DAIRY FOODS TECHNICAL NOTES

Extraction of Whey Proteins with Magnesium and Zinc Salts

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ABSTRACT

A method was developed for the extraction of protein from cottage cheese whey with magnesium and zinc salts. After neutralization of the whey, both magnesium and zinc salts (at 4 and 2% wt/vol, respectively) precipitated most of the protein nitrogen plus some nonprotein nitrogen and lactose. Calcium at these concentrations was only partially effective. Yields of dried precipitate obtained from cottage cheese whey containing approximately 7% solids ranged from 25 to 34 g/L. Precipitates contained 16 to 21% protein and from 11 to 24% lactose; the remainder was ash. Zinc was the preferred reagent because it was effective at neutral pH; magnesium, alone or with calcium, required alkaline pH for thorough precipitation of protein. All reagents tested were effective precipitants of whey protein in a one-step procedure at room temperature.

INTRODUCTION

Over 16 billion kilograms of whey are produced annually by the cheese industry in the United States; about 10% of this comes from cottage cheese manufacture. This whey contains nearly 90 million kilograms of protein, of which little more than half is utilized as feed or food (15). The remainder, including most of the cottage cheese whey, is considered as waste and often causes water pollution (12). Various processes have been developed for the recovery of whey components, but these have not been as universally adopted by the industry as had been projected (10). Many of these processes are both complicated and require considerable capital investment. This research was undertaken to recover efficiently and economically feed grade protein by precipitation from cottage cheese whey utilizing magnesium, zinc, and calcium salts.

MATERIALS AND METHODS

Reagents

All chemicals used were of reagent grade.

Lactose and Nitrogen Determination

Lactose and nitrogen were determined by the phenol-sulfuric acid method (5) and the standard micro-Kjeldahl method (1), respectively. Crude protein was calculated by multiplying the total nitrogen by the factor 6.38. True protein and nonprotein nitrogen (NPN) were determined after separation by dialysis in tubing with a 5000 M_r cutoff (4). Precipitated whey protein nitrogen was calculated as previously described (2). Phosphate was determined by the molybdenum blue micromethod as previously described (3).

Precipitation Procedures

Cottage cheese whey was obtained from a local commercial plant and was produced by fermentation; all operations employed 50-ml batches. Sodium hydroxide was added as one normal solution to adjust pH. Solid reagents were added to the final concentration given in the tables and the mixture stirred for 20 min at room temperature. The reaction mixtures were centrifuged at 1200 rpm for 10 min. Nitrogen and lactose were determined in the supernatant.

Yields and Analysis of Precipitates

The precipitates were washed with acetone and dried at 105°C. Lactose and nitrogen were determined on the dry solids.

For ash determination, dried precipitate was ignited in a muffle furnace at 525°C until the

Received June 24, 1985.
[1] Agricultural Research Service, US Department of Agriculture.

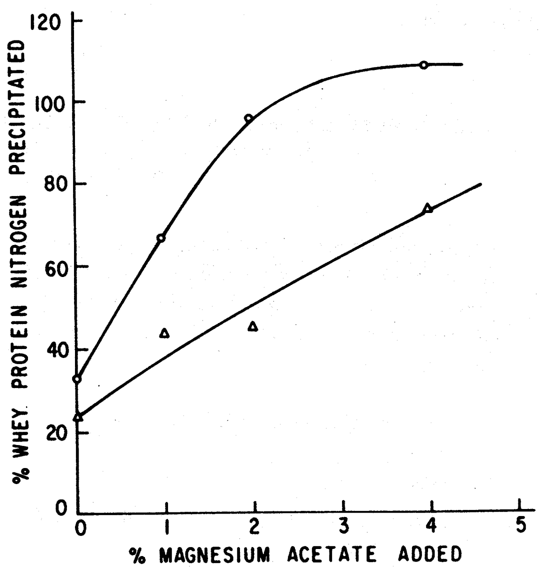

Figure 1. Precipitation of whey protein nitrogen by various concentrations of magnesium acetate at pH 10.5 (○) and 6.7 (△). Precipitation was described in Materials and Methods.

ash was carbon free. The ash was cooled in a desiccator, weighed, and expressed as percent ash.

The results reported represent the average of three trials with three different commercial whey samples.

RESULTS AND DISCUSSION

Magnesium as a Precipitant

Precipitation of whey nitrogen-containing compounds was rapid and complete within 10 min. No additional precipitate formed when the supernatant was allowed to stand for several hours. Neutralization of cottage cheese whey with NaOH without added salts resulted in the precipitation of 20 to 30% of the whey protein nitrogen at pH 6.7 and 10.5, respectively (Figure 1). This may be the result of coprecipitation of the N with the insoluble calcium phosphate formed upon neutralization of the acid whey. By increasing the amount of Mg^{2+}, more whey protein nitrogen was precipitated. At pH 10.5, 4% magnesium acetate was a very effective precipitant of whey protein nitrogen.

Neutralization with $Ca(OH)_2$ in place of NaOH (Table 1) precipitated 54% of the whey protein nitrogen. Data in Table 1 show that a combination of 1% $Ca(OH)_2$ and 1% magnesium acetate precipitates nearly all whey protein nitrogen and is more effective than $Ca(OH)_2$ alone. A reaction mixture containing 2% magnesium acetate and 2% $Ca(OH)_2$ caused nearly complete precipitation of total whey nitrogen. In other experiments, concentrations of magnesium acetate and $Ca(OH)_2$ (4% total) also precipitated NPN-containing substances yielding over 90% of the total whey N in the precipitate.

Zinc as a Precipitant

Addition of zinc acetate to whey resulted in nearly complete precipitation of whey protein N as well as part of NPN; however, precipitation is highly dependent upon pH (Table 2). Maximal precipitation of whey protein occurred near neutrality, preventing the need for alkaline pH that can cause undesirable side reactions between protein and lactose. The neutralized

TABLE 1. Precipitation of nitrogen from whey by calcium and magnesium salts.

		Precipitated, %	
Precipitant	pH	Total nitrogen	Whey protein nitrogen
2% $Ca(OH)_2$	11.6	36.0	54.0
1% Mg acetate[1] + 1% $Ca(OH)_2$	10.9	62.3	98.0
2% Mg acetate + 1% $Ca(OH)_2$	9.9	74.5	100
2% Mg acetate + 2% $Ca(OH)_2$	10.9	83.7 ± 5.4[2]	100
2% Mg acetate, NaOH	9.9	58.6	95.5

[1] $Mg(C_2H_3O_2)_2$"$4H_2O$.

[2] Mean and standard deviation for four trials with four separate wheys.

TABLE 2. Precipitation of nitrogen from whey by zinc salts.

Precipitant	pH	Precipitated, % Total nitrogen	Precipitated, % Whey protein nitrogen
2% Zn acetate	3.5	8.8	14.2
2% Zn acetate + NaOH	5.5	48.7	79.4
2% Zn acetate + NaOH	7.0	69.0	100
2% Zn acetate + NaOH	11.4	46.1	83.5
2% Zn acetate + 1% $Ca(OH)_2$	6.2	89.3 ± 6.9[1]	100
2% Zn acetate + 2% $Ca(OH)_2$	7.1	74.0	100

[1] Mean and standard deviation of three trials with three different wheys.

supernatant would be easier for processing. Therefore, zinc acetate appears more practical as whey protein precipitant than $Ca(OH)_2$ or magnesium acetate. Precipitation is independent of the base used; sodium, potassium, or calcium hydroxide may be employed depending on the desirability of ingredients in the final products; $Ca(OH)_2$ increased total nitrogen precipitation (Table 2), particularly at pH 6.2.

Remaining Supernatant

Total solids and ash contents remaining in the supernatants after precipitation were determined and compared with those of the starting whey (Table 3). Ash content increased in all the supernatants; clearly part of the added inorganic reagents remained. Total solids content of the wheys after protein precipitation was not appreciably changed, probably because of the added salts.

Composition of Precipitates

Total yields of 25 to 34 g/L precipitates were obtained (Table 4). The precipitates contained 16 to 20% protein, up to 24% lactose, and 40 to 50% ash. Use of $Ca(OH)_2$ in place of NaOH yielded a high Ca:P ratio, which could be beneficial if other feed components have a high phosphate content. The zinc acetate-$Ca(OH)_2$ precipitate contained nearly 15% zinc and is thus comparable in metal content to zinc proteinate (specified to contain at least 9% zinc), which is commercially available (Zincpro Corp[2]) as a feed nutrient for livestock and poultry. Dried acid whey composition is included for comparison; note the increased metal ion and protein contents for the products studied here.

Salts of magnesium and zinc with added calcium have been employed for recovery of proteins from acid whey. Precipitation processes are pH-dependent, and adjustments do not require expensive and complex equipment. Zinc is more desirable than magnesium in that the precipitation occurs at neutral pH, whereas magnesium requires high pH, which may result in loss of nutritional quality.

Magnesium, zinc, and calcium are essential nutrients required by all livestock; the amount of zinc needed for nutrition is very small, but its presence (13) is critical for optimum per-

[2] Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

TABLE 3. Solids and ash content in selected supernatants and in acid whey.

	Solids	Ash
	(%)	
Cottage cheese whey[1]	7.1	.88
Magnesium acetate supernatant[2]	7.1	2.47
$ZnCl_2$ Supernatant[3]	8.1	2.00

[1] Obtained from a commercial plant.

[2] 2% Magnesium acetate, 1% $Ca(OH)_2$, pH 9.9.

[3] 2% $ZnCl_2$, 2% $Ca(OH)_2$, pH 7.1.

TABLE 4. Yields and composition of selected precipitates.

Precipitant	Crude protein	Lactose	Ca	Zn	Mg	PO_4	Ash	Yield (g/L)
	(%)							
4% Mg acetate + NaOH, pH 9.9	16.0	21.9	...[1]	...	...	...	43.5	32.0
2% Mg acetate + 1% $Ca(OH)_2$, pH 9.9	17.0	24.0	11.3	...	7.0	5.0	43.0	34.0
2% Zn acetate + NaOH, pH 7.0	20.7	10.9	...	...	...	...	43.2	25.0
2% Zn acetate + 2% $Ca(OH)_2$, pH 7.1	16.5	21.0	12.2	14.6	...	4.7	50.0	34.0
Dried acid whey[2]	11.7	73.5	2.1	<.1	.2	1.3	10.8	...

[1] Means not determined.

[2] Reference (11).

formance by livestock. Zinc is added to feeds to improve weight gain of animals as well as to cure and to prevent parakeratosis (6, 7, 8). Magnesium is essential to prevent grass tetany in cattle and sheep (14). All these nutrients are added to milk replacers. Use of cottage cheese whey protein in this manner could aid in the further utilization of this resource. Modler and Emmons (9) completed similar studies on cottage cheese whey; after neutralization with CaO, remaining supernatants were dried and were acceptable products. The zinc process reported here would lead to recovery of proteins now contributing to pollution. Secondarily, the neutralized supernatant could be more readily recovered and possibly used as a source of nonprotein nitrogen or recycled precipitating agent.

ACKNOWLEDGMENT

The authors would like to thank E. Della-Monica of this laboratory for his analysis of calcium, magnesium, and zinc by atomic adsorption spectroscopy.

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