

temperatures close to and below 0° C., a different procedure involving tempering below 0° C. for longer periods of time will be necessary.

The fat of all three butters exhibited a similar dilatometric behavior, depending on the conditioning procedure, but the butters vary widely in spreadability. Since the degree of unsaturation of the three samples agreed closely, as indicated by the narrow range of iodine values, the SFI values at any temperature were dependent exclusively on the tempering procedure. It may be concluded that dilatometry can not be used for predicting the spreading quality of butter in cases of similar chemical composition of the original milk fat samples.

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INCOMPLETE RELEASE OF CITRATE DURING ACID PRECIPITATION OF CASEIN

In previous publications (2, 4) it was shown that less citric acid was found in acid sera than in skimmilk, and that this was probably caused by a loss of citrate adsorbed on the precipitated casein, and not attributable to an artificially high result with skimmilk. However, the similar citrate content of HCl and TCA acid filtrates (2) indicated a similar degree of citrate adsorption, despite the radically different surface charges of the two caseins (pH 4.5 and <1.0, respectively). This suggested that (i) citrate was bound externally at specific sites, or that (ii) the adsorption was not a peripheral surface phenomenon. The general nature of the adsorption was therefore studied by comparing the effect of trichloroacetic acid (12% final concentration) on removal of citrate adsorbed by a suspended casein, and by solubilized casein subjected to reprecipitation.

In the study with suspended casein, Van Slyke and Baker (6) casein was freed of

other milk components by seven washings in 0.1 M acetate buffer at pH 4.5 (4) and 3.0-g. portions were then suspended in 100 ml. of solution containing: (a) citrate at pH 4.5, (b) citrate + TCA at pH <1.0, (c) citrate + phosphate + lactate + calcium + TCA at pH <1.0, and (d) same as (c), but at pH 4.5. The concentrations of the various components approximated those in actual acid sera, i.e., 10 mM sodium citrate, 30 mM potassium phosphate, 3 mM sodium lactate, and 30 mM calcium chloride per liter. After a 30-min. suspension treatment, the solutions were filtered (No. 2 Whatman) and analyzed for citrate (2, 3).

In the study with solubilized casein, 100 ml. of synthetic milk solutions were prepared by dissolving 3.0 g. of Van Slyke and Baker (6) casein in the presence of (e) citrate at pH 6.7, and (f) citrate + phosphate + lactate + calcium at pH 6.7; the final concentrations of the various components were similar

TABLE 1
Adsorption of citrate by casein under various conditions

Test No.	Treatment	Composition of solution ^a	Citric Acid, $\mu\text{g}/\text{ml}$. ($\pm 1.3\%$)		% Citrate lost
			Before treatment	After treatment	
a	Suspension of casein	Citrate at pH 4.5	1,858	1,747	6.0
b	Suspension of casein	Citrate + TCA at pH <1.0	1,858	1,862	None
c	Suspension of casein	Citrate + phosphate + lactate + calcium + TCA at pH <1.0	1,856	1,853	None
d	Suspension of casein	Same as test c but at pH 4.5	1,878	1,879	None
e	Precipitation by TCA	Citrate + casein dissolved at pH 6.7	1,953	1,816 ^b	7.0
f	Precipitation by TCA	Citrate + phosphate + lactate + calcium + casein dissolved at pH 6.7	1,929	1,836 ^b	4.8

^a Concentrations added to solutions: 10 mM citrate, 30 mM phosphate, 3 mM lactate, and 30 mM calcium per liter; TCA = 12% final concentration; Casein = 3% w/v throughout.

^b Includes a correction ($\times 0.98$) to enable comparison with untreated solution containing 3% solubilized casein (4).

(i.e., within 5%) to those used in the suspension study. Aliquots of the synthetic milk solutions were treated with TCA (12% final concentration) to precipitate the casein (pH <1.0), filtered after 30 min., and the filtrate analyzed for citrate (2, 3).

Results of analyses of all filtrates obtained after the suspension or precipitation studies are given in Table 1, along with the citrate analysis of the initial solutions (4). Although citrate could be adsorbed on the surface of casein suspended at the iso-electric point (pH 4.5), this externally adsorbed citrate was completely displaced by 12% trichloroacetic acid at pH 4.5 or 1.0 (Table 1, Tests a to d). However, when casein from synthetic milk preparations was precipitated with 12% TCA, a significant proportion of the citrate was retained at the interior of the casein agglomerate (Table 1, Tests e and f). The amount of citrate so adsorbed was reduced by the presence of other milk components, but remained within a range consistent with results obtained with actual acid filtrates (2, 4).

These results suggest that 12% TCA displaces citrate superficially adsorbed on casein. However, when the protein coalesces from a milk preparation, the rapid onset of coagulation apparently prevents complete displacement of casein-bound citrate, a fraction which accounts for 8 to 14% of the total citrate in milk (5). A considerable amount of citrate is, therefore, retained within the casein particles, and its removal is difficult by virtue of its inaccessibility to an eluting agent such as TCA. The present authors have previously shown (4) that acid-precipitated casein requires seven resuspensions in equivalent volumes of fresh washing solution for complete removal of citrate. The above results substantiate the previous conclusion (4) that analysis of TCA (or HCl) acid sera does not provide

a true value for total citrate in skimmilk, but yields a result which can be from 4 to 10% low.

In a study of the citric acid method proposed by the present authors (2), Evenhuis (1) concluded that the direct method for skimmilk gave erroneously high results, and also suggested that adsorption of citric acid by precipitated protein was unlikely. However, Evenhuis' conclusion that casein interferes with the determination has not been substantiated by more recent data (4), and the present paper shows that low citrate values are obtained in acid sera. The direct method therefore appears to give a more reliable measurement of the citric acid content of milk than is obtainable by analysis of milk sera.

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