

Application of Steffen Process and Its Modifications to Recovery of Lactose and Proteins from Whey

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The Steffen process, which utilizes CaO for precipitation of sucrose in molasses, was applied to the recovery of lactose from cheese whey. In the cold precipitation step, up to 81% of the total

lactose and 64% of the total nitrogen present were precipitated. Lactose yields were improved (87-95%) when FeCl₃ was used in combination with CaO.

In the beet sugar industry, the Steffen process (Schneider, 1968; Silin, 1964; Steffen, 1878; Terek *et al.*, 1970; Tschersich and Richey, 1971) has been widely used for the recovery of sucrose present in molasses. The process involves precipitation of sucrose with CaO at low temperature (0-10°) as the tricalcium saccharate. Sucrose is recovered in aqueous solution by carbonation of the tricalcium saccharate suspension. The Steffen process, which has not been utilized for the recovery of reducing sugars, was investigated as a possible method for lactose recovery from cheese wheys.

Liquid whey contains about 5-6% lactose, which is a suitable concentration for precipitation by the Steffen process. Reducing sugars such as lactose are more sensitive to alkaline reagents than nonreducing sugars; therefore, the complete process must be carried out at low temperature to avoid the destruction of lactose. Application of the Steffen process, either alone or in combination with FeCl₃, for the recovery of lactose and protein in whey is the subject of this report.

EXPERIMENTAL SECTION

Reagents. All chemicals used were of a reagent grade. CaO was low in fluorine. Preparation of 1:12 ferric polyphosphate reagent (Jones *et al.*, 1972): Fe/P mole ratio 1:12 (0.167 M in Fe). One volume of 0.5 M FeCl₃ was mixed with 2 vol of 3 M Calgon (sodium polyphosphate glass, with the average chain length of 11.2; from Calgon Corporation, Pittsburgh, Pa.). The reagent was stored at 4°.

Lactose and Nitrogen Determination. Lactose was analyzed by the phenol-H₂SO₄ method as described by Marier and Boulet (1959). Nitrogen was analyzed by the standard micro-Kjeldahl method, AOAC (1960); total protein was determined by use of the factor 6.38 × total nitrogen.

Precipitation Procedures. Fresh acid whey (pH 4.7) was cooled to 3-5° and maintained at that temperature for all subsequent operations.

CaO. Two-hundred milliliters of whey (9.5 g of lactose) and 12.5 g of finely powdered CaO (130 parts of CaO to 100 parts of lactose) were mixed together and vigorously stirred with a mechanical stirrer for 45-60 min and then centrifuged. The precipitate was freeze-dried.

CaO + FeCl₃. A 50-ml 10% FeCl₃·6H₂O solution was added to a mixture of whey (200 ml) and CaO (12.5 g) and stirred for 45-60 min; the precipitate was collected by centrifugation and freeze-dried.

FeCl₃. Two-hundred milliliters of acid whey (pH 4.7) was mixed with 2.7 g of FeCl₃·6H₂O to give a pH 2.3 solu-

tion. Precipitation did not occur until the pH was raised to 3-4 by the addition of a 1 N NaOH solution with agitation. Stirring was continued for 10 min, the reaction mixture was centrifuged, and the precipitate was freeze-dried.

Organic Solvents. When acetone or methanol was used as an additional precipitant in the above three methods (CaO, CaO + FeCl₃, FeCl₃), it was added to the reaction mixture 10 min before centrifugation. Usually an equal volume of acetone or methanol was added and the reaction mixtures were stirred for 10 min and centrifuged, and the precipitate was freeze-dried.

CO₂ Treatment. The CaO and CaO + FeCl₃ precipitates, obtained by the above three methods, were suspended in water (approximately 4% concentration) and saturated with CO₂ while maintaining vigorous stirring. The CaCO₃ precipitate was centrifuged off and the aqueous protein-lactose fraction was concentrated to a small volume in a rotatory evaporator at 40° and freeze-dried.

The freeze drying was an expedient for the present research and was not a part of the Steffen process.

RESULTS AND DISCUSSION

The results of all the experiments described above are summarized in Table I.

CaO as a Precipitant. The reaction of CaO with lactose and whey nitrogen-containing compounds was relatively slow and the percent of precipitate was a function of time. Time studies showed that the reaction was not complete even after 75 min of stirring, but a reaction time of 60 min was selected when 66-81% of the lactose and 64% of the total nitrogen were precipitated (Table I). It was observed that additional precipitate was formed in the supernatant several hours after centrifugation. However, the amount of additional precipitate was negligible and was not taken into account. Experiments showed that 60 to 90 min of reaction time was recommended for the precipitation reaction. The yield of lactose from whey was lower than the 88-97% of sucrose recovered from molasses (Rogina *et al.*, 1966; Schneider, 1968; Silin, 1964; Terek *et al.*, 1970).

In contrast to the sugar beet proteins, which remain in the supernatant, most of the whey nitrogen-containing compounds were precipitated with CaO. Deproteinization of whey by dialysis (dialyzable fraction was used) or by heat treatment of whey at 95° for 30 min did not affect the precipitation of lactose.

No difference was observed in lactose yields when CaO was added to whey in a single addition or a stepwise addition; the bulk addition was preferred for convenience.

The yield of lactose was dependent on the concentration of CaO and the temperature of reaction mixture. Usually, 115-130 parts of CaO were required for 100 parts of sucrose in molasses (McGinnis, 1951; Silin, 1964). It was found by present experiments that 130 parts of CaO to 100 parts of lactose was the optimum ratio in whey. Higher

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Table I. Precipitation of Lactose and Nitrogen from Whey

	Precipitant	Precipitated, % of total	
		Lactose	Nitrogen
5% Lactose solution	CaO	59	
	CaO, MeOH	90	
Deproteinized whey ^a	CaO	66-70	
	CaO, MeOH	87	
	CaO, acetone	94-96	
Acid whey	CaO	66-81	64
	CaO, FeCl ₃	87-95	62
	CaO, MeOH	90	91
	CaO, acetone	99.5	82
	CaO, FeCl ₃ , acetone	95-99	82-87
	Ferripolyphosphate ^b	^c	80
Deproteinized whey ^b	CaO	68	60 ^d
	CaO, acetone	99	60 ^d
Acid whey	FeCl ₃ , pH 4.0	Traces	41.0
	FeCl ₃ , pH 5.0	38	89.4
	FeCl ₃ , pH 7.0	64.2	93.6
	FeCl ₃ , pH 9.0	21	51.5
	FeCl ₃ , reagents adjusted to pH 6.5 before mixing	33	59

^a Deproteinized by dialysis or heat treatment. ^b Deproteinized by 1:12 ferripolyphosphate (Jones *et al.*, 1972); 25 ml of 1:12 reagent was added to 100 ml of acid whey. ^c Not detected. ^d Calculated on the basis of nitrogen remaining after treatment of whey with ferripolyphosphate (Jones *et al.*, 1972).

ratios of CaO added to whey lactose (>130:100) did not affect the yield of lactose, but lower ratios decreased the yield of lactose considerably. The optimum reaction temperature for lactose was at 3-5°, similarly as recommended for sucrose (0-10°). When the reaction was carried out at ambient temperatures (25°), the yield of lactose was approximately 30% lower than that obtained at 3-5°. When the Steffen process is applied to molasses mother liquor, an additional yield of sucrose is obtained by heating to 85-90°; however, heating the whey supernatant destroys essentially all of the lactose in a few seconds. Possibly saccharinic acids are formed in this treatment.

The change of lactose concentration in whey was not feasible from the practical point of view. Liquid whey contains about 5% lactose which is the proper concentration for the Steffen process. Dilution of whey would just increase the volume. Evaporation of whey to decrease volume is an additional treatment and is very costly.

CaO + FeCl₃ as Precipitant. Use of CaO as a precipitant does not result in the total precipitation of lactose or nitrogen-containing compounds from whey. In an effort to increase these yields, CaO was used in conjunction with FeCl₃, which has been used as a precipitant for sugars and nitrogen-containing compounds. Traube *et al.* (1933) used FeCl₃ in combination with BaCl₂ and NaOH to precipitate sugars and sugar alcohols. Siegfried (1895) and Block *et al.* (1953) used FeCl₃ to precipitate proteins and polypeptides from whey. The combination of CaO and FeCl₃ increased the lactose yield by 14-21%; however, no increase in total nitrogen precipitation was obtained (Table I). Possibly the pH was too high to precipitate all proteins.

FeCl₃ as Precipitant. Block *et al.* (1953) obtained complete precipitation of whey proteins using FeCl₃; however, the effect of FeCl₃ on lactose precipitation was not reported. The present studies showed that the yields of lactose and proteins precipitated by FeCl₃ were the function of pH and, at neutrality, approximately 64% of lactose could be recovered (Table I). At pH 5-7 the proteins were precipitated completely.

CaO Precipitate. Water in which the CaO precipitate is suspended has a pH 11 and, to avoid undesirable effects on proteins and lactose, the product must be dried immediately or neutralized with H₃PO₄ or CO₂ (carbonation). The freeze-dried CaO precipitate is a grayish powder and contains approximately 35% lactose, 5% protein, and 50% CaO. The H₃PO₄ neutralized product is a white powder with a yield of 225 g/l. of whey and contains approximately 20-25% lactose, 4% proteins, 15-20% Ca, and 10-15% P.

Lactose purification is obtained by carbonation of the fresh CaO precipitate, which neutralizes Ca(OH)₂ to CaCO₃ and leaves lactose in solution. Twenty to twenty-five percent of the total nitrogen is recovered with the lactose and the remainder (75-80%) is precipitated with the CaCO₃. This is in contrast to the sugar beet molasses precipitation, where all nitrogen is precipitated with CaCO₃.

Lactose is stable in the described cold precipitation procedure. Using ion-exchange, thin-layer, and paper chromatographic analysis of the wet CaO precipitate, no sugars other than lactose were present after 24 hr of storage at room temperature (25°). Some H₂S odor was detected when the precipitate was acidified with HCl following 24 hr of storage at room temperature; this reflects degradation of sulfur-containing amino acids.

Addition of Acetone and Methanol to the Reaction Mixture. CaO, FeCl₃, or CaO + FeCl₃ did not precipitate completely either lactose or nitrogen containing compounds from whey. Use of alcohols to precipitate whey proteins and lactose (Morr and Lin, 1970) seemed to be feasible in the present research.

Addition of equal volumes of acetone or methanol to the reaction mixture before centrifugation gave almost total precipitation of lactose and nitrogen from whey (Table I). A combination of CaO treatment with acetone usually precipitated 94-99.5% of lactose and 82% of total nitrogen from solution. Methanol precipitated more nitrogen from whey, but slightly less lactose than did acetone.

Deproteinization of Whey with "Ferripolyphosphate." Jones *et al.* (1972) have used "ferripolyphosphate" to precipitate proteins from whey. They precipitated essentially all of the proteins from acid whey but lactose was left in solution.

In the present research, the Steffen process was applied to the protein-free supernatant obtained after the addition of ferripolyphosphate. Using CaO and acetone, lactose was precipitated completely (99%) (Table I). The freeze-dried product, obtained in this precipitation, was white powder.

SUMMARY

It appears that the Steffen process could be used for the recovery of lactose from cheese whey. In a single precipitation step, the bulk of lactose and nitrogen-containing compounds are removed from whey. Lactose is stable in the cold precipitation step. In comparison with the ultrafiltration and the reverse osmosis methods, the Steffen process does not require expensive and complicated apparatus.

The disadvantages for the Steffen process application to whey are the sensitivity of lactose at higher temperatures, and the incomplete separation of lactose from whey nitrogen-containing compounds in the carbonation process. Precipitates containing lactose, nitrogen-containing compounds, and CaO cannot be kept indefinitely. To avoid possible destruction of nitrogen-containing compounds and possibly, lactose, the CaO precipitates must be either neutralized or carbonated shortly after the precipitation step is completed.

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Whey Solids as Agricultural Foam Stabilizers

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"Chemifoams" were custom-tailored using whey solids and a mixture of whey solids and animal hide glue as stabilizers for protecting row crops from frost and freeze damage, as carriers for herbicides, fungicides, defoliant, and the like, as foam covers after soil fumigation with gaseous fumigants, and as foam markers. A foam containing 1.5% whey solids and 2% Hi-X, a surfactant, was a satisfactory carrier for the herbicide Karmex. A heavier more durable foam containing 1% whey solids, 1% animal hide glue, 0.3% Natrosol, and 2% Hi-X, called the "standard formulation,"

showed improved persistence of 200% at 36°F, 386% at 68°F, and 400% at 73°F. Several variables affecting the standard were examined. Reduction in temperatures caused an orderly increase in persistence of the foam. Maximum persistence was obtained at 67 and 73°F for 2-day-old formulations and at 36°F for 3-day-old formulations. Adjustment of pH to any but the normal pH of the standard was found unnecessary. Optimum concentrations of Natrosol and surfactant were determined and possible dilution of standard was examined.

Agricultural foams ("chemifoams") are generated to give frost and freeze protection to row crops, to act as carriers for herbicides, fungicides, defoliant, and the like, to serve as soil covers after soil injection with volatile fumigants, and to act as farm markers. Application of foams for these purposes *via* ground-operated equipment minimizes pollution in that chemicals are not carried through the air by wind, prevents drift, and reduces total chemicals required. For the above purposes, foams generally contain stabilizers to give them strength, form, the capability of producing skins, extended use life, self-destructiveness of foam and skin, absence of phytotoxicity, and economic feasibility.

Whey solids recovered from whey, the byproduct of cheese manufacture, have potential as a stabilizer for chemifoams. Whey has a high biological oxygen demand and has long been a prime pollution problem in areas where large cheese manufacturers are operating (Chem. Eng. News, 1971). The environmental problem of disposing of whey from cheese manufacture became sharply critical in 1970. Published statistics on whey utilization (Mathis, 1970) show that approximately 11 billion lb of whey out of a total of 21.4 billion lb produced annually are returned to farms for feed or fertilizer, or they are wasted, the last amounting to nearly half of the whey returned. Profitable new uses are urgently needed. Basically, the

components present are protein (lactalbumin), lactose, and salts. It would be preferable to utilize this material in foods and feeds but apparently the current market suffers from a lack of outlets and other outlets, even industrial ones, should be considered.

Whey is dried by spraying into hot air or by coating it on the surface of steam-heated rollers. The least expensive of the products is roller dried and is suitable only for animal feed (Webb, 1970). It is this last product which has been found utilizable in chemifoams either alone or in combination with other materials. This paper presents data to show that inedible whey solids can be substituted for gelatin (Braud and Chesness, 1968) or animal glue (Lambou *et al.*, 1972) in the preparation of long-lasting chemifoams, shows that their performance can be improved greatly by combining with additional protein-containing materials, and discusses some of the parameters and their effects on the characteristics of these foams.

EXPERIMENTAL SECTION

Materials. Listed in Table I are products from seven companies recovering both edible and inedible whey solids in a range of prices and composition. Edible whey solids are spray dried and most feed-grade whey solids are roller dried. Actually all of the whey solids listed produce good foams. An inedible whey product was selected for further study because of its price differential and ease of handling in evaluating a number of variables affecting pertinent agricultural foam characteristics.

Also selected for study were: surfactant Hi-X (a modified alkylsulfate), Walter Kidde & Co., Inc.; viscosity-builder Natrosol (hydroxyethylcellulose), Hercules, Inc.; and herbicide Karmex [3-(3,4-dichlorophenyl)-1,1-dimethylurea], E. I. Dupont de Nemours & Co., Inc.

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