XVII. A STUDY OF SEASONAL VARIATION IN BUTTER FAT.

II. A SEASONAL SPECTROSCOPIC VARIATION IN THE FATTY ACID FRACTION.

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In previous communications [Booth *et al.*, 1933, 1, 2], the seasonal variations in the carotene and vitamin A contents of the butter fat produced from typical English milk were described, and a seasonal variation in the power of butter fat to inhibit the reaction between vitamin A and antimony trichloride was established. We also mentioned that the fatty acids prepared from summer and winter butter fats appeared to show certain differences, namely (1) acids from summer butter fats showed more intense absorption at $230m\mu$ than those from winter butter fats, and (2) summer acids turned brown more rapidly than winter acids when treated with the antimony trichloride reagent. It was not clear at the time whether or not these two properties were related to the greater inhibitory power of summer butter fat in the antimony trichloride test. Further work has now demonstrated that neither the $230m\mu$ absorption nor the rate of brown colour formation can be correlated with the inhibitory power. The rate of brown colour formation, indeed, is greatly increased in aged or oxidised fats, and any decision as to whether a seasonal variation of this property does actually exist must be postponed until one is in a better position to understand the nature of the reaction involved. On the other hand we have satisfied ourselves that the variation in absorption at $230m\mu$ is of a genuinely seasonal character. Data in support of this view are given below.

EXPERIMENTAL.

Collection of butter fat etc. 15 specimens of butter fat were examined, selected from a large number of samples accumulated between April 1932 and May 1933. The period investigated thus included two occasions on which the cows were turned out to grass after stall feeding during the winter. The fatty acids were prepared by the following routine method: 5 g. of the butter fat were boiled for 2 minutes with 2.2 ml. of saturated aqueous KOH and 10 ml. of alcohol. The mixture was then diluted with 40 ml. of water and extracted twice with 75 ml. of ether each time, in order to remove the unsaponifiable matter. 20 ml. of 10 % sulphuric acid were then added and the free fatty acids extracted with 75 ml. of ether. The ethereal solution was washed twice with 10 ml. of water each time (with vigorous shaking) and once with 50 ml. of water (with gentle agitation) and dried by filtering through anhydrous sodium sulphate. The ether was evaporated under partial vacuum, and the residual fatty acids were transferred to a specimen tube.

Spectroscopic examination of the fatty acids. For the spectroscopic examination a Hilger E 3 quartz spectrograph with echelon cell photometer was used throughout. Solutions of the different samples of fatty acid were made in pure cyclohexane for the spectroscopic examination. A suitable concentration was found to be about 25 mg. in 10 ml. of the solvent.

Seasonal variation of intensity of absorption by the fatty acids at 230 m μ .

The variation in the intensity of absorption at 230 m μ is shown in Fig. 1 (continuous curve). It will be seen that for winter butters $E_{1\text{ cm.}}^{1\%}$ for the acids has a value of 10 or slightly less (minimum value observed April 19–20, 1933 = 8.4) whereas the value for acids from summer butters is increased to 20 or more (maximum value observed, May 12–13, 1932 = 25.0)¹. The increase in absorption, as judged from data obtained in the springs of 1932 and 1933, takes place coincidentally with the cows obtaining access to grass. In both seasons significant increases were observed after one week of pasture feeding.

The effect of prolonged saponification on the absorption of the butter fat acids.

In our experiments the only departure from the standard method of saponification occurred during the early stages of the work, when a few of the specimens were saponified for the unnecessarily long period of 1 hour, instead of the usual

Fig. 1. --- Inhibitory power of butter fat. — Absorptive power of acids of butter fat.

period of 2 minutes. To rule out any possibility of a simulation of a seasonal variation through differences in the duration of saponification, acids prepared from typical winter and summer butter fats were submitted to prolonged boiling under reflux (15 hours) in alcoholic potash. A slight increase in the absorption

¹ Slightly higher figures were obtained in subsequent determinations outside the series from which Fig. 1 was drawn.

of the winter butter acids was observed. It was, however, quite inadequate to account for the disparity between the absorption of typical winter and summer acids. A slight increase, probably falling within the range of experimental error, was also observed in the absorption of the summer acids.

	1 min. sap.	15 hrs. sap.
Winter acids, Feb. 24-27, 1933	12.6	15
Summer acids, May 19-22, 1933	27.1	30.0

Differentiation of 230m μ absorption and inhibitory power in SbCl₃ reaction.

In Fig. 1 the inhibitory power of the butter fat is indicated by the broken curve and the absorption of the acids by the continuous curve. Except for a few samples (notably those of May 3-4, 1932, marked *A* in Fig. 1, and Oct. 31, Nov. 3, 1932 marked *B*) there is a fair degree of parallelism between 230m μ absorption and inhibitory power. To test this correlation samples of a typical summer butter fat (May 19-22, 1933) were submitted to various treatments and the effects on absorption and inhibitory power compared. It was found that whereas ageing and irradiation of the fat led to a marked decrease in inhibitory power, the absorption at 230m μ was only slightly decreased. Aeration of the hot fat on the other hand caused reductions both in absorption and inhibitory power. To indicate the degree of severity of the various treatments iodine value determinations were carried out.

Table I. *Inhibitory power of butter fat.*

Butter fat of May 19-22, 1933. The inhibitory power has been expressed in arbitrary units by noting the depression in the blue reading caused by adding 0.5 ml. of the fat to 8 γ of B.D.H. vitamin A concentrate before the addition of 2 ml. of SbCl₃ reagent. The blue reading was taken immediately after adding the reagent. In the case of the aged, irradiated and oxidised specimens brown colorations developed on standing much more rapidly than with the untreated fat. Iodine values were determined by the Wijs method.

Treatment	Details	$E_{1\text{ cm.}}^{1\%}$ 230m μ	Blue reading in absence of fat	Blue reading in pre- sence of fat	Inhi- bitory power	I.V.
Untreated	—	25.9	7	2	5	48.5
Ageing	Fat was kept for several weeks in shallow layer in bottle on laboratory bench	18.8	7	5	2	46.9
Irradiation	Fat was exposed for 20 mins. to irradiation by mercury arc lamp at distance of 20 cm.	21.5	7	5	2	46.5
Oxidation	Fat was melted on water-bath and aerated by passing a current of air for about 24 hrs.	11.0	7	5	2	43.4

DISCUSSION.

230m μ absorption. The fact that the mixed fatty acids of butter fat show an absorption maximum at 230m μ was first pointed out by Gillam *et al.* [1931]. The band, however, is by no means specific and is observed in varying intensity in most fatty acids, *e.g.* in the mixed fatty acids of cod-liver oil [Edisbury *et al.*, 1933]. The main interest of the present work lies in the fact that the magnitude of the seasonal increase in absorption cannot be accounted for on the basis of our

accepted knowledge of the relative composition of winter and summer butter fats and of the extinction coefficients of their known constituents. Since Ramart-Lucas *et al.* [1930] have shown that all saturated acids have very small and similar extinction coefficients at $230 m\mu$, our attention, in attempting to account for the absorption of the mixed butter acids, may be restricted to oleic and linoleic acids, which according to Hilditch and Sleightholme [1930] are the main unsaturated components. In Table II the percentage of acids present has been taken from data obtained by Hilditch and Sleightholme with butters similar to those examined in the present work. The extinction coefficients for oleic and linoleic acids were observed by Dann and Moore [1933], using commercial preparations of acids, and may be taken as maximum values which probably would exceed those shown by the pure freshly prepared acids.

Table II.

	Absorbing component	% present in mixed acids	$E_{1\text{ cm.}}^{1\%}$ $230 m\mu$	Contribution towards E of mixed acids
Winter butter acid (coconut cake feeding)	Oleic	31.7	16.5	5.23
	Linoleic	2.4	9.6	0.23
	Total calculated E			5.46
	Observed			8.4
	Excess of observed over calculated			54 %
Early summer butter acids	Oleic	40.9	16.5	6.75
	Linoleic	4.1	9.6	0.39
	Total calculated E			7.14
	Observed			25.0
	Excess of observed over calculated			250 %

Two explanations for the excess of the observed over the calculated absorption seem possible. (1) Small amounts of unknown acids having very high extinction coefficients may be present in butter fat, the concentration being relatively low in winter and considerably increased in summer. (2) One or more of the known acids may be present in butter fat in a more highly absorptive form than that in which it exists in specimens prepared from vegetable sources. The second of these explanations is made attractive by our observation, reported in an accompanying communication [Dann *et al.*, 1935] that when certain oils are fed to the cow there is evidence of the appearance in the butter fat of acids having much higher extinction coefficients than the acids originally present in the oils. Although no definite decision can be reached as to the exact nature of the acids causing absorption, it seems probable that acids of the linoleic type, and possibly also more highly unsaturated acids, are involved.

Differentiation of $230 m\mu$ absorption and inhibitory power of the $SbCl_5$ reaction. Although the above experiments permit no direct advance in our knowledge of the substances present in summer butter fat which cause inhibition of the blue coloration in the vitamin A test, it may be inferred that these substances, whatever their chemical character may prove to be, are extremely sensitive to oxidation and to irradiation. In these respects they are much less stable than the substances responsible for absorption at $230 m\mu$, which are affected only by more vigorous treatment.

SUMMARY.

1. The absorption of the fatty acids of butter fat at $230 m\mu$ shows a pronounced seasonal variation, being more than doubled during the summer months when the cows have access to pasture.

2. The increase in absorption runs approximately parallel with the increased inhibitory power of the butter fat in the antimony trichloride reaction for vitamin A as previously described. The substances responsible for inhibitory power, however, are much more sensitive to oxidation and irradiation than those responsible for $230m\mu$ absorption.

3. The increased absorption of the acids from summer butter fat must be ascribed either to the presence of small amounts of unrecognised acids possessing high extinction coefficients or to the presence of modified forms of known unsaturated acids in a highly absorbing state.

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