to be inadequate for some present commercial considerations, particularly in regard to the effect of additives on actual fat stability as compared to predicted stability by the AOM procedure.

In 1946, Eckey (4) proposed an oxygen absorption method, and in 1957 Gearhart, Stuckey, and Austin (4) proposed a modification of the ASTM Bomb Method (5) for evaluating the stability of fats. Both of these methods are based on the direct absorption of oxygen by the fat, but the selected conditions of measurement differ.

The conditions employed in the three methods are as follows:

1) The AOM involves bubbling 2.33 cc of purified air per second through 20 ml of oil at 97.8C until a peroxide value of 100 meq per kg of fat is attained.

2) The Eckey Oxygen Absorption involves suspending one g of oil on 12.5 g of sand in a closed vessel containing air at atmospheric pressure and heating at 80C until a pressure drop of 40 mm of mercury is attained.

3) The modified ASTM Oxygen Bomb involves placing 6 g of oil on inert tissue dispersant in a sealed bomb at 50 psi oxygen pressure and maintaining at 100C until a pressure drop of 2 psi per hr is attained.

All determinations are reported as the number of hours required to reach the selected endpoint.

Experimental

A comparison of the AOM and Eckey Oxygen Absorption methods was undertaken in our laboratory before the bomb method was proposed. This comparison was made using two samples each of crystal modified lard, lard, and hydrogenated vegetable oil. Treatments employed included 0 and 5% of monoglycerides and 0.000, 0.005, and 0.010% of three different antioxidant mixtures. The antioxidants employed were: 1) Propyl gallate (PG). 2) Propyl gallate (PG) plus butylated hydroxy anisole (BHA). 3) Butylated hydroxy anisole (BHA) plus butylated hydroxy toluene (BHT). See Table I.

The data from this complete factorial experiment have been subjected to statistical analyses. The following conclusions were indicated and are demonstrated graphically.

Crystal Modified Lard

As shown in Figure 1 the addition of 5% monoglycerides significantly lowered the oxygen absorption values and significantly increased the AOM values. Increasing the level of the stabilizers increased both the oxygen absorption and AOM values, but the increase was not the same for all the antioxidant mixtures. As indicated by the AOM, the BHA + PG

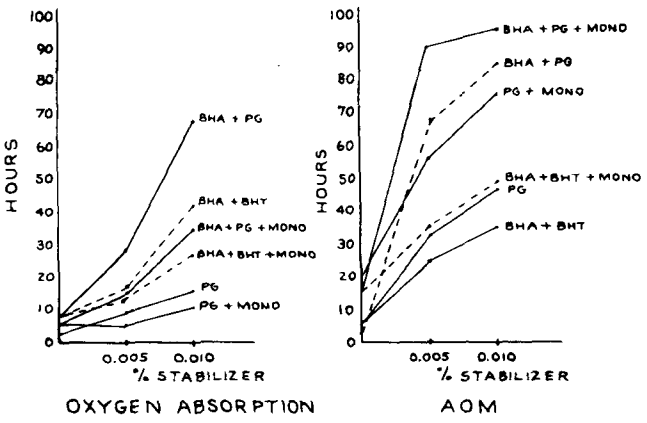

FIG. 1. Crystal modified lard.

TABLE I

Summary of Data Oxygen Absorption and AOM Analyses

Antioxidant		Hour for test											
		BHA + BHT 1 + 1				BHA + PG 1 + 1				PG			
Method		Ox A		AOM		Ox A		AOM		Ox A		AOM	
Product	% of Anti-oxidant	Sample		Sample		Sample		Sample		Sample		Sample	
		1	2	1	2	1	2	1	2	1	2	1	2
Hydrogenated veg. oil	0.000	69	75	61	77	69	75	68	87	70	75	64	87
	0.005	91	86	85	93	90	89	91	100	74	76	100	121
	0.010	98	102	82	83	126	118	136	130	88	85	123	136
Hydrogenated veg. oil + 5%monoglyc.	0.000	23	26	68	71	22	22	69	69	23	24	68	70
	0.005	32	36	86	84	32	32	110	91	27	26	95	101
	0.010	33	39	83	86	50	45	144	152	31	38	117	130
Lard	0.000	3.8	3.0	7	3	4.0	3.1	8	4	3.7	2.9	9	4
	0.005	20	19	40	30	30	9	100	44	10	5.2	60	43
	0.010	45	42	70	50	72	24	130	49	22	12	95	7.8
Lard + 5% monoglyc.	0.000	5.1	4.9	25	43	5.0	4.2	26	20	4.8	3.8	26	32
	0.005	9	12	50	40	17	4.8	111	52	10	4.8	98	65
	0.010	18	22	68	54	34	13	128	85	17	10	122	80
Modified lard	0.000	4.6	4.7	10	2	4.2	3.8	6	3	4.3	3.6	8	3
	0.005	17	17	27	20	34	26	51	78	8.3	6.4	33	30
	0.010	44	42	37	30	69	66	75	93	12	15	50	47
Modified lard + 5% monoglyc.	0.000	5.7	5.3	13	17	6.3	5.0	15	18	6.0	5.6	14	20
	0.005	13	12	30	38	13	15	75	105	6.3	6.0	55	55
	0.010	24	26	35	63	28	49	88	106	10	13	77	77

mixture showed the greatest effect of increasing the level and also gave the highest values; whereas, the BHA + BHT mixture showed the least effect of level increases and gave the lowest values. Propyl gallate was intermediate in effect. The oxygen absorption method also showed the BHA + PG treated fat to be the most affected by level changes, the BHA + BHT treated fat was intermediate, and the propyl gallate treated fat was least affected by changes in level and gave the lowest values.

Lard

Figure 2 reveals that the same general trends evidenced in the crystal modified lard samples were observed with the lard samples, except that the AOM did not indicate any significant difference between propyl gallate and the BHA + PG mixture.

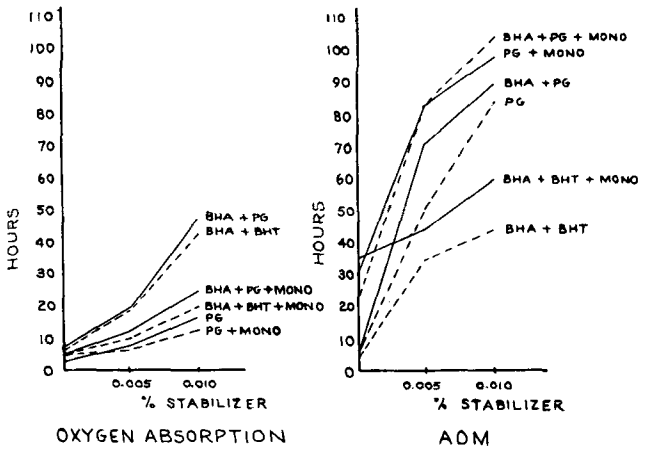

FIG. 2. Lard.

Hydrogenated Vegetable Oil

Figure 3 illustrates several factors. The addition of 5% monoglycerides very significantly lowered the oxygen absorption values, more markedly than in the other two fats. However, the AOM method did not indicate any significant effect of adding monoglycerides. With added monoglycerides there were no significant differences among the oxygen absorption values for the three stabilizer mixtures. Without monoglycerides, however, the oxygen absorption differences between the stabilizers were the same as evidenced in the other fats—propyl gallate lowest and BHA + PG highest.

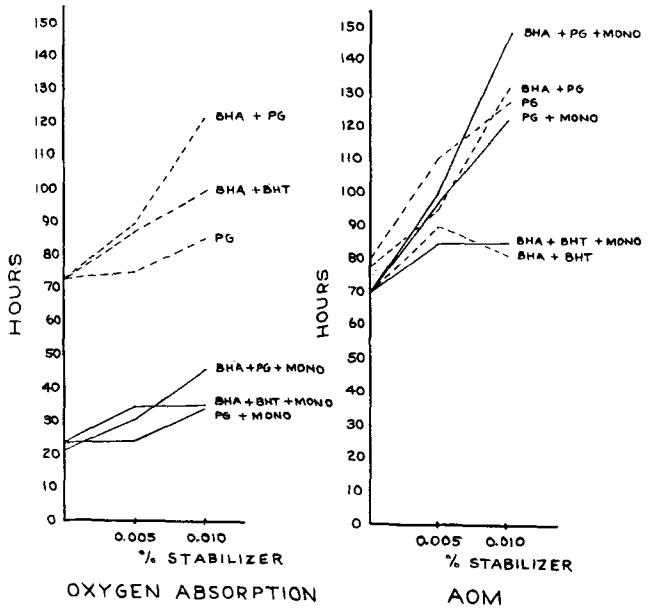

FIG. 3. Hydrogenated vegetable oil.

Increasing the level of stabilizer increased both oxygen absorption and AOM values and at different rates for different mixtures. The oxygen absorption values indicated that increasing the level of propyl gallate had less effect than increasing the level of the other two mixtures. However, the AOM values indicated that increasing the level of propyl gallate or BHA + PG had a greater effect than increasing the level of the BHA + BHT mixture.

The precision of the oxygen absorption method was significantly better than the AOM method as shown in Table II.

TABLE II

Precision Estimates of Oxygen Absorption and AOM Methods

	Oxygen absorption	AOM
Standard deviation	0.89	5.6
Mean	18.85	29.7
Coefficient of variability	4.7 %	18.9%

This estimate was based on duplicate analyses of twelve samples which contained no stabilizers.

Another estimate of analytical variability, which can be expected to be larger than estimates from known duplicate analyses, results from a replication variability based on the failure of the various treatment effects to have been the same on the two samples of the fats. These estimates are contained in Table III.

TABLE III

Additional Precision Estimates of Oxygen Absorption and AOM Methods

	Oxygen absorption	AOM
Standard deviation	5.6	12.8
Mean	37.8	63.4
Coefficient of variability	14.8%	20.2%

The precision of the oxygen absorption method was superior to the AOM by either criterion.

Overall, the addition of monoglycerides increases the AOM values and decreases the oxygen absorption values. The AOM data suggest that propyl gallate is a better stabilizer than BHA + BHT while the oxygen absorption data contradict this view. The effects indicated by the oxygen absorption findings appear more realistic, when related to practical experience in the commercial fat and oil field, and further substantiate the prior research of Gearhart and Stuckey (6).

For control purposes it may, at times, be desirable to reduce the time required for evaluating the more stable fats if the anticipated loss of precision can be tolerated. Accordingly, the effect of temperature on elapsed method time was studied on a series of fats at 100 and 110C. The results are shown in Table IV.

As might be expected the increase in temperature

TABLE IV

The Effect of Temperature Change on the Eckey Oxygen Absorption Method

Shortenings	AOM, hr	Oxygen absorption, hr			
		100C		110C	
Lard	3	1.4	1.5	0.8	0.7
Stabilized lard	28	11.0	11.6	5.4	5.9
Stabilized lard base	42	22.6	21.2	10.6	9.7
Tallow base	55	27.5	27.6	13.5	12.7
Hydrogenated veg. oil	60	30.2	28.1	14.8	15.9
Hydrogenated veg. oil	135	40.2	43.3	21.0	23.1
Means	.....	22.1		11.2	
Standard deviation	.....	1.1		0.8	
Coefficient of variability	.....	5.0%		7.1%	

TABLE V
Summary of Data for AOM Oxygen Absorption and Bomb Methods

Type of fat	AOM		Oxygen absorption at 100C. hr		Bomb at 100C and 50 lb pressure		Bomb at 100C and 100 lb pressure	
Meat fat	1	1	1.3	1.3	1.5	1.5	.....	.....
Meat fat	1	1	1.4	1.3	1.7	1.4	.....	.....
Meat fat	38	45	16.3	17.7	13.3	16.1	14.2	15.8
Meat fat	26	30	11.5	11.6	14.1	14.3	13.7	14.7
Meat fat	40	40	4.8	4.8	12.2	11.2	11.7	13.1
Meat fat	50	40	5.1	5.1	12.2	11.9	13.2	11.7
Meat fat	47	53	25.4	25.5	23.8	22.9	20.8	23.1
Meat fat	87	87	25.8	26.4	28.4	32.1	23.8	25.8
Meat-vegetable fat	48	40	13.6	11.8	20.7	17.8	18.3	19.2
Meat fat	....[a]	....[a]	16.3	17.6	15.7	15.7	13.2	13.1
Meat-vegetable fat	145	150	15.9	14.1	38.0	39.2	35.5	32.2
Vegetable	55	50	23.4	23.0	19.7	21.7	17.3	19.7
Vegetable	57	55	16.9	17.9	13.7	15.1	14.9	14.1
Vegetable	108	106	22.5	22.6	29.2	24.7	23.8	25.7
Vegetable	135	140	41.0	44.5	40.4	39.4	35.7	39.1
Meat-vegetable fat	96	100	31.6	31.5	44.9	39.6	34.9	34.4
Meat-vegetable fat	10	14	15.6	15.1	14.2	14.3	11.2	12.6
Meat-vegetable fat	100	95	27.1	27.6	31.3	27.9	26.5	26.8
Meat-vegetable fat	106	100	29.9	33.8	32.2	32.4	27.3	29.6

Because of great activity the first two samples were not tested at 100 lb pressure but at 25 lb pressure. End point came at 1.6 and 1.7 hr.
[a] Foamed out of tube.

of 10C results in about one-half reaction time with a satisfactory precision.

The foregoing investigations were undertaken prior to the proposal of the modified ASTM Bomb method (4). Since that time, a series of commercially available fats and shortenings have been compared by the AOM, Eckey Oxygen Absorption at 100C and Modified ASTM Oxygen Bomb procedures. The data are contained in Table V.

The following conclusions are possible from interpretation of these data. There was a high degree of correlation between the oxygen absorption methods (Eckey and Bomb) which might be expected, since the principles of measurement are similar. A marked increase in oxygen pressure resulted in only a slight decrease in the time required for making bomb determinations. No single, all inclusive, correlation was obtained between the AOM and either of the oxygen absorption methods. This might be anticipated since the end points of the methods are based on different phenomena.

While it is believed from our knowledge of the stability of the shortening used in this study that oxygen absorption may serve as a better index of actual shelf stability than AOM, this cannot be established without carefully conducted storage studies. These should be carried out in conjunction with evaluation of chemical methods and organoleptic panel findings on a variety of fats and oils. A research project in this area is now in progress at our laboratory and is expected to provide answers to many of the questions raised here.

REFERENCES

1. Official and Tentative Methods of the AOCS, Active Oxygen Method Cd 12-57.
2. King, A. E., H. L. Roschen, and W. H. Irwin, Oil and Soap, *10*, 105 (1933).
3. Eckey, E. W., Oil and Soap, *23*, 38 (1946).
4. Gearhart, W. M., B. N. Stuckey, and J. J. Austin, JAOCS, *34*, 427 (1957).
5. A.S.T.M. Standards, 1958, Part 7, Petroleum Products and Lubricants, Method D525-55 p. 268.
6. Gearhart, W. M., and B. N. Stuckey. JAOCS, *32*, 386 (1955).

[Received November 3, 1961]

Effect of Diet and Encephalomalacia on the Fatty Acid Composition of the Brain of Young and Old Chickens[1]

L. J. MACHLIN, G. J. MARCO, and R. S. GORDON, Monsanto Chemical Company, St. Louis, Missouri

Abstract

Encephalomalacia was induced in chickens more than 64 days old by feeding a high linoleic acid diet with an antioxidant (ethoxyquin) for 64 days and then deleting the antioxidant.

The cerebella of young chickens fed linoleic acid contained greater proportions of linoleic acid, arachidonic acid, and fatty acids with retention times corresponding to C-22 polyunsaturated fatty acids than chickens fed low linoleic acid diets. The cerebella of chickens with encephalomalacia were higher in linoleic acid and an unknown acid than the cerebella from control chickens fed antioxidant. Other fatty acids were not significantly affected by the disease.

The cerebella of hens fed a high linoleic acid diet for 12 weeks, starting from 500 days old, contained a higher proportion of linoleic acid and C-20 triene than hens fed a low linoleic acid diet. In contrast to chicks, the % of arachidonic acid or fatty acids with retention times greater than arachidonic were not affected by diet.

[1] Presented at the Spring Meeting, American Oil Chemists' Society, t. Louis, Mo., May 1–3, 1961.

Introduction

ENCEPHALOMALACIA is a disease characterized by cerebellar degeneration. It occurs in young chickens fed diets low in biologically active antioxidants and high in linoleic acid (1). The brains of chickens fed linoloeic acid contain higher proportions of linoleic acid and arachidonic acid than brains of chickens fed low-linoleic acid diets (2,3,4). It has been suggested (1) that the initial cause of encephalomalacia is peroxidation of fatty acids of the linoleic acid family [i.e., fatty acids have the structure $CH_3(CH_2)_4$-$(CH{=}CH{-}CH_2)_{2\text{-}6}(CH_2)_xCOOH$] in the brain as a result of increased concentrations of such fatty acids in the brain concomitant with depletion of antioxidant from this tissue.

Destruction of a fatty acid by peroxidation should result in a decreased amount of that fatty acid. In the present studies the fatty acid composition of the brains of normal chickens, and those with encephalo-