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Review Article

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# Optimization of Lactic Acid Production from Dairy Whey Using Indigenous Lactic Acid Bacteria

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**Abstract**

*Sweet whey from cheese production is an environmentally challenging but nutrient-rich, underutilized resource. This study converted sweet whey from three dairy processors in Northern Rwanda into lactic acid using indigenous lactic acid bacteria (LAB). LAB-4 (Lactobacillus sp.) was selected for its superior acidification performance, and fermentation conditions were optimized using Response Surface Methodology. Optimal conditions of 37 °C, initial pH 6.0, and 10% (v/v) inoculum produced 43.2 ± 1.2 g/L lactic acid after 48 h, with a yield of 0.873 g/g and fermentation efficiency of 75.4%. The process reduced COD by 67.3%, while calcium lactate precipitation achieved 75.0% extraction yield and 82.5% purity. Production cost was estimated at USD 2.10/kg at 500 L/day. These findings demonstrate a practical and sustainable strategy for reducing whey-related pollution while generating valuable lactic acid for food and industrial applications and supporting dairy-waste valorization in Rwanda.*

**Keywords:** Dairy Whey, Lactic Acid Bacteria, Response Surface Methodology, Whey Valorization, Circular Economy

## 1. Introduction

The dairy industry generates large volumes of sweet whey as the primary liquid by-product of cheese manufacture. Approximately 9–10 L of whey are produced per kilogram of cheese, retaining 85–95% of the original milk volume [1]. Globally, whey generation exceeds 190 million tonnes annually [2]. Sweet whey contains 4.5–5.5% lactose, 0.6–1.1% proteins, minerals and vitamins, yet its high biochemical oxygen demand (BOD 30–60 g/L) and chemical oxygen demand (COD 50–102 g/L) make untreated disposal environmentally damaging [3].

In Rwanda, the dairy sector has expanded rapidly under the Girinka programme and the Rwanda Dairy Development Project, with national milk production reaching 1.152 million tonnes in 2024/2025 [4,5]. Cheese production is concentrated in the Northern Province, where processors such as Burera Dairy Limited, Mukamira Dairy Ltd and Fromagerie La Lumière generate substantial volumes of untreated sweet whey. Direct discharge or low-value use as animal feed conflicts with Rwanda's Vision 2050 and circular-economy policies [6].

Microbial fermentation using lactic acid bacteria (LAB) offers a practical route to convert whey lactose into lactic acid, a versatile platform chemical used in food preservation, pharmaceuticals, cosmetics and biodegradable plastics [7]. Indigenous LAB isolated from traditional fermented products are well adapted to local substrates and reduce dependence on imported cultures [8]. Systematic optimisation of fermentation parameters with local whey streams and native strains remains limited in East Africa.

The objectives of this study were to (i) characterize sweet whey from selected Northern Province processors, (ii) isolate and select efficient indigenous LAB, (iii) optimise temperature, initial pH and inoculum size by Response Surface Methodology (RSM), and (iv) evaluate lactic acid yield, recovery efficiency and preliminary economic feasibility.

## 2. Materials and Methods

### 2.1. Raw Materials and Sample Collection

Sweet whey was collected aseptically from three cheese processors in Northern Rwanda: Burera Dairy Limited (Burera District), Mukamira Dairy Ltd (Nyabihu District) and Fromagerie La Lumière (Musanze District). Samples were transported under refrigeration (4 °C) to the Biotechnology Laboratory at INES-Ruhengeri and analysed within 24 h following standard dairy sampling guidelines [9]. Local yoghurt was purchased from the Musanze market for LAB isolation.

### 2.2. Physicochemical Characterization of Whey

pH was measured with a calibrated digital pH meter (Hanna HI 2211) according to AOAC Official Method 981.12 (Dr. George W Latimer, 2023). Titratable acidity was determined by titration with 0.1 N NaOH using phenolphthalein indicator and expressed as % lactic acid [10]. Total solids were determined by oven drying at 102 ± 2 °C (ISO 6731: 2010).Lactose was quantified by the Lane–Eynon method using Fehling's solutions. Protein was determined by the Kjeldahl method (N × 6.38) following. Fat was analysed by the Röse–Gottlieb gravimetric method as described in the Food Safety and Standards Authority of India manual [11]. The ash content of liquid whey was determined by the dry ashing gravimetric method described in (ISO/IDF 258: 2026). Vitamin C was measured by HPLC as described by [12]. COD and $BOD_5$ were determined according to APHA Standard Methods 5220 and 5210, respectively [13]. All analyses were performed in triplicate.

### 2.3. Microbiological Characterization

Total viable count (TVC) was enumerated on Plate Count Agar incubated at 30 °C for 48 h following. LAB were counted on MRS agar under anaerobic conditions at 30 °C for 48–72 h [13,14]. Coliforms were enumerated on Violet Red Bile Agar, faecal coliforms and E. coli on selective media according to, Enterobacteriaceae on VRBG agar, yeasts and moulds on DRBC agar, and psychrotrophic bacteria on Plate Count Agar incubated at 7 °C for 10 days [13,15].

### 2.4. Isolation and Selection of Indigenous LAB

Yoghurt samples were serially diluted and plated on MRS agar as described by (Karenzi et al., 2013) [8]. Colonies were purified, Gram-stained and tested for catalase activity according to standard morphological and biochemical protocols [16]. Acidification capacity was evaluated in sterile whey by monitoring pH over 72 h following the procedure of (Panesar & Kennedy, 2012) [17]. Strain LAB-4 (Lactobacillus sp.), which reduced pH to 3.8 within 72 h, was selected for fermentation trials and maintained on MRS agar at 4 °C.

### 2.5. Experimental Design and Fermentation

A Central Composite Design (CCD) with three factors (temperature 30–45 °C, initial pH 5.5–6.5, inoculum size 5–15% v/v) and five centre-point replicates was generated using Design-Expert software (Stat-Ease Inc.) as recommended for fermentation optimization [18]. Twenty runs were performed in 250 mL Erlenmeyer flasks containing 100 mL sterile whey. Inoculum was prepared by growing LAB-4 in MRS broth overnight and adjusting to approximately $10^8$ CFU/mL [17]. Flasks were incubated under static conditions. Samples were withdrawn at 0, 6, 12, 24, 36, 48 and 72 h for pH, titratable acidity, residual lactose and lactic acid determination [19]. Lactic acid was quantified by titration after clarification and expressed as g/L [20].

### 2.6. Lactic Acid Recovery

After fermentation, the broth was centrifuged (4000 rpm, 15 min). Calcium hydroxide was added to precipitate calcium lactate following the method described by (Abdel-Rahman et al., 2013; Xu & Xu, 2014) [21, 22]. The precipitate was filtered, washed and acidified with HCl to liberate free lactic acid. Overall recovery yield and purity were calculated from mass balance.

### 2.7. Statistical Analysis

RSM model fitting, analysis of variance (ANOVA), lack-of-fit tests and response-surface plots were performed with Design-Expert [23]. Significance was set at $p < 0.05$. Physicochemical and microbiological data are presented as mean ± standard deviation of triplicate measurements.

## 3. Results and Discussion

### 3.1. Physicochemical Characteristics of Sweet Whey

Whey from the three processors exhibited pH values of 6.08–6.35, titratable acidity of 0.15–0.20% lactic acid, total solids of 6.2–6.9%, lactose of 4.68–5.15% and protein of 0.72–0.92% (Table 1). These values fall within the ranges reported for sweet whey in the literature [24]. COD ranged from 54 to 72 g $O_2$/L and $BOD_5$ from 38 to 52 g $O_2$/L, confirming high organic load consistent with previous reports [24]. Mukamira Dairy whey showed the highest lactose and lowest microbial load, consistent with more advanced processing hygiene.

| Parameter | Burera Dairy | Mukamira Dairy | Fromagerie La Lumière | Overall range |
|---|---|---|---|---|
| pH | 6.12 ± 0.04 | 6.35 ± 0.03 | 6.07 ± 0.05 | 6.08–6.35 |
| Titratable acidity (% LA) | 0.18 ± 0.01 | 0.15 ± 0.01 | 0.19 ± 0.02 | 0.15–0.20 |
| Total solids (%) | 6.44 ± 0.12 | 6.86 ± 0.06 | 6.28 ± 0.12 | 6.2–6.9 |
| Lactose (%) | 4.81 ± 0.05 | 5.15 ± 0.06 | 4.72 ± 0.09 | 4.68–5.15 |
| Protein (%) | 0.76 ± 0.03 | 0.94 ± 0.03 | 0.73 ± 0.02 | 0.72–0.92 |

| | | | | |
|---|---|---|---|---|
| COD (g $O_2$/L) | 57.4 ± 1.0 | 72.3 ± 1.3 | 54.2 ± 1.9 | 54–72 |
| $BOD_5$ (g $O_2$/L) | 43.3 ± 1.7 | 51.3 ± 1.1 | 37.8 ± 1.8 | 38–52 |

**Table 1: Physicochemical Characteristics of Sweet Whey from Three Dairy Processors in Northern Rwanda (mean ± SD, n = 3)**

### 3.2. Microbiological Profile

Total viable counts ranged from 5.15 to 6.15 $\log_{10}$ CFU/mL. LAB counts were 4.1–5.0 $\log_{10}$ CFU/mL. Coliforms, faecal coliforms and E. coli were present at higher levels in samples from semi-automated facilities (Burera and Fromagerie La Lumière) than from Mukamira Dairy, indicating the influence of processing hygiene on whey microbial quality, as previously observed in East African dairy processors [25].

### 3.3. LAB Isolation and Selection

Twelve isolates were obtained from local yoghurt. All were Gram-positive rods or cocci and catalase-negative, in agreement with the typical characteristics of LAB [26]. Strain LAB-4 exhibited the fastest acidification, reducing whey pH from 6.2 to 3.8 within 72 h, and was therefore selected for subsequent optimization trials. Similar acidification performance has been reported for indigenous Lactobacillus strains isolated from traditional African fermented milk [8].

### 3.4. Response Surface Optimization

The quadratic model for lactic acid production was highly significant ($F = 36.85$, $p < 0.0001$) with $R^2 = 0.9685$ and adjusted $R^2 = 0.9421$. Lack-of-fit was non-significant ($p = 0.1856$), confirming model adequacy [27]. Temperature and initial pH were the most influential factors ($p < 0.0001$), followed by inoculum size ($p = 0.0012$). Significant quadratic and interaction terms indicated curvature and synergistic effects. The optimal predicted conditions were 37 °C, pH 6.0 and 10% (v/v) inoculum, yielding a predicted lactic acid concentration of 42.9 g/L. These optima are consistent with the physiological preferences of mesophilic Lactobacillus species reported in whey fermentation studies [17].

### 3.5. Fermentation Performance Under Optimized Conditions

Validation experiments confirmed a lactic acid concentration of 43.2 ± 1.2 g/L after 48 h. Lactose consumption reached approximately 62%, corresponding to a substrate-to-product yield (Yp/s) of 0.873 g/g and a fermentation efficiency of 75.4%. Final pH declined to approximately 3.6 and titratable acidity rose to 2.0% lactic acid. COD was reduced by 67.3%, demonstrating simultaneous waste treatment and product formation. The lactic acid concentration and yield obtained are competitive with values reported for whey-based fermentations using laboratory or commercial strains under non-buffered conditions [17, 21].

| Parameter | Value |
|---|---|
| Optimal temperature (°C) | 37 |
| Optimal initial pH | 6.0 |
| Optimal inoculum size (% v/v) | 10 |
| Lactic acid concentration at 48 h (g/L) | 43.2 ± 1.2 |
| Substrate-to-product yield Yp/s (g/g) | 0.873 |
| Fermentation efficiency (%) | 75.4 |
| COD reduction (%) | 67.3 |
| Overall recovery yield (%) | 75.0 |
| Product purity (%) | 82.5 |

**Table 2: Optimized Fermentation Performance Indicators (mean ± SD)**

### 3.6. Recovery and Economic Assessment

Calcium lactate precipitation followed by acidification recovered lactic acid with an overall extraction yield of 75.0% and product purity of 82.5%. These recovery figures are comparable to those reported for similar precipitation-based methods [21]. Preliminary cost estimation at a laboratory scale equivalent to 500 L/day indicated a unit production cost of approximately USD 2.10 per kg lactic acid and a simple payback period of 1.8 years under optimistic market assumptions.

The physicochemical composition of the studied whey is consistent with literature values for sweet whey and confirms its suitability as a fermentation substrate [10]. The high lactose content (4.7–5.2%) provides an adequate carbon source for homofermentative LAB, while residual proteins and minerals support microbial growth without additional nutrient supplementation.

Indigenous strain LAB-4 performed comparably to commercial Lactobacillus strains reported in the literature for whey fermentation. The optimum temperature of 37 °C and initial pH of 6.0 align with the physiological preferences of many mesophilic Lactobacillus species [17]. The high $R^2$ and non-significant lack-of-fit of the RSM model indicate reliable prediction within the experimental domain.

The achieved lactic acid concentration (43.2 g/L) and yield (0.873 g/g) are competitive with values reported for whey-based fermentations using laboratory or commercial strains under non-buffered conditions. The concurrent 67% COD reduction highlights the dual benefit of pollution abatement and product recovery, which is particularly relevant for small- and medium-scale processors in developing regions that lack advanced wastewater treatment infrastructure.

Calcium lactate precipitation is a simple, low-capital recovery method suitable for the scale of Rwandan dairy processors. Although purity (82.5%) is lower than that obtained by advanced chromatographic or membrane techniques, it is adequate for many food and technical applications. The estimated production cost of USD 2.10/kg is within the range of market prices for technical-grade lactic acid, supporting preliminary economic feasibility.

Limitations of the present study include the use of batch flask fermentation, the absence of pH control during fermentation, and the restriction to a single recovery method. Scale-up studies in controlled bioreactors, continuous or fed-batch operation, and techno-economic and life-cycle assessments are recommended before industrial implementation.

In summary, sweet whey from cheese processors in Northern Rwanda is a suitable substrate for lactic acid production. Indigenous Lactobacillus strain LAB-4, isolated from local yoghurt, efficiently converted whey lactose into lactic acid. Response Surface Methodology identified optimal conditions of 37 °C, initial pH 6.0 and 10% inoculum, producing 43.2 g/L lactic acid with 75.4% fermentation efficiency and 67.3% COD reduction. Calcium lactate precipitation recovered the product at 75% overall yield and 82.5% purity. The process offers a practical, low-cost route for simultaneous whey valorisation and environmental remediation in the Rwandan dairy sector and is economically promising at modest scale.

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## References

1. Pires, A. F., Marnotes, N. G., Rubio, O. D., Garcia, A. C., & Pereira, C. D. (2021). Dairy by-products: A review on the valorization of whey and second cheese whey. *Foods, 10*(5), 1067.
2. Buchanan, D., Martindale, W., Romeih, E., & Hebishy, E. (2023). Recent advances in whey processing and valorisation: Technological and environmental perspectives. *International Journal of Dairy Technology, 76*(2), 291-312.
3. El-Aidie, S. A., & Khalifa, G. S. (2024). Innovative applications of whey protein for sustainable dairy industry: Environmental and technological perspectives—A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety, 23*(2), e13319.
4. Ministry of Agriculture and Animal Resources. (2020). Feed the Future Innovation Lab for Livestock Systems: The livestock system in Rwanda—An overview. 15–35.
5. Shapiro, B. I., Gebru, G., Desta, S., & Nigussie, K. (2017). Rwanda livestock master plan.
6. Twagirayezu, G., Nizeyimana, I., Irumva, O., Uwimpaye, F., Uwimana, A., Birame, C. S., ... & Bakunzibake, P. (2026). Road to a circular economy: a systematic analysis of municipal solid waste management in Rwanda. *Environment Systems and Decisions, 46*(3), 53.
7. Ryan, M. P., & Walsh, G. (2016). The biotechnological potential of whey. *Reviews in Environmental Science and Bio/Technology, 15*(3), 479-498.
8. Karenzi, E., Mashaku, A., Nshimiyimana, A. M., Munyanganizi, B. T., & Thonart, P. (2013). Kivuguto traditional fermented milk and the dairy industry in Rwanda. A review. *Biotechnologie, agronomie, société et environnement, 17*(2).
9. Duarte, R. V., Moreira, S. A., Fernandes, P. A., Santos, D. I., Inácio, R. S., Alves, S. P., ... & Saraiva, J. A. (2017). Whey cheese longer shelf-life achievement at variable uncontrolled room temperature and comparison to refrigeration. *Journal of food Processing and Preservation, 41*(6), e13307.
10. Barukčić, I., Jakopović, K. L., Herceg, Z., Karlović, S., & Božanić, R. (2015). Influence of high intensity ultrasound on microbial reduction, physico-chemical characteristics and fermentation of sweet whey. *Innovative Food Science & Emerging Technologies, 27*, 94-101.
11. Food Safety and Standards Authority of India. (2016). Manual of methods of analysis of foods: Milk and milk products. 19–22.
12. Romeu-Nadal, M., Morera-Pons, S., Castellote, A. I., & Lopez-Sabater, M. C. (2006). Rapid high-performance liquid chromatographic method for Vitamin C determination in human milk versus an enzymatic method. *Journal of chromatography B, 830*(1), 41-46.
13. Wehr, H. M., & Frank, J. F. (Eds.). (2004). *Standard methods for the examination of dairy products*. American Public Health Association.
14. de Man, J. D., Rogosa, D., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of applied microbiology, 23*(1), 130-135.
15. American Public Health Association, Water Environment Federation, & American Water Works Association. (1999). Standard methods for the examination of water and wastewater. American Public Health Association.
16. Gupta, K., Chatterjee, C., & Gupta, B. (2012). Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India. *Biologia, 67*(5), 827-836.
17. Panesar, P. S., & Kennedy, J. F. (2012). Biotechnological approaches for the value addition of whey. *Critical reviews in*

*biotechnology, 32*(4), 327-348.
18. Kumar, M., Jain, A. K., Ghosh, M., & Ganguli, A. (2012). Statistical optimization of physical parameters for enhanced bacteriocin production by L. casei. *Biotechnology and Bioprocess Engineering, 17*(3), 606-616.
19. Flutto, T., Thedy, L., Zenato, S., Pramotton, R., Vernetti-Prot, L., & Valentini, S. (2024). Method For Preparing a Skin Repairing Ingredient from Cheese Whey Following Lactobacillus Fermentation. *JOURNAL OF DERMATOLOGY, 6*(2), 9-17.
20. George Jr, W. L. (2012). Official Methods of Analysis of AOAC International 19th edition, volume II. *Rockville, USA, AOAC International*, 20-25.
21. Abdel-Rahman, M. A., Tashiro, Y., & Sonomoto, K. (2013). Recent advances in lactic acid production by microbial fermentation processes. *Biotechnology advances, 31*(6), 877-902.
22. Xu, K., & Xu, P. (2014). Efficient calcium lactate production by fermentation coupled with crystallization-based in situ product removal. *Bioresource technology, 163*, 33-39.
23. Behera, S. K., Meena, H., Chakraborty, S., & Meikap, B. C. (2018). Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal. *International Journal of Mining Science and Technology, 28*(4), 621-629.
24. Mirzakulova, A., Sarsembaeva, T., Suleimenova, Z., Kowalski, Ł., Gajdzik, B., Wolniak, R., & Bembenek, M. (2025). Whey: Composition, processing, application, and prospects in functional and nutritional beverages—A review. *Foods, 14*(18), 3245.
25. Djobo, O., Sina, H., Tagba, S., Ahyi, V., Savadogo, A., Adjanohoun, A., ... & Baba-Moussa, L. (2022). Bio-production of lactic and lactobionic acids using whey from the production of cow's milk Wagashi cheese in Benin. *Frontiers in nutrition, 9*, 1020934.
26. Goyal, R., Dhingra, H., Bajpai, P., & Joshi, N. (2012). Characterization of the Lactobacillus isolated from different curd samples. *African journal of biotechnology, 11*(79), 14448-14452.
27. Wang, J., Huang, J., Laffend, H., Jiang, S., Zhang, J., Ning, Y., ... & Liu, S. (2020). Optimization of immobilized Lactobacillus pentosus cell fermentation for lactic acid production. *Bioresources and bioprocessing, 7*(1), 15.

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