that perhaps the difference in assessed values in the Kies and Fox study (1971) was due to a superior ability of the rat compared to humans to utilize D-methionine.

Conventional amino acid analysis of the soy products revealed small changes in amino acid patterns for soy isolate compared to soy flour (Tables III and IV). Either these changes were too small to cause differences in nitrogen retention or they were counteracted by other factors. Feeding soy isolate supplemented with amino acids to match the amino acid pattern of flour or supplemented with small amounts of methionine and cystine led to nitrogen retentions which were not statistically different from retention with flour and isolate alone. Of course, this should not be interpreted to mean that supplementation at higher levels would not improve retention.

In summary, under the conditions of this study there were no detectable differences in protein quality for human subjects between the soy flours, soy isolate, and supplemented soy isolate as measured by the nitrogen balance technique with approximately 5 g total nitrogen intake. Obviously, similar comparisons of other flours and isolates might reveal significant differences in protein value. However, the evidence of these studies clearly indicates that it should not be automatically assumed that either soy flour or soy isolate would be significantly better in protein quality for humans, especially since processing conditions vary widely (Mattil, 1974).

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Precipitation of Proteins from Whey with Bentonite and Lignosulfonate

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A method is presented for the recovery of protein in cheese whey (0.7–0.9%) by precipitation with bentonite and lignosulfonate used separately and in combination. These reagents are compared with the commonly employed precipitant hexametaphosphate. Both bentonite and lignosulfonate precipitated most of the crude protein plus some of the nonprotein nitrogen and lactose from cottage cheese whey, pH 4.6. Precipitation with 3% bentonite yielded 38.5 g/L of dried precipitate containing 5.6 g or 92% of the crude protein, while 1% lignosulfonate yielded 12.5 g/L with 5.2 g or 85% of the crude protein. Hence, both reagents are effective precipitants of whey protein in a one-step procedure carried out at room temperature.

Over 30 billion pounds of whey result from cheese making each year, and only about half of this whey is used as animal or human food; the remainder (15 billion pounds) is wasted and often causes water pollution (Woychik, 1975). Proteins have been recovered from whey by a variety of techniques (Morr, 1976; Woychik, 1975); they have been precipitated with sodium hexametaphosphate (Hartman and Swanson, 1966; Richert, 1973; Hidalgo et al., 1973), carboxymethyl cellulose (Hidalgo and

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Table I. Precipitation of Nitrogen from Whey by Lignosulfonate at 25 °C

Percent of precipitant	pH	Precipitated, % of total nitrogen $\bar{x} \pm \sigma$	Precipitated, % of whey protein nitrogen
1	3.0	63.3 ± 1.5	87
0.5	4.6	55.3 ± 1.1	78
2	4.6	62.7 ± 5.9	90
1	9.0	15	20

Hansen, 1971), ferripolyphosphate (Jones et al., 1972), and calcium oxide (Cerbulis, 1973). The subject of this report is the recovery of proteins from whey by the application of bentonite and sodium lignosulfonate used either separately or in combination. Their effectiveness as protein precipitants is compared with hexametaphosphate.

EXPERIMENTAL SECTION

Reagents. All chemicals used were of reagent grade. Sodium bentonite (Volclay) powder was from American Colloid Co., Skokie, Ill.; sodium lignosulfonate was from Pfaltz and Bauer, Inc., Flushing, N.Y.; and sodium hexametaphosphate was from the J. T. Baker Chemical Co., Phillipsburg, N.J.

Nitrogen Determination. Nitrogen was determined by the standard micro-Kjeldahl method (AOAC, 1960); crude protein was calculated by the use of the factor 6.38 × total nitrogen. True protein and nonprotein nitrogen (NPN) were determined on the whey by dialysis employing tubing with a 5000 molecular weight cutoff (Cerbulis et al., 1972). The precipitated whey protein nitrogen was calculated assuming that only protein nitrogen would be precipitated. Therefore: whey protein nitrogen precipitated = (total whey N − supernatant N). The percent of whey protein nitrogen precipitated is defined as: (total whey N − supernatant N)/true protein N of whey. Direct analysis of nitrogen in the precipitates gave yields consistent with this differential analysis of the supernatants.

Precipitation Procedures. Cottage cheese whey (50 mL) was adjusted to the desired pH with 1 N NaOH or 1 N H_3PO_4 and stirred with the precipitants for 50 min at 25 °C. The amounts of precipitant added ranged from 0.5–3.0% by weight. The mixture was centrifuged at 1200 rpm for 10 min to separate solids. Supernatants were decanted, and Kjeldahl N was determined on each supernatant. The precipitates were washed twice with acetone, then air-dried and analyzed for total N, moisture, ash, and lactose. The weights of precipitates are reported as g/L of whey.

Ash. For ash determination, the dried precipitate was ignited in a muffle furnace at 525 °C until the ash was carbon free. The ash was cooled in a desiccator, weighed, and calculated as percent ash.

Lactose. Lactose was determined by the phenol–sulfuric acid method described by Marier and Boulet (1959).

RESULTS

Hexametaphosphate as a Precipitant. Sodium hexametaphosphate when used at 0.5 to 3% as a whey protein precipitant at pH 2.3–2.8 and 25 °C precipitated almost all of the whey protein nitrogen (88–99%) and between 63–71% of the total whey nitrogen. The present results confirm those of Hartman and Swanson (1966) and Hidalgo et al. (1973) who reported that hexametaphosphate precipitated more than 90% of the whey protein nitrogen. In the experiments reported here only 10% of the whey protein nitrogen (6.4% total) was precipitated by sodium hexametaphosphate at pH 4.6.

Table II. Precipitation of Nitrogen from Whey by Bentonite at 25 °C

Percent of precipitant	pH	Precipitated, % of total nitrogen $\bar{x} \pm \sigma$	Precipitated, % of whey protein nitrogen
2	3.0	35.5 ± 0.8	50
1	4.6	35.3 ± 1.5	54
2	4.6	61.7 ± 5.9	88
3	4.6	71.0 ± 6.5	112
1	7.0–8.3	30	41

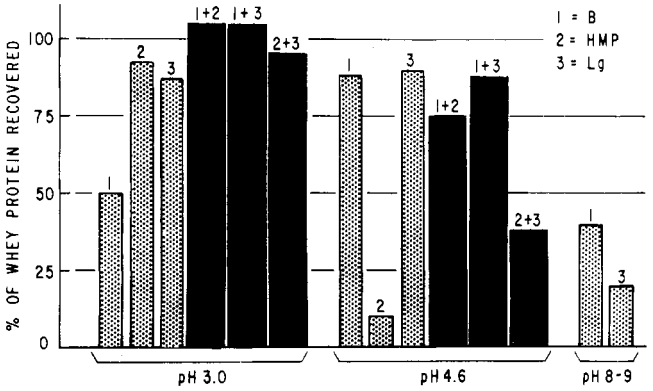

Figure 1. Precipitation of whey protein nitrogen by bentonite (B, 1), hexametaphosphate (HMP, 2), and lignosulfonate (Lg, 3) at various pHs alone at 2% or in combination (pH 3.0, 1% each; pH 4.6, 0.5% each). Details of experiments are described in the Methods and Materials section.

Lignosulfonate as a Precipitant. The results of the experiments using lignosulfonate as a precipitant are given in Table I. The reaction of lignosulfonate is pH dependent. At acid pH (3.0 and 4.6), this reagent precipitated 87–90% of whey protein nitrogen, but only 20% at pH 9.0. At pH 3, the reagent is nearly as effective as hexametaphosphate. But at pH 4.6, the natural pH of cottage cheese whey, lignosulfonate which precipitated 80–90% of whey protein nitrogen is a much better reagent than hexametaphosphate which precipitated only 10% of whey protein nitrogen. These data indicate that lignosulfonate could be used directly on cottage cheese whey to recover protein without altering the pH.

Bentonite as a Precipitant. At 25 °C, 1% bentonite applied to whey at pH 2.7, pH 4.6, or pH 7.0–8.3 was not an effective precipitant of whey protein nitrogen (Table II). When the bentonite concentration in the pH 4.6 reaction mixture was increased to 3%, the estimated yield of whey protein nitrogen was increased to over 100% (72% of total whey nitrogen). Bentonite apparently also removed most of the riboflavin from the whey leaving a colorless supernatant.

Combination of Precipitants. The precipitants were used in pairs at low concentrations; these data are compared with the values obtained for the reagents alone (2%) in Figure 1. At pH 4.6 lignosulfonate and hexametaphosphate are apparently antagonistic; a combination of 0.5% bentonite with hexametaphosphate (0.5%) decreased to 74% while bentonite (0.5%) with lignosulfonate (0.5%) increased the amount of whey protein nitrogen recovered to 87%. At pH 3.0, 1% bentonite in combination with either 1% hexametaphosphate or lignosulfonate gave 105% precipitation of whey protein nitrogen, indicating the precipitation of some nonprotein nitrogen. The combination of lignosulfonate and hexametaphosphate was almost equal to the others at pH 3.0. From the data in Figure 1 it appears as though the reagents, when used in

Table III. Composition of Dried Precipitates

	3% B,[a] pH 4.6	1% Lg,[b] pH 4.6	0.5% Lg, 1% B, pH 3.0	1% HMP,[c] 1% B, pH 3.0	1% HMP, pH 3.0
Moisture, %	1.9	3.0	4.1	4.3	2.4
Ash, %	69.3	8.1	45.0	57.9	12.3
Crude proteins, %	14.7	41.5	25.5	30.3	72.5
Lactose, %	11.8	23.0	15.0	9.7	10.8
Others, %	2.3	24.4	10.4	3.8	2.0
Yield of precipitate, g/L	38.5	12.5	23.0	20.0	8.3
Estimated protein recov., g/L	5.6	5.2	5.9	6.0	6.0

[a] B = bentonite. [b] Lg = lignosulfonate. [c] HMP = hexametaphosphate.

concert, are more effective precipitants of whey protein nitrogen at lower concentrations than are the reagents employed alone at comparable or higher concentrations.

Analysis of Precipitates. The compositions of selected precipitates were studied in detail. The results along with the yields are summarized in Table III. The protein recovery obtained at 3% bentonite was estimated to be 100%. The composition of the precipitate was as follows: crude protein 14.7%, lactose 11.8%, ash 69.3%, and moisture 1.9%. Nearly all of the bentonite occurs in the precipitate as evidenced by the high ash content of the precipitate.

One percent lignosulfonate at pH 4.6 removed 90% of whey protein nitrogen in a single precipitation step at room temperature. This product has a lower total yield due primarily to a lower ash content. Since lignosulfonate has a low nitrogen and ash content, its contribution to the precipitate appears in the "others" column. In the case of bentonite the high ash content comes primarily from the reagent. The lignosulfonate product contains a somewhat higher lactose content than the other products. The composition of the products obtained from combinations of precipitants are what might be predicted from the data obtained when the precipitants were used alone.

DISCUSSION

Cottage cheese whey protein nitrogen was completely precipitated by 3% bentonite at pH 4.6. Nearly all of the bentonite was found in the precipitate, leaving a colorless lactose-containing supernatant. One percent lignosulfonate at pH 4.6 approaches the effectiveness of 3% bentonite. Hexametaphosphate by comparison did not appreciably precipitate whey protein nitrogen unless the pH was decreased below 3. Disposal of the highly acidic supernatant could pose difficulties.

All of the products obtained in these studies contain an equivalent amount of protein nitrogen, although the percent protein varies because the amount of precipitant carried over into the product varies. The occurrence of the precipitants in the product may not be harmful, but could be beneficial. All three reagents have been used as feed or food additives and the toxicity should not be a problem provided that the components of feeds are mixed to keep the final concentration of precipitants at their optimal levels for the animals.

Hexametaphosphate is a common additive to human food (Chapman and Pugsley, 1971); no reports were found in the literature about the limits of its use in animal feed, although the LD_{50} for female rats was reported to be 2.9 g/kg.

Bentonite has been widely used as a binder, emulsifier, plasticizer, and clarifying agent (American Colloid Co., 1970). In the food industry bentonite has been used as a clarifying agent in wineries, a binder in feed pelleting, and even as a feed ingredient (*Chem. Eng. News*, 1973). At 1–3% dietary levels bentonite improved the performance of broilers, but at 5–10% depressed chick performance, indicating that bentonite is not a source of energy (Day et al., 1970). The beneficial effect (maintaining fat test and increasing milk yield) of bentonite added at the 5% level to a high grain ration for dairy cattle may result from a slower rate of passage of the feed through the digestive tract (Brindge and Schultz, 1969; Rindsig et al., 1969). Also the increased feed efficiency observed in poultry from bentonite addition could be due to a decreased rate of passage (Kurnick and Reid, 1960). Hence, the bentonite–whey protein product may have a potential as a supplement in feed for livestock. Brethour (1976) reported that bentonite improved cattle weight gain and increased nitrogen absorption.

Lignosulfonate has been used in industry as an adhesive binder (Goheen et al., 1967) and as a binder for pelleting of feed (*Fed. Regist.*, 1969). Rosen (1974) reported that sodium lignosulfonate (alprecin) was used in the Alwatech process to recover fat and protein from poultry processing plants' waste waters. The process was used in 18 units in Western Europe. The Alwatech process was introduced in the United States under the name ALTRA process (Crocco, 1975). In a related study Herstad and Hvidsten (1973) reported that with 13% lignosulfonic acid in the diet, the weight gain of chicks was considerably reduced. However, Conrad (1972) found that up to 2% ammonium salts of lignosulfonates could be used in dairy cattle rations without adversely affecting the feed intake. Thus the lignosulfonate–protein product could also be used as a feed. An additional value of lignosulfonate as a precipitant may arise from the fact that the protein and sulfur occur together, since lignosulfonate has been used a a source of dietary sulfur for lactating cows (Bouchard and Conrad, 1973).

In comparison with ultrafiltration and reverse osmosis, the present precipitation methods do not require expensive and complex equipment and could be applied to acid whey by small manufacturing plants who do not now process whey and may have to pay a penalty to discharge it. The precipitate may be filtered, centrifuged, or even allowed to sediment by gravity and then the supernatant, which now contains a lowered nitrogen content, may be decanted. Bentonite and lignosulfonate may be used directly without pH adjustment and in themselves increase the utilization of the recovered nitrogen by the methods discussed above. Although a feed grade product is produced, bentonite is relatively inexpensive and offers an alternative to pollution. Further research including feeding trials will be necessary in order to prove the effectiveness of these products.

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Protein Precipitation Method for the Quantitative Determination of Tannins

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The tannin content of crude plant extracts or of purified preparations was determined by adding the sample to a standard solution of protein, isolating the insoluble tannin–protein complex, dissolving it in alkaline solution, and measuring the absorbance at 510 nm after adding ferric chloride. Plots of absorbance as a function of the amount of tannin are linear for tannic acid and partially purified sorghum tannins for amounts of tannin ranging from 0.20 to 1.0 mg. Nontannin components of crude methanolic extracts of sorghum and cowpeas do not interfere with the assay. The results of the precipitation method are qualitatively similar to results obtained with the vanillin assay. The precipitation assay can be used to study the effects of pH and other parameters on tannin–protein interactions.

Tannins are polyphenolic compounds which form insoluble complexes with proteins (Swain, 1965). They are present in a wide variety of plants used for foods and feeds including sorghum (Bate-Smith and Rasper, 1969), beans (Martin-Tanguy et al., 1977), barley (Bate-Smith and Rasper, 1969), millet (Ramachandra et al., 1977), and some legume forage species (Jones et al., 1976). The interactions of tannins with proteins may play a role in the antinutritional effects of tannin-containing feeds which have been observed in nonruminants (Tamir and Alumot, 1970; Jambunathan and Mertz, 1973; Schaffert et al., 1974; Martin-Tanguy et al., 1977); tannin-containing forages may be useful in the control of bloat in ruminants (Driedger and Hatfield, 1972).

Tannin content is usually determined by assays such as the vanillin test (Burns, 1971), the Prussian Blue test (Price and Butler, 1977), and the Folin-Denis test (Burns, 1963), which are based on the chemical characteristics of tannins. An assay for tannins based on their ability to precipitate proteins might provide useful information about the nutritional value of foods and feeds which contain tannin. Existing precipitation methods are of limited value. The official method of the Association of Agricultural Chemists (1965) for the determination of tannins in tea, based on the precipitation of gelatin, has been reported to be of little value for the determination of tannin in sorghum grain (Maxson and Rooney, 1972). Bate-Smith (1973) has suggested an assay based on the precipitation of hemoglobin by tannins. This method is inconvenient because freshly drawn blood is used, and saponins and other plant metabolites interfere with the assay (Bate-Smith, 1977). Goldstein and Swain (1965) have suggested a method based on the ability of tannins to inhibit the enzymatic activity of β-glucosidase, but the results of the assay are difficult to interpret, because the relationship between enzymatic activity and the formation of insoluble complexes is not fully understood.

The precipitation method described here does not suffer from the above disadvantages. It is rapid, reproducible and can be used with either condensed or hydrolyzable tannins. Purified extracts containing only polyphenolic components or crude extracts containing phenolic and nonphenolic components can be analyzed with this technique.

MATERIALS AND METHODS

Materials. Reagent grade chemicals were used throughout. The standard protein solution, 1.0 mg/mL

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