XCVII. THE CAROTENOIDS OF BUTTER.

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IN a series of papers [Gillam *et al.*, 1933; Watson *et al.*, 1933; 1934; Gillam, 1934] data have been collected on the carotene and vitamin A contents of the butter of several breeds of cattle fed under various conditions. In view of the fact, however, that carotene exists in more than one isomeric form [Kuhn and Lederer, 1931; Kuhn and Brockmann, 1933] and that, of these, the α - and γ -forms are physiologically only half as active as the β -isomeride [Kuhn, Brockmann *et al.*, 1933] it is obviously important to ascertain precisely in which form the pigment is present in butter. The present paper describes the results of a chromatographic examination of the carotene fractions of a number of ordinary and colostrual butter samples with a view to elucidating this problem. The colostrual butters, which became available during a larger experiment at present in progress, were especially useful in this investigation on account of their exceedingly high carotene content [*cf.* Dann, 1933; Semb *et al.*, 1934].

The butters (100–500 g.) were saponified as previously described [Gillam *et al.*, 1933] and the unsaponifiable material, after removal of the major part of the sterols by crystallisation from methyl alcohol, separated into carotene and xanthophyll fractions by partition between light petroleum and 90 % methyl alcohol. The methyl alcoholic solution, containing the major part of the vitamin A and all the xanthophyll, was discarded. The light petroleum solution (30–40 ml.) was washed with water to free it from methyl alcohol, dried over sodium sulphate and subjected to the Tswett chromatographic process using a 3×20 cm. column of alumina (Brockmann's activated Al_2O_3 (Merck) diluted 1 : 2 with inactive Al_2O_3). The chromatogram was developed by washing successively with light petroleum of B.P. 40–60° and 60–80°. The separated zones were eluted individually with light petroleum containing methyl alcohol, filtered from adsorbent and examined spectroscopically in either light petroleum or carbon disulphide.

The principal pigments.

The majority of the samples exhibited two main coloured zones which washed down the columns separately, the lower one being yellow or buff in colour whilst the upper one was brown or pink. A similar separation was observed when calcium hydroxide was employed in place of alumina [Karrer and Walker, 1933]. The spectroscopic values are shown in the accompanying table, together with the absorption maxima recorded for α - and β -carotene in the literature. As Häusser (quoted by Kuhn, 1931) has shown that different spectroscopic techniques give slightly different values for the absorption maxima of carotene solutions, we have also made chromatographic and spectroscopic examinations of carotene samples definitely known to contain the α - and β -isomerides, under conditions exactly comparable with those used in the case of the butter pigment, employing for this purpose:

- (a) the unsaponifiable matter of palm oil [Kuhn and Brockmann, 1931], and
- (b) a sample of crystalline carrot carotene, m.p. 178° [Kuhn and Lederer, 1931; Mackinney, 1935].

Table I.

Sample	Adsorbent	Colour of zone on adsorbent	Lower zone (α -carotene)				Upper zone (β -carotene)				Ratio of $\alpha : \beta$ carotene present	
			Absorption maxima				Absorption maxima					
			Petrol 70-80° $m\mu$		CS ₂ $m\mu$		Petrol 70-80° $m\mu$		CS ₂ $m\mu$			
Ordinary butters:												
English (1)	Al ₂ O ₃	Pink-buff	470	445	—		Red-brown	480	451	—		1 : 2.7
Shorthorn (2)	Ca(OH) ₂	Brown-yellow	474	446	510	478	Red-brown	—		—		About 9 : 1
English	Al ₂ O ₃	Buff	475	447	505	479	Salmon-pink	483	453	513	482	1 : 2.2
Guernsey	Al ₂ O ₃	Buff	473	447	—		Pink	482	452	—		1 : 4
Danish	Al ₂ O ₃	Buff	475	447	506	477	Red	483	453	513	480	1 : 2.2
New Zealand	Al ₂ O ₃											
Colostrum butters:												
Shorthorn	Al ₂ O ₃	Yellow	477	448	510	477	Pink-brown	481	452	—		4 : 1
Shorthorn	Al ₂ O ₃	Yellow	473	446	508	477	Pink	482	452	514	481	1 : 4.4
Friesian	Al ₂ O ₃	Yellow	478	448	510	478	Brown	—		—		7 : 1
Guernsey	Ca(OH) ₂	Yellow	475	447	509	478	Pink-brown	480	450	—		Mainly β -form
Ayrshire	Al ₂ O ₃	Yellow	473	446	—		Deep red	482	452	—		1 : 2.7
Ayrshire	Al ₂ O ₃	Yellow	474	445	—		Salmon-pink	482	452	—		1 : 1.8
Carotene extracts of known composition:												
Cryst. carrot carotene m.p. 178°	Al ₂ O ₃	Yellow	477	448	508	477	Red-brown	483	453	513	482	—
Palm oil unsaponifiable matter	Al ₂ O ₃ (also Ca(OH) ₂)	Bright yellow	476	447	507	475	Red-brown	481	451	513	481	—
Data on pure pigments given in the literature	Ca(OH) ₂	Yellow	478	447.5	509	477	—	—		—		Karrer and Walker [1933]
	—	—	478	447.5	511	478	—	483.5	452	521	485.5	Kuhn and Brockmann [1932]
	MgO	—	—		507	476	—	—		511	485	Kuhn and Lederer [1931]
												Strain [1934]

A comparison of the data (see Table I) with the corresponding butter values definitely proves that butter contains both α - and β -carotene and that the relative amounts of these isomerides vary considerably in different samples. The ratio of the two substances present in each butter was determined as follows. The separately eluted pigments were made up to equal volumes in light petroleum B.P. 70-80°, and the intensity of absorption of light of each solution was determined at the wave length of maximum absorption (447 $m\mu$ for α -carotene, 452 $m\mu$ for β -carotene). When equal thicknesses of solution are examined the relative amounts of pigment present are directly proportional to the respective intensities of absorption recorded.

Pigments present in only small amount.

In addition to α - and β -carotene, several of the more highly pigmented butters were found to contain minor amounts of other petrol-phasic lipid pigments, whose presence was indicated by the appearance of one or more coloured bands (usually a yellow-brown ring with a reddish violet ring just over it) situated above the β -carotene on the developed chromatogram. The yellow pigment was definitely characterised as kryptoxanthin by the following properties: (a) the yellow-brown colour of the ring and the fact that it is adsorbed just above the β -carotene; (b) absorption maxima in light petroleum at 481 and 451.5 $m\mu$. Kuhn and Grundmann [1933], using a grating spectroscope instead of our prism spectroscope, record maxima for kryptoxanthin at 485.5 and 452 $m\mu$; (c) the pigment, although epiphasic in light petroleum to 90 % methyl alcohol, is

partially extracted by 95 % methyl alcohol; (d) the pigment is adsorbed only with difficulty on calcium carbonate.

In the case of one Ayrshire colostral butter the amount of this pigment was estimated to constitute approximately 3 % (*i.e.* 0.15 mg. per 100 g. fat) of the total carotene fraction.

The reddish violet band was usually present in too small quantity for definite characterisation, but in the case of the casual Danish butter sample, which was much richer than usual in both these subsidiary pigments, it was found possible to separate it almost completely from the kryptoxanthin by repeated adsorption on alumina. The pigment is characterised by absorption bands at 503, 470 and 442 $m\mu$, which agree with the maxima of lycopene recorded by Kuhn and Brockmann [1932] who give 506, 474 and 446 $m\mu$. The slight discrepancy in the wave lengths is probably due to incomplete separation of all the kryptoxanthin, and in this connection Kuhn and Grundmann have reported that these two carotenoids are not sharply separable on alumina. In this particular sample of butter, the kryptoxanthin and lycopene were present in approximately equal amounts, and together constituted nearly half of the total petrol-phasic colouring matter.

In the light of these results it was argued that as the carotenoids of milk fat must come from the food ingested by the cow *via* the blood, then the blood serum carotenoids might be expected to be similar to those of butter. A preliminary examination of the lipid pigments of the blood serum of cows indicates a striking similarity to those of butter. We are at present examining the matter in more detail.

SUMMARY.

The petrol-phasic carotenoid fractions of the unsaponifiable matter of a number of ordinary and colostral butters have been examined by combined chromatographic and spectroscopic methods. It has been found that the carotene present is a mixture of α - and β -forms, the relative amounts of which vary considerably in different samples. Kryptoxanthin and lycopene have also been detected in small amount in several of the more pigmented samples of butter.

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