A Weighing Method for Measuring the Induction Period of Marine and Other Oils[1]

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THE TECHNIQUE of following the rate of oxidation of oils by weighing small samples at intervals during storage has been used from time to time for at least 75 years (1, 2, 3, 4). However most workers studying the stability of edible oils have relied instead upon manometric measurements of oxygen absorption or upon the development of peroxides or carbonyl compounds (4, 5, 6). We have found the weighing procedure to be a convenient method for estimating the relative effectiveness of antioxidants in marine and other oils and purified fatty esters. The purpose of this communication is to describe details of the technique being used and to present a brief discussion of its advantages and disadvantages.

Procedure

To a series of 30-ml. beakers are added accurately measured amounts of the antioxidants to be studied (for example, 0.2 ml. of a solution of propyl gallate containing 1 mg. per ml. in absolute alcohol). The solvent is carefully removed in a stream of nitrogen. The beakers are then weighed, and to each is added 1.000 ± 0.003 g. of oil (from a dropper). Each beaker is accurately reweighed. The beakers are then placed in a constant temperature oven (30° to 80°C., depending upon the information required), and after they have come to temperature (10–20 min.), the contents of the beakers are rotated sufficiently to insure uniform distribution of the antioxidant in the oil. They are kept covered with watch glasses. At daily intervals the beakers are removed from the oven, allowed to cool at room temperature for 15 to 30 min., weighed (to ± 0.1 mg.), and replaced in the oven.

A limiting factor with respect to the volume of work possible is the time required for weighing, hence an accurate rapid-weighing balance is expeditious. We use a Sartorious semi-micro balance. With foreknowledge of the approximate weight a weighing can be completed in 30 seconds to 1 minute.

Comments on Procedure. The amount of oil used and the size of container are arbitrary. We obtain satisfactory data with 200 ± 0.6 mg. samples of oil in 10-ml. beakers as well as with the larger amounts cited above (Table I). It is obviously desirable to keep the ratio of surface to volume high. Data illustrating the effects of container and sample size and comparing draft and convection oven incubation are presented in Tables II and III. These and other results emphasize the variability that may be encountered (for example, the unexpectedly low value for the 0.5-g. sample in the 30-ml. beaker, Table II) and the necessity for replication of experiment.

The volatility of some antioxidants requires that care be used in the removal of the solvent. This was particularly true of 6-ethyl-2,2,4-trimethyl-1,2-dihydroquinoline (EMQ) (Santoquin), 2-(or 3-)tertiarybutyl-p-cresol (BHA), and 2,6-di-tertiarybutylparacresol (BHT). For example, approximately 50% of 0.2 mg. of EMQ was lost from a 30-ml. beaker held without cover in a constant temperature draft oven at 50°C. for 20 min. An obvious alternate method is to use solutions of antioxidant in oil prepared in larger amounts or obtained by dilution of accurately prepared, more concentrated solutions.

Still another alternative is the following. The oil is weighed into the beaker, and the additives are then put in as aliquots of solutions in volatile solvents. In constant draft ovens at 50° or 60°C. absolute alcohol or iso-octane is removed quantitatively from the uncovered beakers in less than 15 hrs., as judged by constant weight in subsequent weighings. This procedure eliminates the step of evaporating the solvent prior to the addition of oil, also minimizes the loss of volatile antioxidants. It is the current method of choice.

Samples of oil containing residual solvent or other volatile materials are carefully heated *in vacuo* to remove these, otherwise there is an initial drop in weight. A rotating evaporator is useful for this

TABLE I

Apparent Change in Weight of Various Oils During Incubation[a]

Days	Menhaden oil + 0.1% EMQ (50°C.)	Trilinolein +0.025% EMQ (50°C.)	Lard + 0.01% BHT (60°C.)	Lard (60°C.)	Cottonseed oil (60°C.)
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	0	−0.1	0	0.2	0
2	0	0	0	1.5 R	−0.3
3	0.1	0.1	0.1	3.3	0
4	0.1	0.1	0.1	—	0.2
5	0.2	0.1	0.1	—	0.2
6	0.8 R[b]	0.2	0.9 R	—	—
7	4.7	0.3	2.6	—	0.3
8	—	0.1	—	—	0.4
9	—	0.4	—	—	0.2
10	—	1.3 R	—	—	0.8 R
11	—	4.0	—	—	6.2

[a] 200-mg. samples of oil. Without additive the menhaden oil gained 5.4 mg. in 18 hrs.; the trilinolein gained 2.5 mg. in 15 hrs.
[b] R, definitely rancid by odor.

TABLE II

Effect of Container and Sample Size on Induction Period[a]

Beaker		Induction period	
Capacity	Cross sectional area	0.5-g. sample	1.0-g. sample
ml.	*sq. cm.*	*days*	*days*
5	2.6	55	61
10	4.0	39	46
20	6.6	40	42
30	7.6	31	42
50	12.0	25	36

[a] The substrate was a preparation of distilled ethyl esters of menhaden oil fatty acids containing 0.05% EMQ held at 40°C. in a draft oven. The induction period was the time required for the sample to gain 0.4% in weight.

TABLE III

Effect of Sample Size and Type of Oven on Induction Period[a]

Sample size	Oven	Induction period
g.		*days*
0.1	draft	5
0.2	draft	6
0.5	draft	9
0.5	convection	10
1.0	draft	10
1.0	convection	11

[a] The substrate was a sample of tuna oil containing 0.02% EMQ, held in 30-ml. beakers at 50°C. The induction period was the time required for the samples to gain 0.4% in weight, to the nearest day.

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purpose. Esters of fatty acids, such as ethyl oleate, methyl linoleate, and linolenate, and ethyl esters prepared from marine oils, lose weight in uncovered beakers at a small but regular rate in the draft ovens. If the beakers are covered with cover glasses, the rate of evaporation is negligible at 40°C.

Concentrations of antioxidants and temperatures are usually chosen so that the induction periods are within the range of 3 to 20 days and the one-half to one-hour interruption for the daily weighing will be of minor significance in estimating the length of the induction period.

The reproducibility of the method may be illustrated as follows. Over a period of three and one-half months the induction period of a refined cottonseed oil at 60°C. was found to be 12, 11, 10.5, 11.5, 10.5, 10, 11, 10, and 10 days (Table I). The induction period of a menhaden oil containing 0.05% EMQ was 8, 10.5, and 9 days, and with 0.1% EMQ, 24, 17.5, and 18 days all in runs started on separate days (50°C.). A lard sample (200 mg.) had gained weight as follows at 60°: by the second day, 1.5, 1.7, 0.4, and 1.3 mg., and by the third day, 3.3, 3.7, 1.0, and 3.6 mg., respectively, in four runs started several days apart (Table I). These data are of the same order of duplicability as that noted by other workers in this field.

We have used the weighing technique to compare the effects of different antioxidants and mixtures on the following oils and esters: menhaden, tuna, pilchard, salmon, whale, sable, anchovy, herring, cottonseed, corn, soybean, and olive, ethyl oleate, methyl linoleate, linolenate and arachidonate, and trilinolein. Examples of the type of data obtained are presented in Table I and in Figure 1. The results of other observations will be published separately.

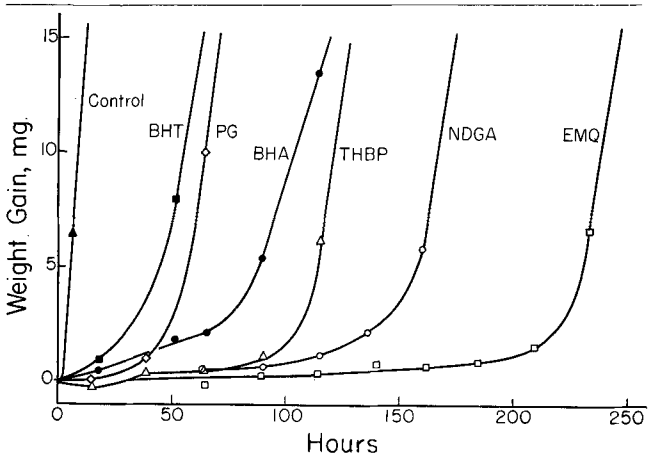

FIG. 1. Effect of antioxidants (0.04%) on crude menhaden oil (1 g.) at 50°C. BHT, butylated hydroxytoluene; PG, propyl gallate; BHA, butylated hydroxyanisole; THBP, 2,4,5-trihydroxybutyrophenone; NDGA, nordihydroguaiaretic acid; EMQ, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline.

Discussion

It is now generally accepted that at least with some substrates the addition of oxygen to form peroxides is reasonably quantitative during the initial stages of autoxidation (5, 6, 7, 8, 9) hence the measurement of an induction period by following the changes in weight is sound theoretically. It is not to be expected that these will be markedly affected by losses of water, carbon dioxide, aldehydes, or other volatile decomposition products until oxidation has proceeded to or close to the relatively rapid stage. With fats in which a fairly well-defined induction period is followed by rapid autoxidation, the weighing procedure should be as useful as any other. On the other hand, where absorption of oxygen is slow and the transition to rapid oxidation is less sharp, the weighing method would have the same drawbacks as others in defining the end of the induction period (the BHA curve in Figure 1).

Marine oils have a fairly sharp increase in weight at the end of the induction period and are frankly rancid in odor by the time they have gained 0.3–0.5% in weight (at 30–60°C.). This value may be expected to differ with different oils, temperatures of incubation, and with the anti- or pro-oxidants added. Data may be presented by figures showing the shape of the weight increase-time curve, or, to conserve space, by using an arbitrary cut-off point of 0.4%–0.5% increase in weight as marking the end of the induction period (Table I and Figure 1).

In a brief discussion of this method of measuring susceptibility to oxidation Lea (4) suggested that it was not sufficiently sensitive for the investigation of the earlier stages of oxidation in the more saturated fats, that a loss of weight was often recorded during the induction period in spite of the fact that the fat was absorbing oxygen, presumably because of volatile products lost, and that an error may be introduced by hygroscopic products of oxidation taking up moisture from the air.

Our observations indicate that these points are not particularly serious. The sensitivity is limited only by that of the balance used. It is true that the more saturated fats do not absorb as much oxygen, nor do they have as abrupt an increase in weight at the end of the induction period as do those of marine oils, for example, but the increases are readily measured. With respect to losses in weight during the induction period, these have been inconsiderable in our hands and, where they have occurred, have been accountable for in terms of residual volatile solvent or volatility of the substrate. Highly stabilized oils have remained at their original weight ± 0.01% for many weeks. Finally we have seen no evidence of moisture being absorbed by hygroscopic by-products. In a study of the length of the induction period the many complicated reactions which occur once it is passed are of no particular consequence.

Summary

A convenient method for measuring the length of the induction period of marine and other oils and unsaturated fatty acid esters depends upon determining the increases in weight. Small amounts of oil with or without additives are held in constant temperature ovens and weighed at regular intervals. Some of the details of technique are described.

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