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TECHNICAL NOTES

COMPOSITION OF CASEIN IN THE SMALLEST CASEIN-CONTAINING PARTICLES OF MILK

In a previous publication *(1)*, analyses of centrifugally separated casein colloid fractions were presented which showed that over a broad size-range all of the particles contain a phosphoprotein which has the same phosphorus: nitrogen weight ratio, 0.0563. For the 12 samples analyzed, the standard deviation from this average is 0.00035. The smallest casein colloid particles, representing from 5 to 10% of the total casein, were not removed in the centrifuging times used; threfore, the results do not apply to these sizes. It has recently been reported that the constituents of the centrifugable casein micelles are in equilibrium with similar constituents free in solution *(5, 6)*. Therefore, it seemed worthwhile to make a careful analysis of the casein obtained from these smallest particles.

Portions of a sample of skimmilk were centrifuged in a Servall[1] angle-head centrifuge for 30 min., 60 min., and 2 hr., at an average centrifugal force of 27,000 times gravity, and in a 70-cc. capacity air-driven, air-floated bowl rotor for 90 min. at 490 r.p.s. In the latter case, the centrifugal force at the mid-point of the annulus was 39,500 times gravity, the time theoretically sufficient to remove all particles having sedimentation constants greater than 65×10^{-13}. The deposited colloids were discarded, the supernatant liquids from several runs accumulated, and aliquots analyzed. The caseins were then precipitated from the accumulated supernatants, at pH 4.6 in the usual way, redispersed in dilute alkali, reprecipitated, washed with water, dried and analyzed for total nitrogen, total phosphorus, and acid-soluble phosphorus.

Nitrogen was determined by a semimicro-Kjeldahl method *(1)*. Phosphorus was determined by King's semimicro colorimetric method *(2)*, using 1,2,4–aminonaphtholsulfonic acid after liquid digestion with concentrated sulfuric acid and hydrogen peroxide in the presence of selenium as a catalyst, the acid-soluble phosphorus on the hydrochloric acid extract. Separate test comparisons of the colorimetric method with a gravimetric, magnesium pyrophosphate method and a volumetric, ammonium phosphomolybdate method showed it to give results of equal accuracy.

The analyses of the four casein samples prepared as described, and other pertinent data, are given (Table 1).

It is clear that the O.P./T.N. ratio is the same for the noncentrifugable particles as for the others. The persistence of some acid-soluble phosphorus in the caseins suggests that these small particles are not free casein but, referring to the bowl rotor results, simply casein complex particles having sedimentation constants of the order of 65×10^{-13} or lower. This question could be settled by oxalate titrations of centrifuged serums by the Ling *(3)* or Pyne and Ryan *(4)* procedures.

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[1] The use of this trade name is for identification only and does not imply endorsement by the U. S. Department of Agriculture of the product or its manufacturer.

TABLE 1

Results of analyses of caseins prepared from centrifugally depleted portions of a skimmilk

Serum sample			Casein					
Centrifuging time *(min.)*	pH	Approx. % of total casein remaining	T.N.[a]	T.P.[a]	T.P./T.N.[a]	A.S.P.[a]	O.P.[a]	O.P./T.N.[a]
				Servall[b]				
30	6.72	60	15.08	0.916	0.0607	0.069	0.847	0.0562
60	6.60	40	15.23	0.962	0.0632	0.106	0.856	0.0562
120	6.62	25	14.96	0.880	0.0588	0.039	0.841	0.0562
				Bowl rotor				
90	6.72	8	14.08	0.817	0.0580	0.027	0.790	0.0561

[a] T.N. = total nitrogen; T.P. = total phosphorus; A.S.P. = acid-soluble phosphorus; O.P. = organic phosphorus.
[b] See footnote[1] in text.

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