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Effect of liquid Gouda cheese whey inclusion on the physicochemical, microbiological and sensory quality of set yoghurt

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Abstract

Sweet whey from Gouda cheese making is largely discarded or sold cheaply as animal feed in Rwanda, although it retains lactose, soluble proteins and minerals. This study evaluated whether fresh liquid Gouda cheese whey can partly replace milk in set yoghurt without loss of quality. Pasteurised whole milk was replaced with pasteurised liquid whey at 0 (T0), 10 (T1), 20 (T2) and 30% (T3, v/v). Composition, viscosity, minerals, syneresis, pH and titratable acidity during 42 days at 4°C, hygiene indicators and consumer acceptance (n = 30, 9-point hedonic scale) were assessed. Whey inclusion lowered protein (3.23 to 2.68%), fat (4.00 to 3.16%) and total solids (13.94 to 12.65%) ($p < 0.001$) and reduced apparent viscosity by 69% (752 to 233 mPa·s). Syneresis increased with whey level and storage time, reaching 22% (T0) and 30% (T3) on day 42. pH declined slightly faster in whey-containing yoghurts, whereas storage-averaged titratable acidity did not differ among treatments ($p = 0.11$). Total coliforms, *Escherichia coli*, and yeasts and moulds were below 10 CFU/g in all samples. Yoghurt with 10% whey did not differ from the control in appearance, aroma, taste, texture or overall acceptability (8.12 vs 8.23), although mouthfeel was rated lower (7.37 vs 8.40); acceptability declined significantly at 20 and 30% whey. Fresh liquid Gouda cheese whey can therefore replace up to 10% of milk in set yoghurt, giving a safe, well-accepted product and a simple outlet for a dairy by-product; higher levels require added milk solids or stabilisers.

Introduction

Cheese making converts most of the milk volume into whey. Roughly 8–9 L of whey are generated for every 10 L of milk processed, and this liquid retains about half of the nutrients originally present in the milk [1], [2]. Sweet whey from rennet-coagulated cheeses such as Gouda has a near-neutral pH of 6–7 and typically contains 46–52 g/L lactose, 6–10 g/L protein and 2.5–4 g/L minerals [1]. Its proteins, mainly β-lactoglobulin and α-lactalbumin, have high biological value, while lactose and soluble minerals make whey a ready fermentation substrate [3], [4]. Despite this composition, whey has historically been treated as an effluent. Its organic load is very high, with a biochemical oxygen demand of about 35–45 kg/m³ and a chemical oxygen demand of 60–70 kg/m³, so untreated discharge depletes dissolved oxygen in receiving waters and contaminates soils and groundwater [2]. Managing whey is therefore both an environmental obligation and an economic opportunity for the dairy sector [5], [6].

Industrialised dairy systems have largely solved this problem through fractionation. Ultrafiltration, nanofiltration, electrodialysis and spray drying turn whey into whey protein concentrates and isolates, lactose, permeate powders and demineralised ingredients [2], [4]. These routes, however, require capital-intensive equipment, energy and technical skills that small and medium-sized cheese plants in low-income countries rarely possess. For such processors, the most realistic options are direct uses of liquid whey that fit within existing equipment, such as whey beverages, whey cheeses or partial milk replacement in fermented products [7], [8]. Recent studies confirm the potential of these simple routes. Lactose in sweet and acid whey can be converted enzymatically into prebiotic galacto-oligosaccharides [9], products made from sheep and goat second cheese whey fermented with yoghurt cultures were

rated favourably by sensory panels [10], and yoghurt enriched with postbiotics produced in cheese whey showed improved antioxidant activity and acceptability [11]. Acid whey from strained yoghurt has likewise been redirected into food applications rather than disposal [12].

Rwanda illustrates this problem clearly. Milk production has expanded rapidly through national dairy programmes, and most milk is produced on smallholder farms and delivered to processing plants through milk collection centres [13]. The Western Province highlands, including the Gishwati milk shed in Nyabihu and Rubavu districts, supply a large share of the milk processed by formal plants. Several small and medium-sized enterprises in this region produce Gouda-type cheese, and each generates substantial volumes of sweet whey; during this study, the plants reported weekly whey volumes of about 3,500 L (Mukamira Dairy), 3,000 L (Ingabo Dairy) and 2,400 L (Fromagerie la Reine). In practice, most of this whey is sold cheaply to farmers as pig or cattle feed or released untreated into the environment. None of these plants operates membrane or drying equipment, so the nutritional value of the whey is largely lost. At the same time, yoghurt is one of the most widely consumed processed dairy products in Rwanda, and the same plants already operate pasteurisation, homogenisation and incubation lines for yoghurt. Replacing part of the milk in yoghurt with fresh whey would therefore use existing equipment, reduce milk demand per unit of product, and divert part of the whey stream from disposal.

The technological challenge lies in the structure of set yoghurt. In set yoghurt, the inoculated milk is filled into retail containers before fermentation, so the gel forms in the pack and is never stirred. Its firmness, viscosity and resistance to whey separation depend almost entirely on the continuous acid-induced network formed by casein micelles as pH falls towards their isoelectric point [14]. Heat treatment of the yoghurt mix denatures whey proteins, which then associate with κ-casein on the micelle surface and with each other, modifying the network. The casein-to-whey protein ratio and the total protein and solids content are therefore key determinants of yoghurt microstructure, firmness and water-holding capacity [14], [15]. When whey proteins are added as concentrated ingredients, total protein rises and syneresis usually decreases [14], [15]. Liquid whey behaves differently. Because it contains about 93–95% water and less than 1% protein, partial replacement of milk dilutes casein, fat and total solids rather than enriching them. A weaker gel, lower viscosity and greater susceptibility to syneresis can therefore be expected. Hyseni et al. [16] reported that yoghurt made with 25% liquid whey had higher syneresis during cold storage than control yoghurt, although sensory differences were small when fruit was added.

Liquid whey may also change acidification during storage. Lactose and soluble minerals supplied by whey, together with a lower casein buffering capacity, could accelerate post-acidification, the continued production of lactic acid by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* after fermentation [17], [18]. Post-acidification increases sourness, promotes whey expulsion and shortens shelf life. It is a particular concern in tropical and resource-limited settings, where cold chains are often interrupted between plant and consumer [17], [19]. The microbiological quality of whey is a further risk. Whey has high water activity and is rapidly colonised by spoilage organisms when not cooled, so its safe use requires pasteurisation and hygienic handling [2]. This is relevant in Rwanda,

where milk is often transported without refrigeration and hygiene indicator counts can be high before processing [13].

Published work on whey in yoghurt has focused mainly on processed whey ingredients, such as whey protein concentrates, isolates, polymerised or microparticulated whey proteins and whey powders, rather than on fresh liquid whey [4]. The few studies that used liquid whey directly have mostly examined a single inclusion level, stirred or flavoured yoghurt, or acid whey [12], [16]. Information is lacking on how graded levels of fresh sweet whey from Gouda cheese affect the composition, viscosity, syneresis, acidification during prolonged refrigerated storage, hygienic quality and consumer acceptance of plain set yoghurt. No such data exist for East African processing conditions, where milk composition, starter handling and consumer expectations differ from those in Europe or Asia. Processors also need a practical upper inclusion limit that preserves product quality and complies with national standards for fermented milks.

This study therefore evaluated the feasibility of replacing 10, 20 and 30% of milk with fresh liquid Gouda cheese whey in set yoghurt produced under pilot conditions in a Rwandan dairy plant. The specific objectives were to determine the effects of whey inclusion on (i) proximate composition and major minerals, (ii) apparent viscosity, (iii) syneresis, pH and titratable acidity during 42 days of refrigerated storage, (iv) hygiene indicator microorganisms, and (v) consumer sensory acceptance. We hypothesised that whey inclusion would dilute milk solids, weaken the gel and increase syneresis in a dose-dependent manner, but that a low inclusion level would remain acceptable to consumers and microbiologically safe.

Materials and methods

Study site and raw materials

The study was conducted at the pilot yoghurt line of Mukamira Dairy Ltd (Nyabihu District, Western Province, Rwanda), with laboratory analyses at Mukamira Dairy, Rwanda Standards Board (RSB) and the National Industrial Research and Development Agency (NIRDA), Kigali. Fresh bulk cow's milk was not collected by the authors from farms or milk producers; it was obtained from the processing line of Mukamira Dairy Ltd after the plant's routine reception of raw milk supplied from the Gishwati milk shed. Fresh sweet whey was collected from Gouda cheese production at Mukamira Dairy Ltd, Ingabo Dairy and Fromagerie la Reine, pooled, cooled to ≤ 4°C held in cool boxes and used within 1 h 30 min of collection. The composition of the milk and whey, measured with a MilkoScan FTIR analyser (FOSS, Hillerød, Denmark), is shown in Table 1. A freeze-dried thermophilic yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LYOFAST Y 438 B, Sacco System, Cadorago, Italy) was used. White sugar, corn starch stabiliser and vanilla flavour were purchased from Dairy Consulting Ltd (Kigali, Rwanda).

Table 1
Composition of the raw milk and liquid Gouda cheese whey used in the formulations

Parameter	Whole milk	Gouda cheese whey
pH	6.80	6.40
Titratable acidity (% lactic acid)	0.14	0.21
Fat (%)	4.20	0.60
Protein (%)	3.30	0.20
Lactose (%)	4.80	4.20
Solids-not-fat (%)	8.60	7.60
Total solids (%)	12.80	8.20
Density (g/mL)	1.030	1.023

Single determinations by FTIR (MilkoScan) on the day of processing.

Experimental design and yoghurt manufacture

Four formulations were prepared in which pasteurised whole milk was replaced by liquid whey at 0 (T0, control), 10 (T1), 20 (T2) and 30% (T3, v/v) of a 10 L batch (Table 2). Each formulation was prepared in three independent batches (n = 3).

Table 2
Formulation of set yoghurts per 10 L batch

Treatment	Whey inclusion (% v/v)	Whole milk (L)	Liquid whey (L)	Sugar (g)	Corn starch (g)
T0	0	10	0	60	40
T1	10	9	1	60	40
T2	20	8	2	60	40
T3	30	7	3	60	40

All formulations also contained 0.001% (w/v) vanilla flavour and starter culture dosed according to the supplier's instructions.

Raw milk was pasteurised at 85°C for 15 s in a plate heat exchanger (Alfa Laval, Lund, Sweden), homogenised at 180 bar (Shanghai Jiangi Machinery & Equipment Co., China) and cooled to 4°C. Whey was pasteurised separately under the same conditions (85°C for 15 s) before use. Milk, whey, sugar and stabiliser were blended, preheated to 40–45°C, heat-treated at 90°C for 3 min and homogenised at 180

bar. The mixes were cooled to 45°C, inoculated with the starter culture and vanilla flavour, and gently mixed for 3 min. Each mix was filled into ten sterile 1 L containers, incubated at 43 ± 2°C for 7 h (Memmert GmbH + Co. KG, Schwabach, Germany) and then cooled and stored at 4 ± 1°C for 42 days.

Proximate composition and titratable acidity

Protein was determined by the Kjeldahl method, AOAC Official Method 991.20 [20], using a nitrogen-to-protein factor of 6.38. Fat was determined gravimetrically after ammonia–ethanol treatment and extraction with diethyl and petroleum ethers (Röse–Gottlieb principle). Total solids were determined by drying 5 g of sample over a boiling water bath for 30 min and then at 102 ± 2°C to constant mass. Ash was determined by incineration of 2 g of sample at 550°C for 3 h. Lactose and solids-not-fat were measured by FTIR spectroscopy (MilkoScan, FOSS) at RSB. Titratable acidity was measured by titrating 10 g of yoghurt diluted in 30 mL of distilled water with 0.1 N NaOH to a phenolphthalein end point and expressed as % lactic acid. pH was measured on a 1:10 (w/v) yoghurt–water dispersion with a calibrated pH meter (HI 8424, Hanna Instruments, Woonsocket, RI, USA). All compositional determinations were made in triplicate on day 1 of storage.

Major minerals

Calcium, potassium, magnesium and sodium were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 8000, PerkinElmer, Waltham, MA, USA). Two grams of sample were digested with 8 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 in a closed microwave system (Milestone, Sorisole, Italy) at 200°C for 15 min and diluted to 50 mL with ultrapure water. Emission was read at 422.673 nm (Ca), 766.491 nm (K), 383.882 nm (Mg) and 588.995 nm (Na). Concentrations were calculated against external calibration standards and corrected for reagent blanks. Each sample was analysed in triplicate.

Apparent viscosity

Apparent viscosity was measured on undisturbed samples on day 1 with a rotational viscometer (Smart Series L, Fungilab, Barcelona, Spain) fitted with spindle L3 at 50 rpm and 23.5 ± 0.1°C. Readings were taken 30 s after rotation began, with torque between 10 and 100%. Measurements were made in triplicate and expressed in mPa·s.

Syneresis, pH and titratable acidity during storage

Syneresis was determined on days 1, 7, 14, 21, 28, 35 and 42 by centrifuging 10 g of yoghurt at 7870 × g and 4°C for 10 min (Heal Force, Shanghai, China). The separated serum was weighed and expressed as a percentage of the initial sample mass. pH and titratable acidity were measured on the same days as described above.

Microbiological analyses

Total coliforms were enumerated according to ISO 4832 [21] on MacConkey agar incubated at 37°C for 24 h. *Escherichia coli* was enumerated according to ISO 16649-2 [22] on chromogenic agar (Micromaster, Thane, India) incubated at 37°C for 24 h. Yeasts and moulds were enumerated according to ISO 21527-1 [23] on potato dextrose agar (BD, Franklin Lakes, NJ, USA) incubated at 25°C for 5 days. These standard methods were applied with modifications to suit the media available in the laboratory: MacConkey agar was used instead of violet red bile lactose agar for coliforms, *E. coli* plates were incubated at 37°C instead of 44°C, and potato dextrose agar was used instead of dichloran rose bengal chloramphenicol agar for yeasts and moulds. Analyses were performed on day 1 of storage, and results were expressed as CFU/g with a limit of detection of 10 CFU/g.

Sensory evaluation

Thirty untrained consumer panellists (18 men and 12 women, aged 25–50 years) were recruited among staff of Mukamira Dairy Ltd who regularly consume yoghurt. Each panellist received the four yoghurts as 100 mL portions served at about 5°C in cups labelled T0, T1, T2 and T3, and rated appearance, aroma, taste, texture and mouthfeel on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). Panellists rinsed their mouths with potable water between samples. Samples were served in the same order for all panellists, from T0 to T3. Overall acceptability was calculated for each panellist as the mean of the five attribute scores.

Statistical analysis

Raw data were re-analysed in Python 3 using SciPy 1.17 and statsmodels 0.15. Compositional, mineral and viscosity data were analysed by one-way ANOVA, with means separated by Tukey's honestly significant difference (HSD) test. Pearson correlation coefficients (r) were calculated between whey inclusion level and each response. Sensory scores were analysed as a randomised complete block design, with treatment as the fixed factor and panellist as the block, followed by Tukey's HSD. The Friedman test was used to confirm treatment effects without assuming normality. Storage data (syneresis, pH and titratable acidity) were analysed by two-way ANOVA with treatment and storage day as factors. The rate of change of each storage variable was estimated for each treatment by linear regression on storage time. Results are reported as mean ± standard deviation, and differences were considered significant at $p < 0.05$.

Results and discussion

Proximate composition

Replacing milk with liquid whey reduced every major solid component of the yoghurt in a dose-dependent manner (Table 3). Protein fell from 3.23% in the control to 2.68% at 30% whey (− 17%), fat

from 4.00 to 3.16% (− 21%), total solids from 13.94 to 12.65% and lactose from 4.34 to 4.01% (all $p < 0.001$). Each was strongly and negatively correlated with whey level ($r = -0.93$ to -0.99). At 10% whey, solids-not-fat and lactose did not differ from the control, whereas protein, fat and total solids were already slightly but significantly lower.

Table 3
Proximate composition, initial titratable acidity and apparent viscosity of set yoghurts with increasing levels of liquid Gouda cheese whey (day 1)

Parameter	T0 (0%)	T1 (10%)	T2 (20%)	T3 (30%)	F	r
Protein (%)	3.23 ± 0.01^{a}	3.10 ± 0.01^{b}	2.90 ± 0.01^{c}	2.68 ± 0.01^{d}	2580	−0.99
Fat (%)	4.00 ± 0.02^{a}	3.88 ± 0.04^{b}	3.49 ± 0.03^{c}	3.16 ± 0.05^{d}	350	−0.98
Solids-not-fat (%)	9.94 ± 0.03^{a}	9.95 ± 0.02^{a}	9.62 ± 0.03^{b}	9.49 ± 0.02^{c}	325	−0.93
Lactose (%)	4.34 ± 0.02^{a}	4.30 ± 0.02^{a}	4.13 ± 0.03^{b}	4.01 ± 0.03^{c}	129	−0.97
Total solids (%)	13.94 ± 0.03^{a}	13.83 ± 0.03^{b}	13.10 ± 0.03^{c}	12.65 ± 0.07^{d}	649	−0.97
Ash (%)	1.18 ± 0.06^{a}	0.91 ± 0.01^{b}	0.73 ± 0.06^{c}	0.40 ± 0.01^{d}	191	−0.99
Titratable acidity (% lactic acid)	0.74 ± 0.01^{c}	0.75 ± 0.01^{c}	0.77 ± 0.01^{b}	0.81 ± 0.01^{a}	60	0.96
Apparent viscosity (mPa·s)	752.2 ± 1.0^{a}	464.7 ± 0.4^{b}	349.5 ± 0.6^{c}	233.0 ± 0.6^{d}	332 450	−0.97

Values are mean ± SD ($n = 3$). Means in a row with different superscript letters differ significantly (one-way ANOVA, Tukey's HSD, $p < 0.05$); all F tests $p < 0.001$ ($df = 3, 8$). r = Pearson correlation with whey inclusion level ($n = 12$).

These reductions are consistent with simple dilution. Using the milk and whey compositions in Table 1, a 30% replacement predicts about 3.1% fat and 12.4% total solids once the 1% added sugar and starch are included, close to the measured 3.16 and 12.65%. Sweet whey keeps most of the milk lactose and soluble minerals but loses casein and fat to the curd [1], [3], so the losses were largest for fat and protein and smallest for lactose. Whey therefore lowers the nutrient density of yoghurt per unit volume, mainly through loss of casein and fat. The decline in ash, from 1.18 to 0.40%, was much larger than predicted by dilution, because sweet whey retains roughly 0.5–0.7% ash [2]. This result should be interpreted cautiously and confirmed by repeated analysis.

Initial titratable acidity rose from 0.74 to 0.81% as whey inclusion increased (r = 0.96). The whey itself was more acidic than the milk (0.21 vs 0.14%; Table 1), and the extra lactose and soluble nitrogen it supplies may also have stimulated starter activity during the 7 h incubation, since whey is a readily fermentable substrate for lactic acid bacteria [2]. All values remained within the range usually reported for fresh set yoghurt.

Apparent viscosity

Apparent viscosity was the property most sensitive to whey inclusion (Table 3). It fell by 38% at 10% whey (from 752 to 465 mPa·s) and by 69% at 30% whey (233 mPa·s). The loss of viscosity was disproportionate to the loss of protein, which fell by only 4% at 10% whey. The viscosity of an acid milk gel depends on the connectivity of the casein network, not simply on protein mass. Once casein concentration and total solids drop, the number of particle–particle contacts in the network falls sharply, and the gel becomes coarser and more porous [14], [15]. Liquid whey contributes very little denaturable whey protein to compensate. It also adds water, lactose and monovalent ions, which do not take part in network formation. By contrast, studies that fortified yoghurt with concentrated whey proteins observed firmer gels, because total protein increased and denatured whey proteins bridged casein micelles [4], [15]. The present results show that this benefit does not apply to fresh liquid whey added at the expense of milk.

Syneresis during storage

Syneresis increased steadily during storage in all yoghurts and was consistently higher at greater whey levels (Fig. 1a). On day 1, centrifugal syneresis was 2% in T0 and 5% in T3; by day 42 it reached 22, 24, 25 and 30% in T0, T1, T2 and T3. Two-way ANOVA confirmed significant effects of both treatment ($F = 39.5$, $p < 0.001$) and storage time ($F = 117.6$, $p < 0.001$). Averaged over storage, syneresis was 11.1% in T0, 13.1% in T1, 15.1% in T2 and 19.3% in T3. T1 did not differ from T0, T2 differed from T0 but not from T1, and T3 differed from all other treatments (Tukey's HSD = 2.2%). The rate of whey expulsion was also highest in T3 (0.62% per day, $R^2 = 0.96$) compared with 0.47% per day in the control.

Greater syneresis in whey-containing yoghurt is the expected outcome of a weaker, more porous casein network [14]. As the gel continues to rearrange and contract during cold storage, serum held loosely in large pores is expelled more readily. Post-acidification further pushes the gel below the pH of optimal casein hydration and accelerates network contraction [17]. Hyseni et al. [16] similarly found that replacing 25% of milk with liquid whey increased syneresis during cold storage of yoghurt. The 10% level is therefore the practical threshold in this system: its storage-averaged syneresis was statistically indistinguishable from that of the control, whereas 30% whey produced visible wheying-off. Syneresis increased substantially in the control as well, reaching 22% after 42 days. This indicates that the corn-starch stabiliser at 0.4% provided limited water binding over long storage, and the shelf life of all formulations may be shorter than 42 days in practice.

pH and titratable acidity during storage

All yoghurts underwent post-acidification during refrigerated storage (Fig. 1b, c). pH fell from 4.4–4.5 on day 1 to 3.8–4.1 on day 42, while titratable acidity rose from 0.68–0.71% to 0.96–1.01% (storage effect $p < 0.001$ for both). Treatment had a small but significant effect on pH ($F = 4.8$, $p = 0.013$). Storage-averaged pH was 4.29 in T0, 4.23 in T1, 4.19 in T2 and 4.20 in T3, and only T2 and T3 differed from the control. The rate of pH decline increased with whey level, from 0.073 units per week in T0 to 0.087, 0.095 and 0.102 units per week in T1, T2 and T3. Final pH values were 4.1 (T0), 4.0 (T1), 3.8 (T2) and 3.9 (T3). In contrast, storage-averaged titratable acidity did not differ among treatments (0.81–0.83%; $F = 2.3$, $p = 0.11$), and its rate of increase was similar (0.046–0.054% per week).

Post-acidification is driven mainly by the residual β-galactosidase and lactate dehydrogenase activity of *L. delbrueckii* subsp. *bulgaricus*, which tolerates low pH and continues to ferment lactose at refrigeration temperatures [18], [19]. Lactose was not limiting in any formulation (≥ 4.0%; Table 3), so differences in lactic acid production among treatments were expected to be small, as the titratable acidity data show. The faster pH decline in whey-containing yoghurt is more likely explained by lower buffering capacity. Casein and colloidal calcium phosphate provide much of the buffering of milk between pH 4 and 5. Diluting them means that similar amounts of lactic acid lower pH further, so a similar acid load gives a lower pH in T2 and T3 than in the control. This interpretation is consistent with the similar titratable acidity but lower pH of the whey-containing yoghurts. Final pH values below 4.0 in T2 and T3 are associated with excessive sourness and increased whey separation [17], which agrees with the sensory and syneresis results. Because the storage data are single readings with a pH resolution of 0.1 units, small differences among treatments should be interpreted with caution.

Major minerals

Mineral concentrations measured by ICP-OES differed significantly among treatments ($p < 0.001$), but none showed a consistent trend with whey level (Table 4). Calcium was highest in T1 and lowest in T2, potassium and sodium were lowest in T1 and T2 and highest in T0 and T3, and magnesium was lowest in T2. Correlations with whey level were weak ($|r| \leq 0.38$).

Table 4
Major mineral concentrations in the digest solutions of set yoghurts with increasing levels of liquid Gouda cheese whey

Mineral (mg/L in digest solution)	T0 (0%)	T1 (10%)	T2 (20%)	T3 (30%)	F	r
Calcium	4.76 ± 0.02^{b}	5.16 ± 0.02^{a}	4.45 ± 0.02^{c}	4.74 ± 0.02^{b}	966	−0.34
Potassium	5.65 ± 0.02^{b}	5.27 ± 0.02^{c}	5.15 ± 0.02^{d}	5.70 ± 0.02^{a}	918	0.02
Magnesium	0.65 ± 0.02^{a}	0.62 ± 0.02^{b}	0.51 ± 0.00^{c}	0.63 ± 0.01^{ab}	78	−0.38
Sodium	4.59 ± 0.02^{b}	3.68 ± 0.01^{c}	3.61 ± 0.02^{d}	4.65 ± 0.02^{a}	4646	0.03

Values are mean ± SD of triplicate instrument readings. Means in a row with different superscript letters differ significantly (Tukey's HSD, $p < 0.05$). Concentrations are expressed in mg/L (ppm) of digest solution.

On compositional grounds, replacing milk with sweet whey should lower calcium and magnesium, which are largely bound to casein micelles and retained in the curd, and raise or maintain potassium and sodium, which partition into the serum [1], [3]. The absence of such a gradient suggests that variation in the digestion and measurement step was larger than the true treatment effect. Because these values are triplicate readings of single digests, they capture instrument precision rather than sample variability. Mineral results are therefore reported for completeness but are not used to draw nutritional conclusions. Replicate digestions with certified reference materials are needed to quantify the mineral contribution of whey in yoghurt.

Microbiological quality

Total coliforms, *E. coli*, and yeasts and moulds were below the detection limit of 10 CFU/g in all four yoghurts (Table 5).

Table 5
Hygiene indicator microorganisms in set yoghurts with increasing levels of liquid Gouda cheese whey

Microorganism (CFU/g)	T0 (0%)	T1 (10%)	T2 (20%)	T3 (30%)
Total coliforms	< 10	< 10	< 10	< 10
Escherichia coli	< 10	< 10	< 10	< 10
Yeasts and moulds	< 10	< 10	< 10	< 10

Limit of detection 10 CFU/g.

These results show that whey inclusion up to 30% did not compromise hygienic quality when the whey was heat-treated together with the milk mix at 90°C for 3 min and fermented under controlled conditions. This is notable because whey, with its high water activity and nutrient content, spoils rapidly and can carry contaminants from cheese making [2]. The final yoghurt pH of 4.4–4.5, together with lactic acid and other antimicrobial metabolites of the starter, provides an additional barrier to coliforms [17]. For industrial use, however, whey should be cooled immediately after drainage and processed within a defined time, because raw milk in Rwanda can carry high hygiene indicator loads before processing [13]. Yeast and mould counts during later storage, and the survival of starter bacteria, were not monitored and should be included in shelf-life studies.

Sensory acceptance

Whey inclusion significantly affected all sensory attributes (randomised-block ANOVA and Friedman test, $p < 0.001$; Table 6). Yoghurt with 10% whey did not differ from the control in appearance, aroma, taste, texture or overall acceptability. Its appearance (8.50) and aroma (8.53) scores were numerically the highest of all samples, although not significantly different from the control. Mouthfeel was the only attribute in which T1 scored lower than the control (7.37 vs 8.40). At 20 and 30% whey, taste, texture, mouthfeel and overall acceptability were significantly lower, and appearance and aroma also declined at 30%.

Table 6
Hedonic scores of set yoghurts with increasing levels of liquid Gouda cheese whey (n = 30 panellists)

Attribute	T0 (0%)	T1 (10%)	T2 (20%)	T3 (30%)	F	Friedman χ^2
Appearance	8.23 ± 0.68^{a}	8.50 ± 0.57^{a}	8.13 ± 0.57^{a}	7.37 ± 0.56^{b}	18.2	34.0
Aroma	8.20 ± 0.81^{ab}	8.53 ± 0.68^{a}	7.97 ± 0.72^{bc}	7.60 ± 0.89^{c}	10.8	27.0
Taste	8.30 ± 0.79^{a}	8.47 ± 0.63^{a}	7.40 ± 0.86^{b}	6.83 ± 0.70^{c}	33.9	49.5
Texture	8.03 ± 0.85^{a}	7.73 ± 0.69^{a}	6.27 ± 0.91^{b}	6.00 ± 0.79^{b}	56.4	66.6
Mouthfeel	8.40 ± 0.62^{a}	7.37 ± 0.81^{b}	5.77 ± 0.94^{c}	5.30 ± 0.88^{c}	93.6	75.8
Overall acceptability	8.23 ± 0.30^{a}	8.12 ± 0.31^{a}	7.11 ± 0.48^{b}	6.62 ± 0.40^{c}	138.1	76.1

Values are mean ± SD on a 9-point hedonic scale. Means in a row with different superscript letters differ significantly (randomised-block ANOVA with panellist as block, Tukey's HSD, $p < 0.05$; df = 3, 87). All F and Friedman tests $p < 0.001$. Overall acceptability is the mean of the five attribute scores per panellist.

The sensory pattern mirrors the instrumental results. Mouthfeel and texture, the attributes most directly linked to body and creaminess, declined most steeply, in line with the loss of viscosity and total solids. The 38% fall in viscosity at 10% whey was perceptible as a thinner mouthfeel but was not large enough to reduce texture or overall liking. At 20 and 30% whey, viscosity fell below half of the control, syneresis increased, and taste scores declined. The lower taste scores probably reflect the higher sourness associated with faster pH decline and the slightly salty, whey-like notes of the serum phase [2], [12]. The overall acceptability of T1 (8.12) was statistically equivalent to that of the control (8.23), and all four yoghurts remained above 6.5, in the “like slightly” to “like very much” range. Liquid whey at 10% thus gives a product consumers cannot distinguish hedonically from conventional set yoghurt. Higher levels remain acceptable but would need compensation, for example with skimmed milk powder, whey protein concentrate or a more effective stabiliser [14].

Practical implications and limitations

For a small plant, replacing 10% of the milk in set yoghurt with whey diverts 1 L of whey and saves 1 L of milk per 10 L of yoghurt, using existing pasteurisation, homogenisation and incubation equipment. The product retains acceptable composition, hygienic quality and consumer liking, although its viscosity is lower than that of the control. This makes direct whey inclusion a realistic first step for cheese makers that cannot invest in membrane or drying technology [7], [8]. The quantity of whey diverted at this level is modest, however. Valorising larger volumes would require higher inclusion levels combined with texture compensation, or complementary products such as whey beverages [1].

Several limitations should be considered. The study used whey from one season and region, and whey composition varies with milk, cheese type and processing. Storage measurements were single readings at each time point. The sensory panel was drawn from dairy plant staff, who may be more tolerant of dairy flavours than general consumers, and samples were served in a fixed order, so order effects cannot be excluded; cups also carried treatment codes rather than random three-digit codes, so panellists were not blinded to the samples. Starter viability, texture profile, colour and volatile compounds were not measured, and no cost analysis was performed. Future work should use randomised serving orders and a larger consumer panel, test texture-compensation strategies at 20–30% whey, and include a techno-economic and environmental assessment of whey diversion.

Conclusions

Fresh liquid Gouda cheese whey can replace up to 10% of milk in set yoghurt without loss of consumer acceptance or hygienic quality. Whey inclusion diluted protein, fat and total solids in proportion to the replacement level and reduced viscosity sharply, by 38% at 10% and 69% at 30% whey. It also increased syneresis and accelerated the pH decline during 42 days of cold storage. At 10% whey, overall acceptability (8.12 vs 8.23), texture, taste and storage-averaged syneresis did not differ from the control, and only mouthfeel was perceptibly thinner. At 20 and 30% whey, mouthfeel, texture, taste and overall acceptability declined significantly. Coliforms, *E. coli*, and yeasts and moulds remained below 10 CFU/g at all inclusion levels. Small Rwandan cheese makers can therefore adopt 10% whey inclusion with their

existing yoghurt equipment as a low-cost way to recover part of their whey. Higher inclusion levels will require texture compensation, and future work should confirm the mineral contribution of whey, establish shelf life with randomised consumer testing, and quantify the economic and environmental benefits.

Abbreviations

ANOVA
analysis of variance
CFU
colony-forming units
FTIR
Fourier-transform infrared
HSD
honestly significant difference
ICP-OES
inductively coupled plasma optical emission spectrometry
NIRDA
National Industrial Research and Development Agency
RSB
Rwanda Standards Board
SNF
solids-not-fat
TS
total solids
TTA
titratable acidity.

Declarations

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Author contributions. JDN conceived the study, carried out yoghurt production and laboratory analyses, collected the data and wrote the original thesis. FG re-analysed the data, prepared the tables and figures, and wrote and revised the manuscript. JCD co-supervised the laboratory work, contributed to data interpretation and critically reviewed the manuscript. JN supervised the research, contributed to the study design and critically reviewed the manuscript. EK participated in reviewing and critically revised the manuscript. All authors read and approved the final manuscript.

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Data availability. The datasets generated and analysed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate. The study protocol, including the consumer sensory evaluation, was approved by the INES-Ruhengeri Research Committee, Institut d'Enseignement Supérieur de Ruhengeri (INES-Ruhengeri), Musanze, Rwanda, on 12 January 2026 (approval no. INES/098/AC/RW2026). All panellists were adults who received information about the study and gave informed consent before taking part. The study was conducted in accordance with the Declaration of Helsinki. Participation was voluntary, and panellists could withdraw at any time. This study was not a clinical trial, so clinical trial registration is not applicable.

Consent for publication. Not applicable.

Competing interests. The authors declare that they have no competing interests.

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Figures

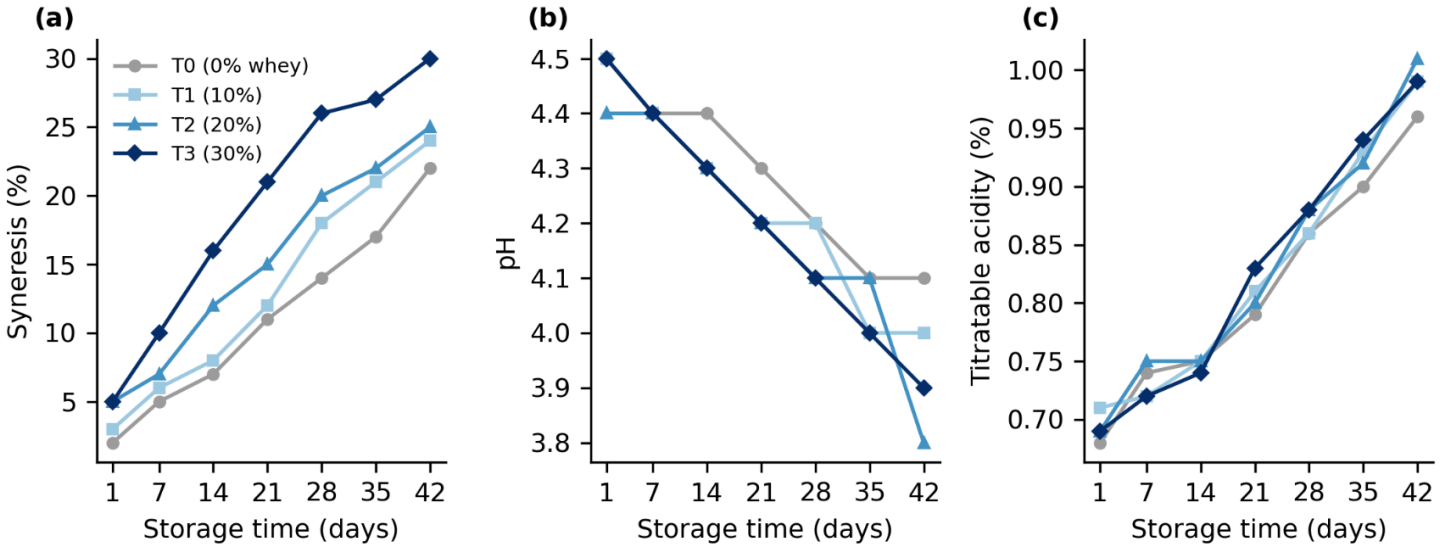


Figure 1

Changes in (a) syneresis, (b) pH and (c) titratable acidity of set yoghurts with 0 (T0), 10 (T1), 20 (T2) and 30% (T3) liquid Gouda cheese whey during 42 days at 4 °C. Two-way ANOVA (treatment × storage day): syneresis, treatment $p < 0.001$; pH, treatment $p = 0.013$; titratable acidity, treatment $p = 0.11$; storage day $p < 0.001$ for all three variables. Values are single measurements at each time point