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# Reduction of Lactic Acid, Nonprotein Nitrogen, and Ash in Lactic Acid Whey by *Candida ingens* Culture

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A simple, efficient procedure for removing lactic acid and for reducing nonprotein nitrogen and ash in lactic acid whey has been developed. The procedure consists of culturing *Candida ingens* on the whey. This organism could assimilate >98% of the lactic acid and approximately 40% of the nonprotein nitrogen. Ash reduction of up to 45% resulted from precipitation of calcium apatite due to the increase in pH from 4.4 to approximately 8.0 which occurred during growth of *C. ingens*. Improved fluxes during laboratory-scale ultrafiltration were obtained for the treated lactic acid whey. *C. ingens* treatment of lactic acid whey appears to facilitate processing of this material to a more useful product.

Whey disposal has traditionally been a problem for the dairy industry. "Sweet" cheese whey can be utilized for the production of lactose or it can be dried, with or without prior demineralization, for use in baby foods, baked goods, and other food products. Lactic acid, or "sour," whey is derived from the manufacture of lactic casein or cottage cheese by the fermentation of approximately 10% of the lactose content of skim milk by lactic starter bacteria. Since lactic acid interferes with the lactose manufacturing process as well as with demineralization procedures (12, 26) and since lactic acid whey has a higher content of ash and nonprotein nitrogen (NPN) than sweet whey, processing lactic acid whey is unattractive. Although procedures for drying lactic acid whey to a nonhygroscopic powder have been developed, markets for the dried product are limited (13).

Soluble whey protein concentrates (WPC) are currently produced commercially from lactic acid whey by ultrafiltration (11), but since the ultrafiltrate contains practically all the lactose, lactic acid, NPN, and minerals of the original whey, the problems of processing this material to utilize its main component, lactose, are of the same magnitude as for the whey. These disadvantages in fully utilizing the total solids in lactic acid whey condemn it or the greater proportion of its dry matter to be a waste product or effluent, at best utilized on farm land by spray irrigation (21). This is the fate of $1.2 \times 10^6$ tonnes of acid whey produced annually in New Zealand and a significant percentage of the $2 \times 10^6$ tonnes of cottage cheese whey produced annually in the United States.

Lee and Merson (14) have reported that the recovery of whey proteins by ultrafiltration from lactic acid whey requires an additional pretreatment step if ultrafiltration processing rates similar to those for sweet whey are to be obtained. The lactic acid-to-protein ratio of the WPC varies inversely with the extent of concentration by ultrafiltration, and further processing (e.g., diafiltration) is necessary for complete lactic acid removal, especially for WPCs of 35 to 50% protein (23).

A solution to the lactic acid whey disposal problem would be to convert it to a more valuable raw material by reducing the lactic acid, NPN, and ash content, thus facilitating its use in current whey processing schemes (2). Henry (9) reported that *Candida ingens* has the capacity to remove significant amounts of nitrogen, calcium, phosphorus, and volatile fatty acids from pig wastes. Under growth conditions resulting in pellicle formation, the cells contained a very high ash content (21%), a major portion of which consisted of calcium (6%) and phosphorus (7%) (10). The organism is known to assimilate lactic acid but not lactose (29).

The objective of this study was to determine if *C. ingens* could reduce the lactic acid, NPN, and ash content of lactic acid whey.

## MATERIALS AND METHODS

**Yeast.** *C. ingens* were obtained from D. P. Henry of the Central Animal Breeding House, University of Queensland, Brisbane, Australia. The organism was maintained on a medium consisting of: 0.4 g of lactic acid, 0.1 g of yeast extract, and 0.3 g of peptone (Difco, Detroit, Mich.); 0.1 g of $KH_2PO_4$; 1.1 g of agar (Davis Gelatine, Christchurch, N.Z.); 100 ml of water. The pH was adjusted to 7.0 with NaOH. Before experimen-

tal use the organism was subcultured twice on pasteurized (70°C, 1 h) lactic acid ultrafiltrate (pH 4.4) in shake flasks at 25°C.

**Media.** Lactic acid whey and ultrafiltrate were obtained from the New Zealand Dairy Research Institute, Palmerston North. The lactic acid whey had been produced from skim milk inoculated with a multiple strain starter composed of four strains of *Streptococcus cremoris* (27). The ultrafiltrate was derived from the operation of a four-stage "stages-in-series" ultrafiltration plant equipped with Abcor HFM 180 tubular membranes (Abcor Inc., Wilmington, Mass.).

A single batch of lactic acid whey and ultrafiltrate was stored frozen until used, then thawed, and filtered (Whatman no. 4 filter paper) to remove the slight sediment that had formed.

**Shake flask cultures.** Cultures were carried out in 1-liter Erlenmeyer flasks containing 400 ml of lactic acid whey or ultrafiltrate at 25°C on an NBS gyrotary shaker, model G10, operating at 250 rpm. The medium had been pasteurized by heating at 70°C for 1 h. The inoculum for each flask was 40 ml of a 72-h *C. ingens* culture grown on either lactic acid whey or ultrafiltrate. Foaming was controlled by the addition, as needed, of 25 to 50 mg of silicone oil per liter (Dow-Corning Antifoam).

**Ultrafiltration.** Experiments were conducted using an Amicon model 52 stirred cell, 65-ml capacity, with a UM 10 membrane (43-mm diameter, 12.5-$cm^2$ area); Amicon Corp., Lexington, Mass.). The stated molecular weight cutoff was 10,000. All samples were centrifuged at 12,000 × *g* for 10 min before ultrafiltration. Initial sample volumes were 50 ml, and all experiments were conducted at room temperature.

**Analytical procedures.** Microbial cell mass was determined by centrifuging the culture medium in a refrigerated centrifuge at 12,000 × *g* for 10 min. The cells were dried to a constant weight by lyophilization. Cells contaminated with calcium apatite were washed with hydrochloric acid at pH 4.5 to remove this material before freeze-drying. The supernatant was collected and frozen for later analysis.

Extracellular protease activity was assayed for by the method of Udaka (28). Sodium dodecyl sulfate-gel electrophoresis was done according to the procedure of Cashmore (3). Solids and ash were determined according to *Official Methods of Analysis* (1). *C. ingens* cells were analyzed for nitrogen by Kjeldahl digestion (1), for calcium by atomic absorption (25), for phosphorus by the procedure of Murphy and Riley (19), and for amino acids by column chromatography in a Beckman 120C amino acid analyzer on samples hydrolyzed at 110°C for 22 h in 6 M HCl (20).

Analyses of the lactic acid whey and various fractions were by the following methods: total nitrogen by a Kjeldahl method on a Kjelfoss Automatic 16210 (A/S N Foss Electric, Hillerod, Denmark), NPN by precipitating the protein with 15% trichloroacetic acid and measuring the nitrogen in the supernatant by the above method, calcium by a titration procedure (22), and inorganic phosphate colorimetrically as a phosphomolybdate complex (30). Lactose was also determined colorimetrically (16), and lactic acid was estimated by a gas chromatographic procedure (7).

## RESULTS

**Reduction of lactic acid, NPN, and ash.** The culturing of *C. ingens* on lactic acid whey or ultrafiltrate resulted in almost complete removal of lactic acid, concurrent with substantial reductions in NPN (40%) and ash (45%), with no loss of lactose from the culture (Table 1). These calculations were based on the NPN and ash contents of the liquid samples. The reporting of the results on a dry-matter basis (Table 1) tends to diminish the differences between the treated and untreated samples due to the fact that all of the lactic acid has been removed from the treated samples. In the liquid whey samples the protein content of the treated whey had actually decreased slightly, but in Table 1 the protein content expressed as percent dry matter was higher. The small protein loss was due to the

TABLE 1. *Effect of C. ingens culture on reducing the lactic acid, NPN, and ash content of lactic acid whey and ultrafiltrate*

| Sample | Content (% dry matter) | | | | | | | Dry matter in liquid samples (g/100 ml) | pH | Cell yield (g/liter) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lactose | Lactic acid | Protein | NPN | Ash | Calcium | Inorganic phosphate | | | |
| Whey | | | | | | | | | | |
| 0 h[a] | 64.5 | 10.33 | 8.66 | 0.67 | 12.45 | 2.08 | 3.22 | 5.52 | 4.4 | |
| 48 h | 77.6 | <0.2 | 8.74 | 0.48 | 8.84 | 0.43 | 0.53 | 4.38 | 8.1 | 2.94 |
| Ultra-filtrate | | | | | | | | | | |
| 0 h | 73.5 | 11.84 | 0.51 | 0.76 | 14.00 | 2.41 | 3.63 | 4.90 | 4.4 | |
| 48 h | 85.6 | 0.45 | 0.73 | 0.39 | 9.66 | 0.53 | 0.37 | 4.37 | 7.8 | 2.69 |
| Commercial samples of acid-type dry whey[b] | | | | | | | | | | |
| Mean | 63.2 | 6.00 | 11.7 | 0.58 | 10.6 | 2.40 | 4.87 | | 4.57 | |
| Range | 58.8–71.7 | 4.30–6.76 | 8.0–12.6 | 0.45–0.73 | 7.3–12.2 | 1.34–3.21 | 2.72–6.40 | | 4.40–4.81 | |

[a] Culture time.

[b] Values are from 33 acid-type dry whey samples obtained from five plants in various geographical areas of the United States (6, 7).

pasteurization step and the precipitation of some of the protein along with the calcium apatite. The reduction in ash was due to the precipitation of calcium apatite, which occurred as the pH increased from 4.4 to 8.1. The composition of the dry, *C. ingens*-treated whey product had only trace amounts of lactic acid present, and the NPN and ash contents were also lower than in commercial, acid-type dry whey. The ash values reported here were still relatively high, but this was due to the fact that the starting whey and ultrafiltrate contained 12.5 and 14.0% ash, respectively. The calcium and phosphate values were much lower than in commercially produced samples. The pH of the reconstituted, treated whey was similarly higher.

**Cell production.** Approximately 40% of the insoluble material recovered from the cultures was cell material, the remainder being calcium apatite (Table 2) with a small amount of whey protein. The cells averaged 6.53% nitrogen and 5.8% ash, of which 4.0% was phosphate and 0.6% was calcium. The amino acid analysis of *C. ingens* (Table 3) shows that it would be equivalent to other yeasts as a protein source (24).

**Ultrafiltration.** Results in Table 4 show that substantial increases in fluxes were obtained from *C. ingens*-cultured lactic acid whey. The flux increase would be due to the slightly reduced protein content of the treated whey, but also to a possible reduction in membrane "fouling" components. The high pH (>8.0) did not result in lower ultrafiltration rates, as had been reported when lactic acid whey was simply neutralized with NaOH (15). The samples listed in Table 4 had been centrifuged at 12,000 × *g*, but it was possible to obtain similar results at much lower "*g*" forces.

**Protease activity.** *C. ingens* gave negative results when it was tested for extracellular protease activity by the method of Udaka (28). This was confirmed by using sodium dodecyl sulfate-gels (Fig. 1). The protein bands in the 48-h cultures, which do not appear in the 0-h cultures, were proteins that had apparently been excreted into the medium by *C. ingens*.

## DISCUSSION

The results show that *C. ingens* can eliminate lactic acid, concurrent with substantial ash and NPN reductions, from lactic acid whey. Reduction of lactic acid in lactic casein whey or acid cheese whey, to less than 2% on a solids basis, by aerobic culture of bakers' yeast (*Saccharomyces* sp.) has been described previously (P. Devos, French patent 1 213 446, March 1960) and commercialized (Gervais-Danone S.A., Stenval, France), but no ash reduction occurs in this process if only spray-dried whole whey is produced. The reduction in ash during *C. ingens* culture was not due to the accumulation of calcium and phosphorus within *C. ingens* cells (10), but rather to the precipitation of calcium apatite from the culture medium (Table 2). The ash, calcium, and phosphate contents of *C. ingens* are considerably lower than those reported by Henry et al. (10) but similar to values normally reported for food yeasts (4, 24). The difference may be due to the different culture conditions used in these experiments and in those reported by Henry et al. (10).

NPN was reduced by approximately 40%. The growth of *C. ingens* on ultrafiltrate as well as on

TABLE 3. *Amino acid analysis of C. ingens grown on lactic acid ultrafiltrate*

| Amino acid | % of total amino acids |
|---|---|
| Aspartic acid | 9.8 |
| Threonine | 5.5 |
| Serine | 7.2 |
| Glutamic acid | 18.6 |
| Proline | 3.5 |
| Glycine | 4.6 |
| Alanine | 7.1 |
| Valine | 5.6 |
| Isoleucine | 5.1 |
| Leucine | 7.5 |
| Tyrosine | 3.5 |
| Phenylalanine | 3.9 |
| Histidine | 2.2 |
| Lysine | 7.4 |
| Arginine | 7.2 |
| Methionine | 1.3 |

TABLE 2. *Analysis of the insoluble fractions obtained from 48-h cultures of C. ingens grown on lactic acid whey or ultrafiltrate*

| *C. ingens* grown on: | Yield (g/liter) | % Dry matter | | | |
|---|---|---|---|---|---|
| | | Total nitrogen | Ash | Calcium | Phosphate |
| Whey | | | | | |
| Biomass | 2.94 | 6.52 | 5.49 | 0.54 | 4.16 |
| Biomass + calcium apatite | 7.83 | 3.42 | 39.36 | 13.74 | 20.76 |
| Ultrafiltrate | | | | | |
| Biomass | 2.69 | 6.54 | 6.11 | 0.65 | 3.79 |
| Biomass + calcium apatite | 7.00 | 2.90 | 45.11 | 15.56 | 18.19 |

TABLE 4. *Ultrafiltration fluxes (UM10 membrane) for pretreated lactic acid whey from 48-h cultures of C. ingens*

| Run no.[a] | Sample | pH | Protein concn (%) | Pressure (kPa) | ultrafiltration rate[b] (ml/min) | Distilled water ultrafiltration rate before samples[c] (ml/min) | Protein concn in ultrafiltrate (%) | Protein concn factor |
|---|---|---|---|---|---|---|---|---|
| 2 | Whey | 4.4 | 0.48 | 172 | 0.30 | 1.03 | 0.75 | 1.56 |
| 3 | Pretreated whey | 8.1 | 0.38 | 172 | 0.35 | 0.98 | 0.66 | 1.74 |
| 6 | Whey | 4.4 | 0.48 | 345 | 0.33 | 1.42 | 0.80 | 1.66 |
| 7 | Pretreated whey | 8.6 | 0.38 | 345 | 0.44 | 1.25 | 0.80 | 2.10 |

[a] Run number indicates the number of times the UM10 membrane was used for ultrafiltration.
[b] Rate determined from a 60-min run.
[c] Rate determined from a 10-min run.

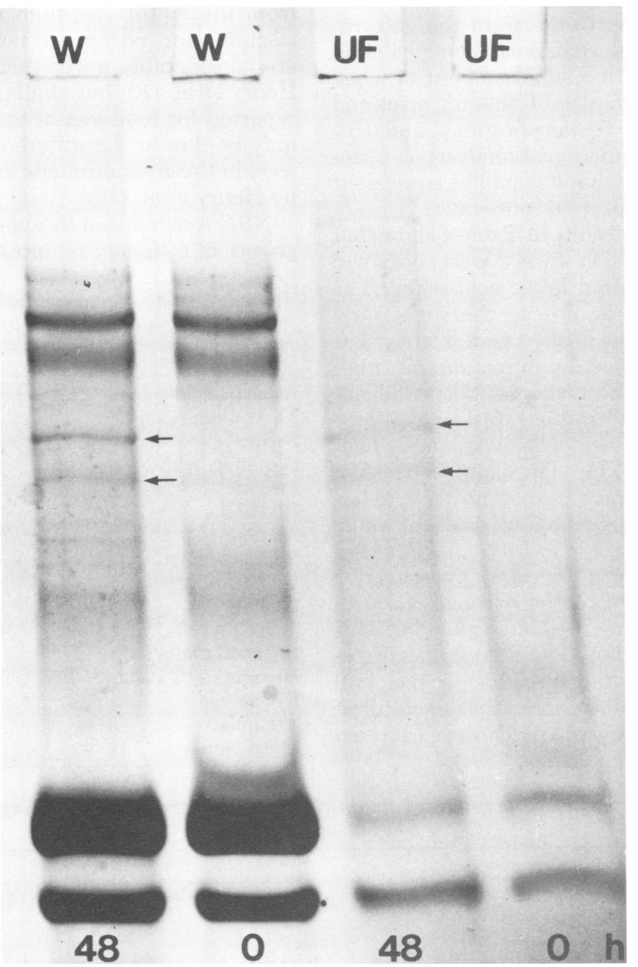


FIG. 1. *Sodium dodecyl sulfate-gel electrophoresis of C. ingens-treated lactic acid whey (W) and ultrafiltrate (UF) before (0 h) and after (48 h) treatment.*

whey indicates that the yeast was not utilizing the whey proteins for growth. This was verified by testing for protease activity. The composition of the whey proteins from *C. ingens* culture differed from untreated whey proteins only by the slight amount of extracellular protein the organism had apparently excreted into solution (Fig. 1).

*C. ingens* treatment of lactic acid whey would produce a substantial biomass if the process was developed commercially. The *C. ingens*-calcium apatite mixture can be easily removed from the culture medium by either centrifugation (1,000 × *g* for 2 min) or filtration (Whatman no. 541 filter paper). Although *C. ingens* cannot normally be filtered from solution, the calcium apatite acted as a filter aid facilitating complete cell removal. The unusually large cell size of *C. ingens* (16) aided in its removal by centrifugation. Because of its high content of nitrogen, calcium, phosphate, and B-group vitamins, the recovered *C. ingens*-calcium apatite mixture could possibly be used as a protein-mineral supplement in animal feeds (17).

The cells can also be easily separated from the calcium apatite by acid washing, making it possible to use the products separately. The recovered yeast could be considered for human consumption. The amino acid analysis of *C. ingens* (Table 3) shows that it would be equivalent to other yeasts as a protein source (24). Henry (8) has reported that *C. ingens* could support growth rates in rats comparable to those obtained with casein when both are in combination with a high-protein cereal grain and that rats fed *C. ingens* for 43 days showed no abnormality at autopsy. This suggests that there would be no problems in utilizing the *C. ingens* products.

The small protein loss from the whey during treatment was due partly to the denaturation of a small amount of protein during the pasteurization step, but mainly to coprecipitation of some of the whey protein with the calcium apatite. This protein was recovered with the yeast fraction. Efforts are being made to minimize this protein loss, since a soluble whey protein fraction obtained by ultrafiltration would be the more valuable product. Extrapolation of the ultrafiltration data from the small-scale experiments reported here to larger-scale production could lead to erroneous conclusions, but it is obvious from the results that *C. ingens*-treated whey would produce a superior product (WPC) containing no lactic acid and having a lower NPN and ash content than a WPC obtained by ultrafiltration of untreated lactic acid whey.

The second shortcoming in treating lactic acid whey with *C. ingens* as reported here was the long culture times. However, equivalent results can be obtained in shorter culture times by using a larger inoculum, adding growth factors, or maintaining an acid pH. The only additive used in the experiments was silicone oil. The development and evaluation of a continuous culture system would indicate the commercial feasibility of the procedure.

Culturing *C. ingens* on lactic acid whey offers an attractive means of removing lactic acid. Simultaneous reduction in NPN and precipitation of calcium apatite due to the rise in pH also increases the suitability of the whey as a raw material for the food processing industry.

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