An Improved Constant Temperature Bath for the Active Oxygen Method Fat Stability Apparatus

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THE ORIGINAL HEATING BATH described for this procedure, at that time known as the Swift Keeping Quality (SKQ) Test (1), consisted of a mineral oil bath surrounded by boiling water. Subsequently other variations have been presented (2, 3, 4), showing how workers have endeavored to eliminate some of the objectionable features of the original apparatus. Precise control of the sample temperature was soon recognized to be one of the critical factors in this test (2), and it was realized that raising the bath temperature from 97.7° to 110° required a correlation factor of 2.5 (5). The usual means of adjusting the temperature of the original type of bath is to add either alcohol or glycerol to the refluxing liquid (6). The original equipment has been commercially available for many years, but recently a much improved apparatus was offered, designed to eliminate many of the undesirable features of the older device. Experience has shown that the following disadvantages are inherent in the original equipment, some of which are still present in the improved design.

a) A continuous flow of cooling water is required to operate the reflux condenser.
b) Variation in atmospheric pressure with altitude, accompanied by weather changes, can significantly change the operating temperature.
c) Agitation of the oil bath is a virtual necessity, further complicating the equipment.
d) The oil bath oxidizes over a period of time, necessitating periodic cleaning and replacement.
e) Sample tubes immersed in the oil bath must be wiped on the outside when removed for sampling, a messy operation at best, and the inevitable residual film complicates the problem of cleaning the tubes after use.
f) Odors from the oil bath interfere with smelling of the effluent air from the sample tubes.

The apparatus next described eliminates the above objections and provides a degree of flexibility not possessed by the original equipment. It has been in constant service for more than four years.

The apparatus (Figure 1) consists of a solid brass block, electrically heated and thermostatically controlled, utilizing commercially available components. The block, cast locally from scrap brass, weighed approximately 225 lbs. before machining. The top surface was milled flat and smooth, the bottom was rough-milled parallel to the top and the $\frac{5}{16}$-in. square slot was routed to provide a press fit for the heating element. The holes were drilled slightly undersize and reamed to dimension diameter. The thermoregulator may be purchased with a metal guard, which was cut off to the length indicated and brazed to the block. The air distribution manifold was attached as indicated, and the block was insulated with 1-in. thick layers of 85% magnesia block. Joints in the successive layers of insulation were staggered and filled with cement to provide the best possible insulation. Each layer was bound with wire, and the outside was covered with cloth.

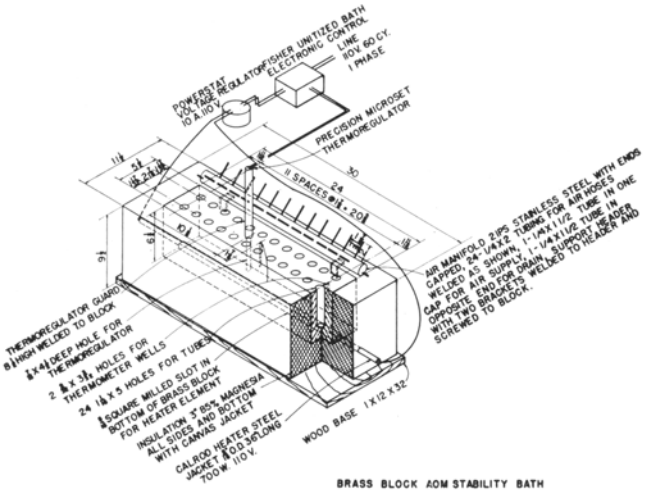

FIG. 1.

The voltage regulator is used to reduce the heat input so that the heat is on approximately 50% of the time.

Temperature Uniformity Throughout the Block

The tube holes are numbered from 1–12, left to right in the front row and 13–24 left to right in the rear row. A sample tube 1 in. in diameter was placed in each hole, a thermometer calibrated to 0.1° intervals was inserted with the bulb resting on the bottom of each tube, and the tubes were then filled with vegetable oil level with the top surface of the block. Maximum temperature variations were recorded at each hole (Table I). This condition will produce wider temperature fluctuations than if the oil were agitated. In fact, merely stirring with the thermometer produced a temperature rise of 0.4° and eliminated practically all the differences between holes.

TABLE I

Temperature Uniformity of Block, Degrees C.

Hole no.	Minimum temperature	Maximum rise	Hole no.	Minimum temperature	Maximum rise
1	97.8	.3	13	97.7	.3
2	97.8	.2	14	97.7	.3
3	97.7	.3	15	97.7	.4
4	97.8	.3	16	97.7	.4
5	97.8	.3	17	97.6	.3
6	97.8	.2	18	97.8	.2
7	97.7	.3	19	97.8	.2
8	97.7	.3	20	97.7	.2
9	97.8	.1	21	97.8	.2
10	97.8	.1	22	97.6	.2
11	97.7	.2	23	97.8	.0
12	97.6	.2	24	97.8	.0

Effect of Changing the Height of the Oil Level in the Block

It was suggested that immersing the sample in the block might provide more uniform temperatures (7). To test this, oil was poured into six tubes to a depth of 80 mm., and the tubes were placed in positions 1, 6, 12, 15, 18, and 22 with a tuft of steel wool alongside the tubes to provide friction so that the level of the oil surface could be adjusted at will. Air was bubbled through the samples at the rate of 150 ml./

min. The thermoregulator was adjusted to hold the sample temperatures as closely as possible to 97.8°, with the oil level even with the top of the block. Temperature uniformity checks were made at this location, with the oil level both 1 in. below and 1 in. above the top surface of the block (Table II).

TABLE II

Effect of Varying the Oil Level in the Block. Temperature in Degrees Centigrade

Hole no.	Level one inch above		Level one inch below		Level even with top	
	Minimum temp.	Maximum rise	Minimum temp.	Maximum rise	Minimum temp.	Maximum rise
1	89.9	.3	101.6	.2	97.5	.1
6	89.9	.4	101.6	.2	97.8	.3
12	89.8	.4	101.6	.2	97.6	.1
15	89.8	.4	101.6	.2	97.6	.2
18	89.8	.4	101.6	.2	97.6	.2
22	89.8	.3	101.6	.2	97.8	.1

Temperature of the block at the center thermometer hole remained 101.7 ± 0.1° throughout the series. Consideration of the data provides the following conclusions.

a) Sample temperatures drop markedly and are erratic as the oil level rises above the block, possibly because of slight differences in oil level or air flow.
b) An oil level 1 in. below the surface provides sample temperatures equal to the block even though no effort has been made to warm the air before passing through the sample.

Because slight variations in air flow materially affect the oil level (and hence the sample temperature) when the oil level is even with or above the block, it is recommended that samples be inserted so that the surface will be at least 1 in. below the surface of the block.

Performance Comparisons

In order to demonstrate the temperature uniformity throughout the block, AOM Stability Tests on six samples each of shortening and liquid vegetable oil were run at the same time (Table III). All-glass sample tubes were used in these tests (H. S. Martin and Company, Evanston, Ill., Cat. M-7920).

Peroxide values of 1.0-g. samples were determined several times during the course of the test. The block temperature was adjusted to maintain 97.8 ± 0.1°, and sample tubes were permitted to rest on the bottom of the holes. In this position the oil level is approximately 1.5 in. below the top of the block. Air was provided from 100 p.s.i. storage through a reducing valve and the purification train to the manifold, in the usual manner. Approximate 1.0-g. samples were placed in iodine value sample cups and weighed to ± .001 g. The milliequivalents of peroxide were determined by titrating with .01 *N* sodium thiosulphate the iodine liberated from potassium iodide.

TABLE III

AOM Stability, Milliequivalents of Peroxide

	Shortening					
Hole no.	1	5	12	13	17	24
AOM hrs.= 21.0	30	26	31	34	39	30
39.0	95	92	91	92	103	92
40.0	110	101	102	109	117	104
	Liquid Vegetable Oil					
Hole no.	2	6	11	14	18	23
AOM hrs.= 9.0	27	28	27	29	28	28
13.5	84	83	82	88	84	85
14.5	110	105	106	115	109	112

If 100 milliequivalents are taken as the end-point, only one shortening sample (hole No. 17) was rancid at 39 hrs. The other five were rancid at 40 hrs. Since previous work did not indicate that hole No. 17 was warmer than any other, it is logical to assume that some factor other than temperature was responsible for this one sample being different from the rest. Similar evaluation of the data for the six liquid oil samples shows that all reached the end-point at the same time (14 hrs.). A great deal of additional data, not presented here in the interest of brevity, provides similar correlation with other fats. Excellent correlation is regularly obtained with several similar baths in other laboratories.

Convenience and Safety

The only utility required for operation of this constant temperature apparatus is 110-volt, 60-cycle electricity. Water supply, water drain, and condenser are eliminated. The block was heated at full capacity for many hours, and the temperature did not exceed 260°C. Although the relay failed on one occasion, the temperature rose to only about 140° because the voltage regulator was set at the usual position of approximately 70 volts. We feel there is virtually no hazard involved in the operation of this equipment.

General Discussion

The success of this apparatus results primarily from its simplicity. It is entirely feasible to modify the apparatus considerably so long as the inherent characteristics are not degraded. For instance, a block of the same size has been made from an aluminum forging, and it is at least equally satisfactory. Another satisfactory block, somewhat smaller and square in plan form, has been made from an aluminum forging, with two heating elements inserted through holes drilled in the block. It is essential that firm contact be maintained between the heater and the block and that there be no local concentration of heat input, such as would be produced by a U-shaped heater or resistance tape wrapped around the sides and ends of the block.

As previously indicated, it is important that the thermoregulator bulb be located at the same depth as the oil samples. Adjustment of the temperature over a wide range is simply accomplished, and the metal blocks have been used for many other reactions at high temperatures.

Brass was used for the original block because its heat conductivity is almost twice as high as aluminum, and its specific heat per unit volume is more than one-third greater than aluminum. On the other hand, aluminum is only about one-third as dense as brass (resulting in a much lighter block). It is relatively easy to obtain a forged piece of any desired size, and its use eliminates a possible source of copper contamination. Newer equipment in this laboratory is made from the lighter metal.

References to the brand names of equipment are for simplicity and clarity; similar equipment of different manufacture can be used with equal facility.

The authors wish to acknowledge that such a heating bath was suggested by the late Alton E. Bailey, and his counsel was most helpful throughout the early part of the development. Comments and suggestions from members of the current AOM Stability Subcommittee of the American Oil Chemists' Society are likewise gratefully acknowledged.

Summary

A constant temperature bath of a type not heretofore reported has been developed. It eliminates the objectionable features of previous apparatus and provides precise control of the very critical temperature factor in AOM stability measurement. The apparatus is easily constructed from readily available materials; it operates reliably, safely and without attention for extended periods of time. Certain matters of technique are discussed, such as submersion of the sample below the top of the bath.

REFERENCES

1. King, A. E., Roschen, H. L., and Irwin, W. H., Oil and Soap, *10*, 105–109 (1933).
2. Freyer, Egbert, *ibid.*, *12*, 139 (1935).
3. Freyer, Egbert, *ibid.*, *13*, 227 (1936).
4. Bates, R. W., and Ast, H. J., Am. Oil Chemists' Soc., *25*, 42–44 (1948).
5. Mehlenbacher, V. C., Oil and Soap, *19*, 137 (1942).
6. Results of questionnaire distributed by AOM Subcommittee of the Am. Oil Chemists' Soc., personal communication.
7. Newby, Wales, personal communication.

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Biological Effects of the Polymeric Residues Isolated from Autoxidized Fats[1,2]

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For a long time it has been recognized that highly autoxidized (rancid) fats are not the nutritional equivalents of fresh, wholesome fats, but the reason for the difference has not been explicitly stated. This loss has been variously attributed to peroxides or oxygenated products, but in our earlier studies (8) no signs of toxicity resulted from the feeding of adequate diets containing 5% of methyl monohydroxystearate, methyl 9,10-dihydroxystearate, methyl *cis*-epoxystearate, or a methyl oleate peroxide concentrate (85% peroxide). These results suggest that simple oxidized substances are not the principal agents responsible for the growth-depressing effects. It has been assumed that the peroxides destroy the vitamins in the fats, but this can be eliminated as a possible cause by separate administration of an adequate supply of vitamins.

The possibility that the nutritional effect is caused by the destruction of essential fatty acids is not tenable because, as we shall show later, the effect is extremely dramatic when the actual growth-depressing ingredients are fed at a high level to animals which have been receiving a nutritionally adequate diet and continue to receive essential fatty acids.

Information which may eventually be of value in clarifying our present-day ideas concerning autoxidized fats developed from two main sources. In studies of the chemistry of drying oils used for paints and varnishes it was slowly recognized that fats are capable of undergoing autoxidatively produced polymerization. At the same time knowledge was accumulating concerning the biological properties of heated fats (1). Both lines of investigation gradually led to the recognition of the importance of fat polymers and to the need for a distinction between polymerization brought about by heat alone and by autoxidation. It is not yet possible however to distinguish clearly between the bio-responses brought on by the two kinds of polymers because it is difficult to say whether the polymers are the only cause of these effects. Nutritional studies in which concentrates of autoxidatively produced polymers were used have not yet been reported. In any discussion of autoxidized fats however the effects caused by oxygen and by heat often overlap.

As an indication of the lack of agreement concerning the nutritional effects of polymers derived from fats, some workers (1a, 10, 11, 12) have stressed the undesirable nutritional properties of partly polymerized fats while others (3, 4, 13, 15) have deliberately polymerized certain oils to stabilize them for human consumption and reported no ill effects. These differences may result, in part, from the fact that the latter group examined partly polymerized fish oils and the others used fats of different origin.

Earlier (19) we had shown that molecular distillation was useful to isolate autoxidatively produced polymers from autoxidized methyl oleate. It was decided therefore to apply this technique to various autoxidized fats with the hope of removing unoxidized and oxidized monomeric triglycerides (molecular weight 1,000 or less) as the distillate fractions, leaving the autoxidatively produced polymers, if any, as the non-volatile residue. This separation technique was found to be satisfactory. It permitted the isolation of polymeric residues from drastically autoxidized lard, drastically autoxidized cottonseed oil, and a hydrogenated fat which had been used in commercial deep-fat frying.

Present Study

Lard aerated at 95°C. for about 200 hrs. and then molecularly distilled yielded an amber-colored polymeric residue which usually amounted to about 17% of the original fat. Cottonseed oil, similarly treated, yielded about 40% of the polymer. It should be noted

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[1] This paper is XXI in the series "Reactions of Fatty Materials with Oxygen." Paper XX is reference 18.

[2] Presented at the Fall meeting of the American Oil Chemists' Society, Philadelphia, Pa., October 10–12, 1955.

[3] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.