XXXV. THE CAROTENE OF MILK FAT (BUTTER)

BY ALBERT EDWARD GILLAM AND
MOHAMMED SHAFIK EL RIDI

From the Chemistry Department, Victoria University, Manchester

(Received 26 December 1936)

THE early experiments of Palmer & Eccles [1914] leave no doubt that the major part of the yellow colour of milk fat (and therefore butter) is due to the hydrocarbon carotenoid, carotene, and further, that this pigment owes its origin to the carotene present in the food of the cow. This dependence of the colour of milk fat on the carotene content of the food ingested by the animal has since been confirmed by many workers. With the discovery that carotene possesses growth-promoting (vitamin A) activity its presence in butter became important from the nutritional aspect, whilst the later discovery that the pigment occurs in several isomeric forms having different vitamin A activities [Kuhn & Lederer, 1931, 1; Kuhn & Brockmann, 1933; Kuhn *et al.* 1933] at once raised the question as to which form was present in butter fat.

In an earlier attempt to answer this question [Gillam & Heilbron, 1935] the carotene fraction of butter fat unsaponifiable matter was adsorbed on alumina; it separated readily into two zones of pigment having adsorption properties and absorption spectra exactly comparable with those of β - and α -carotene respectively. Owing to the small amount of carotene in butter (0.2–2.0 mg. per 100 g. fat) and the difficulty of isolation [cf. Palmer, 1922] these properties were considered sufficient, at the time, to indicate the presence of considerable quantities of α - as well as β -carotene in butter.

It has since been observed, however, that when pure β -carotene is adsorbed repeatedly on alumina it begins to separate into two zones of pigment having absorption spectra exactly comparable with β - and α -carotene respectively [Gillam & El Ridi, 1935]. Further investigation has shown this to be due to an exceptional action of the adsorbent itself in converting the β -carotene into a new isomeric substance, *pseudo*- α -carotene, with properties very similar to those of α -carotene [Gillam & El Ridi, 1936].

In the light of these results the whole question of the presence of α -carotene in butter was left open until the pigment could be isolated in sufficient quantity and sufficiently pure for a determination of its rotation to be made. The presence of optical activity in a carotene is a crucial test for the α -isomeride which has $[\alpha]_{\text{D}} = +380^\circ$ [Kuhn & Lederer, 1931, 1, 2], whereas β -carotene and *pseudo*- α -carotene are inactive; moreover, the rotation results are not open to the objection shown to be peculiar to the chromatographic method as applied to carotenes.

Palmer & Eccles [1914] obtained crystals of carotene from butter for examination by colour and phase tests but they were unable at that time to obtain the crystals free from colourless impurities. The present paper describes the isolation and properties of analytically pure carotene from butter fat and shows that in the bulked sample examined the carotene consists almost entirely of the β -isomeride. Adsorption of the pigment on magnesium oxide gave no evidence of

an α -carotene zone, whilst the rotation, $[\alpha]_{\text{D}} = +1.3^\circ$, although positive, is so small that, in view of the experimental error ($c. \pm 10^\circ$) it is not significant. We must therefore conclude that the α -carotene content of butter fat is negligibly small.¹

EXPERIMENTAL

Butter fat. In order to obtain the maximum amount of pigment from the minimum of butter, colostrum fat, which is noted for its high carotene content, was largely used in place of ordinary butter, whilst to make the sample more representative ordinary and colostrum butters from cows of two different breeds were mixed together as follows:

Cow	Breed	Nature of butter	Wt. of butter fat in g.	Carotene mg./100 g. fat	Mg. of carotene present
1	Shorthorn	Colostrum	170	6.2	10.5
2	Guernsey	Colostrum	445	2.6	11.6
3	Shorthorn	Colostrum	200	2.5	5.0
4	Shorthorn	Colostrum	70	2.4	1.7
5	Guernsey	Ordinary	165	1.6	2.6
6	Shorthorn	Ordinary	170	1.7	2.9
7	Guernsey	Ordinary	190	1.6	2.0
8	Guernsey	Ordinary	150	0.8	1.2
			1560		37.5

Extraction of the pigment. Before proceeding to the saponification of the butter fat it was treated with boiling acetone with the object of precipitating waxy impurities and/or phosphatides in the manner successfully used by Pollard [1936] in the preparation of the carotene of cocksfoot grass.

500 g. portions of the melted fat were poured into boiling acetone (3 l.) and cooled to -6° . The precipitate of fat and waxy impurities was dissolved in a little ether and again precipitated with acetone (1 l.), and cooled as before, the process being repeated until the residue was free from carotenoids. The combined filtrates from three portions of butter fat after treatment in this manner were freed from solvent and the glycerides saponified with alcoholic potash (1.5 ml. 10% KOH per g. fat). The unsaponifiable matter was extracted with ether, transferred to methyl alcohol and cooled to -7° in order to remove some of the sterols (4.4 g.).

The concentrated alcohol extract was diluted with water and the solutes transferred to light petroleum (60–80° B.P.), the solution being subsequently extracted with 90% aqueous methyl alcohol several times to remove xanthophyll and vitamin A. The light petroleum phase was passed through a column of magnesium oxide and Hyflo Supercel [Strain, 1934] and developed with more solvent. No evidence of α -carotene was detected in the chromatogram. The main carotene zone was cut out from the column and eluted, after which a spectrophotometric determination showed that some 25 mg. of the pigment were present. The solution was concentrated and methyl alcohol added and a crop of crystals, obviously containing much sterol mixed with carotene (83 mg., M.P. 120°), was obtained. Careful crystallization from light petroleum yielded good red-black crystals of carotene (4.2 mg., M.P. 180 – 181° , corrected; open tube); and also a second crop (7.2 mg., M.P. 177° corrected).

¹ Strain [1935] reports the detection of a small quantity of α -carotene in Jersey butter (produced under American conditions of management) by adsorption of the pigments on magnesium oxide (found in solution, order of 0.1 mg. α -carotene and 6.0 mg. β -carotene from 6 kg. of fresh butter). 2 mg. of the β -carotene were obtained crystalline, M.P. 179.5° , corrected.

Analysis (on crop 2): C, 89.66; H, 10.27%. $C_{40}H_{56}$ requires: C, 89.5; H, 10.45%.

Rotation. Carried out with red light (Pointolite lamp and Wratten 29 red filter giving only rays near the cadmium line at $643.8m\mu$) on a mixture of the two crops of crystals, 10 cm. tube; $c=0.0967\%$ (benzene); (2 determinations by each of two observers) = $+0.011^\circ$, -0.010° , $+0.010^\circ$ and -0.006° , mean $[\alpha]_{cd} = +1.3 \pm c. 10^\circ$.

Absorption maxima (determined on a Hilger-Nutting Visual Spectrophotometer).

Solvent	Pure β -carotene $m\mu$	Carotene <i>ex</i> butter $m\mu$
CS_2	514, 484	514, 482
Light petroleum	484, 452	482, 452

SUMMARY

Earlier chromatographic adsorption experiments with butter carotene on alumina indicated the probable presence of appreciable quantities of α - as well as β -carotene in butter fat. More recent work, however, has shown that the repeated adsorption of β -carotene itself changes this compound into an α -carotene-like pigment, i.e. *pseudo- α -carotene*. The question whether α -carotene actually occurs in butter to any significant extent had thus to be reconsidered and could only be clearly settled by obtaining the pure crystalline pigment and determining its rotation.

In the present investigation analytically pure butter carotene has been isolated from a mixed sample of colostrum and ordinary milk fat and shown by analysis, melting-point, absorption spectra and optical rotation to be practically pure β -carotene. Adsorption on magnesium oxide as well as the determination of the rotation of the pigment shows that, for the mixed sample of butter fat examined, α -carotene is either absent altogether or is present in negligibly small amounts (less than 0.3% of the total carotene).

The thanks of the authors are due to Prof. I. M. Heilbron, for various facilities placed at their disposal and to Dr S. K. Kon, of the National Institute for Research in Dairying, for kindly supplying the colostrum fat.

REFERENCES

- Gillam & Heilbron (1935). *Biochem. J.* **29**, 834.
 — & El Ridi (1935). *Nature, Lond.*, **136**, 914.
 — — (1936). *Biochem. J.* **30**, 1735.
 Kuhn & Brockmann (1933). *Ber. dtsh. chem. Ges.* **66**, 407.
 — — Scheunert & Schieblich (1933). *Hoppe-Seyl. Z.* **221**, 129.
 — & Lederer (1931, 1). *Hoppe-Seyl. Z.* **200**, 246.
 — — (1931, 2). *Ber. dtsh. chem. Ges.* **64**, 1349.
 Palmer (1922). *Carotenoids and related Pigments*, p. 205.
 — & Eccles (1914). *J. biol. Chem.* **17**, 191, 211, 223, 237, 245.
 Pollard (1936). *Biochem. J.* **30**, 382.
 Strain (1934). *J. biol. Chem.* **105**, 525.
 — (1935). *J. biol. Chem.* **111**, 85.