α-LACTALBUMIN

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I. Principle

α-Lactalbumin is one of a number of proteins that occur in milk whey. This protein is found in the so-called "albumin fraction" of whey, but it is not a true albumin because it is largely insoluble in water and in dilute salt solutions at *p*H 4.0–4.6. It is quite soluble at *p*H values of 6.0 or higher, and it may then be crystallized by salting out with ammonium sulfate at *p*H 6.6–6.7. The method of preparation may be summarized as follows. Casein is separated from skim milk by acidification to *p*H 4.6. The whey proteins are then fractionated by salting out with ammonium sulfate at *p*H 6.0. A crude globulin fraction is removed at about 58% saturation (2.3 *M* ammonium sulfate). The remaining proteins, including β-lactoglobulin and α-lactalbumin, are precipitated as the crude albumin fraction at about 80% saturation (3.3 *M*). Salt is removed from the albumin fraction by dialysis at *p*H 5.2, whereupon the β-lactoglobulin crystallizes. The mother liquor from the crystallization is adjusted to *p*H 4.0, and ammonium sulfate is added to about 33% saturation (1.3 *M*). Crude α-lactalbumin is precipitated. It is dissolved at *p*H 8.0, reprecipitated at *p*H 4.0, dissolved again, and crystallized by the addition of ammonium sulfate to 50–66% saturation at *p*H 6.6; it is recrystallized by repeating these steps.

II. Starting Material

Fresh, raw skim milk can usually be obtained by arrangement with a commercial dairy. The *p*H of unpasteurized skim milk should be about 6.5–6.7.

III. Procedure

Fifteen gallons of fresh, *raw* skim milk are brought to room temperature (25°) by immersing the containers in warm water. The milk is transferred to a 20-gal. earthenware jar. Casein is precipitated by adding approximately 3 l. *N* HCl in a thin stream, the mixture being stirred gently with a wooden paddle. Under these conditions the casein is coagulated in relatively large lumps and its subsequent removal is facilitated. The final *p*H should be 4.6 (*p*H meter). The mixture is allowed to stand for 20–30 minutes, after which time the *p*H should be checked and readjusted if necessary. The casein is then removed by filtration through one or two closely woven cotton bags,[1] and the filtrate is collected in containers roughly calibrated for volume (14-qt. Pyrex glass jars are convenient). The whey is usually turbid; its volume depends on how well it is squeezed out of the bags and may vary from 41 to 52 l.

The whey is returned to the 20-gal. crock, and the *p*H is adjusted to 6.0 by the addition of *N* NH_4OH (about 18 ml. per l. of whey). The quantity of alkali is noted so that the total volume of whey is known. Solid ammonium sulfate[2] is added to a concentration of 2.3 *M* (358 gm. salt per liter of whey) to precipitate the globulin fraction. The salt is added gradually with gentle stirring by means of the wooden paddle.[3] When all the salt has dissolved, the *p*H of the mixture usually falls somewhat. It should be restored to 6.0 by adding a little *N* NH_4OH (about 3 ml. per l. of whey). The mixture is allowed to stand for at least 20 minutes, and it is then filtered by gravity through No. 14 Whatman 50-cm. folded filter papers in 8 large funnels (capacity, 1 gal.). The filtrate is again collected in the calibrated containers. The precipitate of crude globulin is discarded.

[1] One-half-bushel bags, ordinarily described as seed or soil sample bags, may be used.

[2] A good technical grade, such as Baker's Purified, should be used. (Mention of a product does not imply that it is endorsed or recommended by the U. S. Department of Agriculture over similar products not mentioned.)

[3] Mechanical stirring is not recommended because of the tendency of the solution to foam. Metal stirrers or spatulas should not be used in this preparation.

The volume of the filtrates having been noted, the clear solutions are again combined in the crock and ammonium sulfate is added as before to 3.3 *M* to precipitate the crude albumin fraction. The amount of salt necessary is calculated as follows. From the volume of combined filtrates at 2.3 *M*, the grams of ammonium sulfate present are calculated; this figure is multiplied by 0.5 (the specific volume of the salt); the product is subtracted from the volume of filtrate to give the volume of water present; for each liter of water, an additional 200 gm. of ammonium sulfate is needed to adjust the solution at 2.3 *M* to 3.3 *M*. As a useful approximation, assuming that mechanical losses in the preceding operations have not been large, the amount of salt to be added may be calculated on the basis of the original volume of whey, that is, 200 gm. of ammonium sulfate per liter of whey.

The mixture is allowed to stand for at least 20 minutes, and it is then filtered, using the 8 funnels as before. The filtrate is essentially free of protein and is discarded. The precipitated crude albumin fraction contains β-lactoglobulin, α-lactalbumin, and small amounts of other proteins. It should be permitted to drain well on the filter papers, preferably overnight, to remove as much mother liquor as possible. The precipitate must not be allowed to dry out, however; and, if drainage is continued overnight, the funnels must be covered. Each filter paper is spread out on absorbent paper, and the moist precipitate is scraped off with a porcelain spatula or a piece of cardboard and transferred to a 4-l. beaker. The protein adhering to the filter papers may be recovered by extracting them successively with two 500-ml. portions of water. The extracts are filtered through a plug of glass wool, and the combined filtrate is used to dissolve the main precipitate. Another 500 ml. of water may be used for washing during these operations. About 3 l. of cloudy, greenish yellow protein solution are obtained.

All the preceding steps may be carried out at room temperature. The next step, dialysis of the protein solution against distilled water, is best done in the cold (4–7°). Toluene is added

to the dialysis bags[4] as a preservative. Each bag must be filled not more than one-third full because of the increase in volume during dialysis.[5] Dialysis is continued until the water is essentially free of ammonium ion as shown by Nessler's reagent. The protein solution, now somewhat more than 9 l. in volume, is removed from the bags and is filtered by gravity. The clear filtrate has a *p*H of about 6.3. It is brought to *p*H 5.20 ± 0.05 by the dropwise addition of *N* HCl with slow mechanical stirring. About 45 ml. of acid are required. Seed crystals of β-lactoglobulin are added, the solution is returned to the bags, and dialysis is continued until the test for ammonium ion is negative and crystallization is complete.[6] The crystalline β-lactoglobulin is centrifuged.[7]

The volume of the clear supernatant solution is noted. The *p*H is adjusted to 4.0 by the dropwise addition of *N* HCl with slow mechanical stirring. About 35 ml. of acid are required. The solution becomes progressively cloudier, and some precipitate is formed. The precipitate, which contains α-lactalbumin, need not be removed at this point. Solid ammonium sulfate is added fairly slowly to a concentration of 1.3 *M* (187 gm. salt per liter). The *p*H of the mixture increases somewhat (to about 4.5), but no further adjustment is necessary. After about 30 minutes[8] the precipitate is centrifuged and the supernatant liquid is discarded.[9]

[4] Dialysis bags may be made from seamless cellulose tubing (Visking Corporation); the $\frac{36}{32}$-in. (inflated diameter) size is suitable.

[5] The checker found that the bags could be filled three-quarters full if they were cautiously dialyzed against small volumes of water at first. In this way the total volume of solution does not become so great, and the crystallization of β-lactoglobulin is facilitated.

[6] The checker found that 10–14 days of dialysis are required to remove all the ammonium ion and crystallize the β-lactoglobulin completely. Incomplete removal of β-lactoglobulin appears to hinder the subsequent crystallization of α-lactalbumin.

[7] β-Lactoglobulin can be prepared by the method of Larson and Jenness (see p. 23), in which the dialyzed solution is concentrated by pervaporation.

[8] If the precipitate is allowed to settle overnight, most of the supernatant liquid may be siphoned off without centrifugation.

[9] The supernatant fluid contains additional α-lactalbumin. If maximal yields are sought, the ammonium sulfate concentration may be increased to 50% satura