CXLV. IRRADIATION OF FATS.

I. A STANDARDISED METHOD OF USE OF ULTRA-VIOLET LIGHT.

BY LESLIE HERBERT LAMPITT, NORMAN DARBY SYLVESTER
AND PHILIP BILHAM.

(From the Lyons Laboratories.)

(Received March 14th, 1935.)

DURING the last few years an increasing amount of work on oxidative changes in fats has been recorded, and one of the causative factors of this type of change, namely light, has received some attention. Unfortunately however very little effort has been made to study the activity of light from a quantitative standpoint. Two examples only are of sufficient importance to be noted.

Rogers and Taylor [1926] investigated the effect of light on the oxidation of linseed oil under various conditions, using a quartz mercury lamp as the source of illumination. Their statements with regard to the light energy absorbed are however based on calculations and not on direct measurements.

Read and Bailey [1933] examined the effect of ultraviolet light from a quartz mercury lamp on the development of rancidity in crackers and in shortening fats. The incident energy was measured by means of a fluorescent photometer, but no attempt was made to obtain an accurate estimate of the total light energy. Quantitative estimates of the oxidative changes were not made.

Investigations of such actions, in order to be of definite use, necessitate standard methods of evaluating the changes by chemical tests, correlation of the results obtained with observations of taste and smell if desired and a defined technique whereby figures representative of the energy of the light acting on the sample under examination can be obtained.

The work described herein deals with one of these requisites, namely the standardisation of the method of irradiation, and certain results of experiments on butter fat are recorded as examples of the applicability of the method.

Obviously it is of importance to differentiate between the total light and that which is absorbed, but no work has yet been carried out by the present authors to attempt to indicate their respective values. A complete standardisation of the conditions would involve the measurement of intensity at all wave-lengths emanating from the source of irradiation. It was found however when the absorption spectrum of butter fat in ether was determined spectroscopically (Plate III, fig. 1) that no selective absorption could be observed except that in the region 5000–4300 Å., which is due to the pigment of the butter fat, whilst the absorption spectrum (Plate III, fig. 2) of an ethereal solution of butter fat which had been irradiated until colourless showed the absence of the above band. There was however in both cases general absorption throughout the ultra-violet region of the spectrum, and it seems unnecessary therefore to exclude any wave-lengths emitted by the arc in the measurement of the light intensity incident on the fat.

The statement by Gortner [1929] and attributed by him to Holm (no other reference has been found) that the greatest effect occurs when the light has a

wave-length of approximately 3600 Å., is not upheld by these absorption spectra, since there is no predominating absorption at this wave-length.

It appeared therefore that the control of the total light energy of the source of irradiation was sufficient for the purposes of the present investigation.

The possibility of the occurrence of changes in distribution of the light energy emitted from an ageing lamp was not investigated, in view of the definite statement by Coblenz *et al.* [1926] that such changes do not occur. In the present experiments lamps have been used which have previously been burned for about 100 hours. It will be seen that the method of irradiation described makes allowance for any small changes which may occur in the quantity of light emitted from the lamp.

EXPERIMENTAL.

(a) Electrical equipment.

After several abortive attempts to obtain standard and reproducible experimental conditions, the lay-out of apparatus shown in Fig. 1 was finally evolved.

Fig. 1.

The fat to be irradiated was contained in a cylindrical glass vessel which fitted into a metal holder, supported from the cover of a thermostatically controlled water-bath. The mercury quartz arc lamp (Kelvin, Bottomley and Baird) was clamped in a fixed position, the centre of the arc being vertically above the tube containing the fat.

A measure of the light intensity of the lamp was obtained by means of a photo-electric cell (U.N.G. 7 type G.E.C.) having an activated sodium cathode in a glass bulb with a quartz window. A constant potential of 74 v. was applied to the cell from accumulators¹, and the photo-current was measured by means of a micro-ammeter.

The current for the lamp was supplied by a rectifier from the A.C. mains. Two variable resistances were placed in series with the lamp, and the major

¹ In earlier experiments the cell potential was applied from the A.C. mains through a rectifier; difficulties due to fluctuation of the mains potential however made this method impracticable.

Fig. 1. Absorption spectrum of fresh butter fat in ether.

Fig. 2. Absorption spectrum of irradiated butter fat in ether.

alterations in intensity were effected by means of the "rough" resistance, the light from the lamp being approximately adjusted to any required intensity (as indicated by the micro-ammeter reading), accurate adjustments being made with the "fine" resistance.

During the progress of irradiation it was necessary to make periodic adjustments to the fine resistance in order to restore the micro-ammeter reading to the required magnitude. These corrections were necessitated by fluctuations in the A.C. voltage, and they were required at all times of the day and night. Fortunately the major fluctuations occurred at fairly definite times and could be anticipated.

The fat was stirred with an up-and-down motion by means of a glass rod stirrer, shown in Fig. 2, which was designed so that the obstruction of the light would be at a minimum. The stirrer was guided by movement through a vertical glass tube, and was actuated by an A.C. induction motor, the speed of which varied between 63 and 68 revolutions per minute.

Irradiation experiments could then be carried out at any fixed light intensity which was indicated by the micro-ammeter reading, and the effect of slight variations in the A.C. supply, as well as that due to ageing of the lamp, were corrected by periodic adjustments to the resistance.

Fig. 2. Glass stirrer used in irradiation apparatus.

(b) *Measurement of the light intensity in absolute units.*

In order that the micro-ammeter readings obtained with the cell in the remote position might be related to the actual light energy falling on the surface of the fat, it was necessary to determine a conversion factor. The cell responded only to a limited wave-band and therefore the standardisation of the cell against a standard of radiation was made through the medium of a thermopile.

Calibration of the thermopile. The thermopile was used, without its collecting funnel, in conjunction with a micro-ammeter. The calibration of this combination was made with a carbon lamp, standardised by the National Physical Laboratory and used, under the conditions specified in their certificate, as the source of radiation. The response obtained was 0.155 micro-amp. for an incident radiation of 5.864×10^3 ergs per second per cm^2 . A reading of 1.0 micro-amp. would therefore be equivalent to 3.78×10^4 ergs per second per cm^2 radiation on the surface of the thermopile.

This figure was roughly checked by the use of a Leslie cube. The intensity of the radiation R on a spherical wave front from the black face of the cube is given by the formula:

$$R = \frac{\sigma(T^4 - t^4) \times E \times A}{2\pi r^2} \text{ ergs per second per cm}^2,$$

where σ = Stefan's constant = 5.72×10^{-5} ,

T = temperature of cube = 95° ,

t = temperature of air = 19.5° ,

E = efficiency of radiation from cube = 0.9 [Poynting and Thomson, 1919],

A = area of black face = $20.5 \text{ cm.} \times 20.5 \text{ cm.}$,

r = radius of spherical wave front = 50 cm.

When the values were as stated above the response given by the thermopile was 0.34 micro-amp. It may be calculated from these figures that the incident radiation for a response of 1.0 micro-amp. would have been 4.45×10^4 ergs per second per cm.²

This figure is subject to some inaccuracy, due to departure from the inverse square law, owing to the extended surface of the source as compared with its distance from the thermopile. It served as a check on the first figure which has been used in all subsequent calculations.

The conversion factor can be determined in two ways:

1. *The standardisation of the cell against the calibrated thermopile and the measurement of the ratio of the micro-ammeter reading to a hypothetical reading which would be obtained if the cell occupied the position of the irradiated fat.*

The comparison of the responses of the calibrated thermopile and the cell to the light of the arc was carried out by rapidly substituting one instrument for the other when the arc was burning steadily, the surface of the thermopile occupying the same position as the outer surface of the quartz window of the cell. The responses obtained were 3.04 micro-amperes and 1.01 micro-amperes for cell and thermopile respectively and therefore the radiation from the arc which would give a reading of 1.0 micro-amp. when incident on the window of the cell would be 1.25×10^4 ergs per second per cm.²

The ratio of the actual to the hypothetical micro-ammeter readings was determined by direct experiment. When the outer surface of the cell window occupied the same position as the fat surface, the light was so intense that the cell would have given a current far in excess of its normal working range and not proportional to the light intensity. To avoid this error the incident light was reduced by a non-selective filter consisting of a tube on each end of which was tightly stretched and soldered fine mesh wire gauze. Two such filters were used having transmissions of 6.25 and 12.2 %, which factors, combined with the responses, gave values of 8.0 and 7.65 for the ratio. The mean value of 7.8 has been taken.

Using the figures obtained in the above experiments, it is possible to calculate the energy per second incident on the fat surface of area 9.2 cm.² when a reading of 1.0 micro-amp. is given by the cell in the remote position. The result is 0.090×10^7 ergs per second.

2. *The measurement of the energy at the fat surface with the calibrated thermopile and the correlation of this with the response of the cell in the remote position.*

When the cell in the remote position was giving a response of 3.9 micro-amperes, the thermopile was placed so that its surface occupied the same plane as that normally occupied by the fat and gave a response of 8.6 micro-amperes. The light energy per second incident on the surface of the fat when the cell is giving a reading of 1.0 micro-amp. in the remote position can be calculated from the previous calibration of the thermopile. The result is 0.077×10^7 ergs per second.

Since neither method can be considered the more accurate, the mean of the two results, viz. 0.084×10^7 ergs per second, has been used for calculating the total incident light energy in the irradiation experiments to be described.

(c) *Standard procedure.*

The following are the summarised conditions of standard experiments:

Distance, centre of arc to surface of fat	...	...	25.5 cm.
Area of fat surface exposed	...	...	9.2 cm. ²
Weight of fat	...	...	50 g.
Depth of fat	...	...	6.0 cm.
Distance of photo-electric cell from arc	...	...	91.5 cm.

Under these conditions the effects of the irradiation of the butter fat were reproducible, and the chemical determinations satisfactory, even when duplicate experiments were carried out after an interval of as much as 14 days.

Results obtained using the standard irradiation apparatus.

The experiments, the results of which are given in subsequent tables, were carried out on pure butter fat, prepared from best quality Australian unsalted butter. The fat was separated after melting the butter at a low temperature, filtered twice through filter-paper and stored in vacuum jars in the dark.

A point of interest is concerned with the amount of fat taken. Since the extent of oxidation of the fat is not proportional to the time of irradiation, it follows that if, for example, 25 g. of butter fat were taken, the oxidation would not be twice that observed in the normal experiment using 50 g. of fat. It was decided however to carry out experiments in which the weight of fat was reduced to 25 g., the period of irradiation being also reduced to one-half. The other experimental conditions such as area of fat exposed, distance from the lamp, temperature and intensity of the incident light were the same for both experiments.

The results obtained are given in Table I.

Table I.

Time of irradiation	15 hours	30 hours
Weight of fat	25 g.	50 g.
Issoglio value	41.3	41.4

The above results were confirmed by duplicate experiments. It appears that the oxidative changes in the fat are not appreciably affected by a variation in the amount of fat taken, provided that the time of irradiation is proportionately altered. It is obvious that the depth of the fat is an important factor, and that the above conclusion will not be true when the depth of the fat is so decreased that an appreciable proportion of the active light is transmitted by the fat without exercising any photochemical effect.

Irradiations were made under various conditions of time, temperature and light intensity. The other experimental conditions were those which have already been described.

Table II. *Effect of light intensity.*

Time of irradiation constant at 20 hours. Temperature 40°.

Micro-ammeter reading	—	1.5	2.0	3.0	3.5	4.0			
Watt-secs.	Nil	9,000	12,000	18,000	21,000	24,000			
Peroxide value	1.3	3.8	3.7	4.3	6.2	5.8	8.6	12.5	13.9
Issoglio value	2.1	1.95	2.05	6.0	13.3	12.1	15.6	20.7	19.6
Kreis test	Neg.	4	4	10	35	70	100	100	

Table III. *Effect of time.*

Light intensity constant at 2.75 watts per cm.² (micro-ammeter reading 3.0).

Temperature 40°.

Irradiation time	Nil	10 hrs.	20 hrs.		30 hrs.		40 hrs.		50 hrs.	70 hrs.
Watt-secs.	Nil	9,000	18,000		27,000		36,000		45,000	63,000
Peroxide value	0.8	3.5	3.1	5.2	4.9	32.0	26.5	45.5	47.5	68 120
Issoglio value	1.9	2.7	2.4	10.2	10.5	32.0	31.0	—	—	116
Kreis test	Neg.	5	5	18	20	200	200	650	1300	2650

Table IV. *Effect of time.*Light intensity constant at 2.75 watts per cm.² (micro-ammeter reading 3.0).

Temperature 60°.

Irradiation time	Nil	10 hrs.	20 hrs.	25 hrs.	30 hrs.	40 hrs.	50 hrs.
Watt-secs.	Nil	9000	18,000	22,500	27,000	36,000	45,000
Peroxide value	0.8	2.4	8.0 8.6	23.5 23.0	39.0 46.5	78	117
Issoglio value	1.9	3.4	19.0 19.8	30.0 30.5	47.0 46.0	83	—
Kreis test	Neg.	4	50 50	170	650	2000	4000

In the above tables where two sets of figures occur in one column, they represent the results of duplicate irradiations carried out in some cases after an interval of 2 weeks. The duplication of the results is seen to be satisfactory.

Fig. 3. Effect of light intensity.

Fig. 4. Effect of time and temperature on Issoglio value.

Peroxide values, and Issoglio values are both expressed as mg. O₂ per 100 g. fat, and the Kreis test is expressed as the first dilution (in light petroleum) at

which a positive test is not obtained. The methods of analysis will form the subject of a separate paper.

The results are shown graphically in Figs. 3, 4, 5 and 6.

Fig. 5. Effect of time and temperature on peroxide value.

Fig. 6. Effect of time and temperature on Kreis test.

DISCUSSION.

The utility of such an apparatus as has been described is obviously not restricted to the type of experiment described in this paper, and this method of irradiation has, for example, been used by the authors in an investigation on the development of tallowiness in milk. Moreover, it could be applied to determinations of the induction period of a fat with a view to assessing its potential keeping properties, and such factors as the addition of pro- and anti-oxidants, variations in methods of fat processing *etc.* could be examined with regard to their effects on the susceptibility of the fat to oxidative deterioration. Such determinations can be made with the certainty that the experimental conditions can be reproduced and the results confirmed.

The results which have been given on the irradiation of butter fat have been included principally to illustrate the utility of the technique which has been described, and they are not presented as a definite contribution to the subject of oxidative changes in fats.

A consideration of the figures will show, however, that although the three determinations increase concurrently, and in a similar manner, there is not a strict parallelism between any two of them.

The effect of temperature is clearly demonstrated and has an effect on the magnitude of all the determinations. It is seen further that any relationship which might exist, say, between the Kreis test and the Issoglio value is changed by temperature. Lea [1931] has made a similar observation with regard to the Kreis test and peroxide value and the present results afford confirmation of his assertion.

SUMMARY.

1. An irradiation technique has been worked out suitable for experiments on oils and other liquids, such that good reproducibility of results can be obtained.
2. A method has been indicated whereby measurements made in a position other than that of the sample have been correlated with the intensity, in absolute units, of the light incident on the fat.
3. Butter fat has been irradiated under standardised and reproducible conditions. The oxidative changes have been measured by chemical tests, and an approximate determination of the incident light energy has been made.

The authors wish to thank Messrs J. Lyons and Co., Ltd., in whose laboratories this work was conducted, for permission to publish this paper.

REFERENCES.

- Coblentz, Dorcas and Hughes (1926). *U.S. Bureau of Standards Scientific Paper*, No. 539, p. 545.
Gortner (1929). *Outlines of biochemistry*, p. 641. (John Wiley and Sons, New York.)
Lea (1931). *Proc. Roy. Soc. Lond.* B **108**, 175.
Poynting and Thompson (1919). *Heat* (5th edition), p. 321. (Griffin, London.)
Read and Bailey (1933). *Cereal Chem.* **10**, 99.
Rogers and Taylor (1926). *J. Physical Chem.* **30**, 1334.