XCV. THE PHYSICAL AND CHEMICAL PROPERTIES OF CASEIN FAT

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(Received 18 March 1937)

WHEN cream is separated mechanically from milk, an appreciable percentage of fat remains in the separated milk, even under the best conditions. Most of this residual fat is precipitated with the "curd" during the manufacture of commercial casein; rather less is carried down by the calcium caseinate of rennet casein than by the caseinogen of self-soured (or lactic acid) casein. The lipid content of the latter, when it has been made with careful attention to optimum conditions, is still always over 1% and may rise to 2.5%. This represents from 1 to 2.5% of the milk fat, and from 0.03% to 0.08% of the weight of the original milk.

This fat would not be expected to differ materially in composition from milk fat, since its association with the casein seems to be a purely mechanical one. It is, indeed, only possible to determine quantitatively the amount of this fat by procedures involving complete hydrolysis of the casein with strong acid; it seems that the fat is mainly enclosed in the hard granules of casein. Nevertheless, the possibility of some differential adsorption of individual fat components is not to be overlooked; the concentration of phospholipins in buttermilk is well established and suggests a possible analogy.

As far as we are aware, the only recorded investigation into the chemical nature of "casein fat" is that of Kon & Funk [1924]. By repeated recrystallizations from alcohol they obtained a crystalline substance and very surprisingly characterized it as the anhydride of a hydroxystearic acid. This work has neither been confirmed nor disputed. Detailed examination of the glycerides of butter fat [cf. Hilditch, 1936] has only once revealed the presence of a hydroxy-acid [Bosworth & Helz, 1935-36]. This was held to be monohydroxypalmitic acid; it was optically active. It was separated via the mixed methyl esters only after a very laborious fractionation.

It seemed to us, therefore, that it would be worth while to carry out on "casein fat" some of the analytical determinations usually made to characterize natural fats, since the values for these determinations on butter fat are well known and a comparison with them might throw some light on the findings of Kon & Funk. It was the easier for us to do this, because considerable quantities of "casein fat" were available to us.

We have for some time been preparing a "fat-free caseinogen" mainly for use in vitamin A-free basal diets. The starting material is a special lactic-acid casein, whose content of mineral matter and water-soluble vitamins has been reduced as far as practicable by thorough washing of the wet curd with dilute acetic acid. Otherwise the manufacturing procedure does not differ from the normal practice adopted in making high-grade commercial casein of the self-soured type. The dried casein is ground to pass about 80 mesh, and is then repeatedly extracted with hot alcohol (95% industrial methylated spirit), with

a consequent reduction of "fat" content from an average figure of 2.0% to as low as 0.15% in some instances.

The alcoholic extract on cooling deposits a considerable amount of a white flocculent precipitate, which gives a strong positive reaction for nitrogen and is obviously of a protein-like nature. When the filtered extract is concentrated and cooled, further similar material separates.

The alcoholic extract was therefore evaporated practically to dryness and the residue repeatedly extracted with ether until no more dissolved. The ethereal solution was filtered and concentrated, and there was left a soft fatty residue, completely soluble in alcohol, ether and light petroleum, and substantially free from protein or nitrogenous protein degradation products.

This material was carefully analysed, in order to compare its chemical properties on the one hand with the average figures for butter fat, from which it was presumably derived, and on the other with those that would be expected in an anhydride of the type described by Kon & Funk. The results are given in Table I.

Table I

	Ether-soluble material from casein	Butter fat	Hydroxystearic anhydride $C_{36}H_{70}O_5$	Material of Kon & Funk
Appearance	Yellow fat	Yellow fat	—	Colourless glistening plates c. 49
Melting-point, °C.	22-39	28-33	—	—
Refractive index at 40°	1.4572	1.4531-1.4568	—	—
Reaction	Slightly acid	Slightly acid	Neutral	Neutral
Solubility in alcohol	Sol. hot, less sol. cold	Sol. hot, less sol. cold	—	Sol. hot, insol. cold
Composition: Carbon	—	—	74.15	73.90, 74.45
Hydrogen	—	—	12.11	12.48, 12.05
Mol. wt. (mean or actual)	—	(Approx. 800)	582.6	580.5, 530
Iodine value	35.2	32-45	0	—
Saponification value	222	219-233	192 (—COOH); 384 (—OH and —COOH)	—
Insoluble fatty acids + un- saponifiable matter	85.5%	87%	100%	101%
Mol. wt. of free fatty acids (mean or actual)	256	About 250	300.3	302
Reichert value	27.4	24-32	0	—
Polenske value	3.7	1.2-3.0		
Kirschner value	22.1	19-25		
Unsaponifiable matter	1.66%, 1.65%	0.5%*	0	—
Sterols	0.53%	0.2%*	0	—
Iodine value of unsaponi- fiable matter	39.5	64-70	0	—
Phosphorus	<0.0005%	—	0	—

* 0.48 and 0.21% for actual sample (N.Z.).

The absence of phosphorus from our material suggests that practically all the phospholipins pass into the cream during separation. In most other respects our material is indistinguishable from butter fat. It is, of course, certain that frequent recrystallization of butter fat from alcohol would lead to a partial fractionation and separation of the glycerides of higher saturated acids, but it is contrary to all experience for this to result in the isolation from a natural fat of a single pure constituent. We are, therefore, unable to accept the view that our material would yield a pure hydroxystearic anhydride by such treatment, and we find it difficult to believe that any is present.

Dr Kon, with whom we have had the privilege of discussing these results, thought it highly probable that the casein used by him and Funk may have aged considerably before they undertook the examination of its lipoid constituent. This might cause the small amount of "fat" spread over the surface of the granules (as distinct from the imprisoned "fat") to undergo marked chemical changes, including not only oxidation but also hydrolysis and dehydration. Such changes, we agree, are sufficiently probable to be accepted provisionally as explaining the presence of the substance isolated by Kon & Funk, which then, however, is hardly to be described as a constituent of casein fat, but rather as a decomposition product of such constituent.

We have encountered one rather curious result, the apparent threefold concentration of unsaponifiable matter in the casein fat, following the purely mechanical separation of most of the fat from the original milk. The liquid unsaponifiable matter, moreover, has been concentrated to a somewhat greater extent than the sterols, which constitute only 32% thereof, compared with over 40% in butter fat. We think this apparent "affinity" of a protein for the unsaponifiable matter of a fat may be of some interest and even of physiological significance.

REFERENCES

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