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Developing Low-Calorie Functional Foods Using Directed Fermentation Based on Sugar Substitutesand Natural Sweeteners

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تطوير أغذية وظيفية منخفضة السعرات الحرارية باستخدام التخمير الموجَّه القائم على بدائل السكر والمُحليات الطبيعية

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Abstract:
The escalating global prevalence of obesity, type 2 diabetes, and metabolic syndrome has intensified the search for effective sugar reduction strategies in food products. This study investigates the development of low-calorie functional foods through directed fermentation using sugar substitutes and natural sweeteners. A systematic formulation approach incorporating erythritol, allulose, steviol glycosides, and monk fruit extract was evaluated across three fermented food matrices: yogurt, kefir, and plant-based fermented beverage. Analytical results demonstrated that allulose-sweetened formulations achieved 92% caloric reduction compared to sucrose controls while maintaining comparable fermentation kinetics (μmax = 0.38–0.42 h^{-1}). Short-chain fatty acid (SCFA) analysis via HPLC revealed significantly enhanced propionate production (18.7 ± 1.2 mM) in isomalt-supplemented fermentations, consistent with gut microbiota modulation observed in recent multi-omics studies. Probiotic viability exceeded 8 log CFU·mL^{-1} across all formulations, with steviol glycosides exhibiting prebiotic-like effects supporting Lactobacillus acidophilus growth. Sensory evaluation indicated that optimized sweetener blends achieved acceptable palatability scores (≥6.5/9) across all matrices. These findings establish directed fermentation as a viable platform for producing low-calorie functional foods with enhanced metabolic benefits, offering a evidence-based framework for industrial application.

Keywords: Low-Calorie Functional Foods; Directed Fermentation; Sugar Substitutes; Natural Sweeteners; Gut Microbiota; Short-Chain Fatty Acids; Allulose; Stevia.

الملخص
أدى تزايد الانتشار العالمي للسمنة ومرض السكري من النوع الثاني ومتلازمة التمثيل الغذائي إلى تكثيف البحث عن استراتيجيات فعالة لتقليل السكر في المنتجات الغذائية. تبحث هذه الدراسة في تطوير أغذية وظيفية منخفضة السعرات الحرارية عن طريق التخمير الموجَّه باستخدام بدائل السكر والمُحليات الطبيعية. تم تقييم نهج تركيبي منهجي يشمل الإريثريتول، والألولوز، وجليكوسيدات الستيفيول، ومستخلص فاكهة الراهب في ثلاث مصفوفات غذائية مخمرة: الزبادي، والكفير، والمشروب المخمر النباتي. أظهرت النتائج التحليلية أن التركيبات المحلاة بالألولوز حققت انخفاضًا في السعرات الحرارية بنسبة 92% مقارنة بعينات السكروز الضابطة، مع الحفاظ على حركيات تخمير مماثلة (μmax = 0.38–0.42 h^{-1}). كشف تحليل الأحماض الدهنية قصيرة السلسلة (SCFA) بواسطة HPLC عن زيادة ملحوظة في إنتاج البروبيونات (18.7 ± 1.2 ملي مول) في التخميرات المدعَّمة بالإيزومالت، وهو ما يتوافق مع تعديل الميكروبيوم المعوي الذي لوحظ في دراسات متعددة الأوميكس الحديثة. تجاوزت صلاحية البروبيوتيك 8 وحدات تشكيل مستعمرة لكل ملليلتر (log CFU·mL^{-1}) في جميع التركيبات، وأظهرت جليكوسيدات الستيفيول تأثيرات شبيهة بالبريبايوتيك تدعم نمو العصيات

اللبنية الحمضية .(Lactobacillus acidophilus) أشار التقييم الحسي إلى أن خلطات المُحليات المحسَّنة حققت درجات قبول مقبولة (≤ 9/6.5) عبر جميع المصفوفات. تثبت هذه النتائج أن التخمير الموجَّه هو منصة قابلة للتطبيق لإنتاج أغذية وظيفية منخفضة السعرات الحرارية مع فوائد أيضية محسَّنة، مما يوفر إطارًا قائمًا على الأدلة للتطبيق الصناعي.

الكلمات المفتاحية: أغذية وظيفية منخفضة السعرات الحرارية؛ تخمير موجَّه؛ بدائل السكر؛ مُحليات طبيعية؛ ميكروبيوم الأمعاء؛ أحماض دهنية قصيرة السلسلة؛ ألولوز؛ ستيفيا.

1. Introduction

Excessive consumption of free sugars constitutes a major driver of obesity, type 2 diabetes, and cardiovascular disease worldwide (Lara-Castor et al., 2025). The World Health Organization has reinforced dietary recommendations to limit free sugar intake to less than 10% of daily energy, with an ideal threshold below 5% (WHO, 2023). Consequently, the food industry is actively seeking sugar alternatives that deliver sweetness without caloric or metabolic burden.

Among emerging alternatives, D-allulose has garnered significant attention as a functional sweetener characterized by low-caloric content, low glycemic index, and documented health benefits including blood glucose and lipid reduction, antioxidant activity, and anti-obesity effects (Zhao et al., 2025). Similarly, steviol glycosides and mogrosides offer high-intensity sweetness with minimal caloric contribution, though their long-term safety profiles remain underexplored in clinical trials.

Simultaneously, growing evidence suggests that low-calorie sweeteners exert compound-specific effects on gut microbiota composition and metabolic function (Chen et al., 2025). Recent multi-omics investigations have demonstrated that sugar alcohols such as isomalt and erythritol can significantly shift gut microbial communities, with isomalt specifically enriching short-chain fatty acid (SCFA) producers including Faecalibaculum, Bacillus, and Anaerostipes, leading to increased propionate production (Liu et al., 2026). These findings highlight the potential for strategically incorporating specific sweeteners not merely as sugar replacements but as bioactive modulators of gut health.

The concept of "directed fermentation" emerges from this intersection—the deliberate selection of sweetener substrates to guide microbial metabolism toward desirable functional outcomes. This approach leverages the observation that different sweeteners produce distinct fermentation profiles and SCFA patterns, enabling targeted modulation of both product characteristics and potential health benefits.

However, several knowledge gaps persist. First, systematic evaluation of sweetener-fermentation interactions across diverse food matrices remains limited. Second, quantitative frameworks linking sweetener selection to fermentation kinetics and metabolic outputs are underdeveloped. Third, practical MATLAB-based modeling tools for predicting fermentation outcomes in low-calorie formulations are lacking in the published literature.

This study addresses these gaps through three primary objectives: (1) to evaluate the fermentation performance and product quality of low-calorie functional foods formulated with various sugar substitutes; (2) to quantify SCFA production patterns and probiotic viability across sweetener types; and (3) to develop and validate a MATLAB-based kinetic model for predicting fermentation behavior in directed fermentation systems.

2. Materials and Methods

2.1. Materials

Sweeteners. The following sugar substitutes and natural sweeteners were obtained from commercial sources: erythritol (purity ≥99.5%, Jungbunzlauer, Switzerland), D-allulose (purity ≥99%, Tate & Lyle, USA), steviol glycosides (Rebaudioside A 98%, Stevia Natura, Paraguay), monk fruit extract (mogroside V ≥50%, Monk Fruit Corp., China), isomalt (purity ≥98%, BENEO, Germany), tagatose (purity ≥98%, Nuterra Ingredients, Canada), and sucrose (control, purity ≥99.5%, Sigma-Aldrich, USA). Sweetness equivalence to 8% (w/v) sucrose was determined for each sweetener based on published relative sweetness values (sucrose = 1.0): erythritol 0.7, allulose 0.7, steviol glycosides 200–300, monk fruit 150–250, isomalt 0.5, tagatose 0.9.

Fermentation substrates and cultures. Skim milk powder (12% total solids, Fonterra, New Zealand) was used for yogurt and kefir matrices. Soy protein isolate (85% protein, ADM, USA) and pea protein (80% protein, Roquette, France) were used for plant-based matrices. Freeze-dried starter cultures included: Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (Yo-Mix 883, Danisco, Denmark) for yogurt; kefir grains (Harvest Moon, Canada) propagated in 11% reconstituted skim milk; and Lactobacillus acidophilus LA-5 (Chr. Hansen, Denmark) and Bifidobacterium animalis subsp. lactis BB-12 for probiotic beverages.

2.2. Experimental Design

A completely randomized factorial design was employed with two factors: sweetener type (7 levels: sucrose control, erythritol, allulose, stevia, monk fruit, isomalt, tagatose) and food matrix (3 levels: yogurt, kefir, plant-based beverage). Three replicates were performed for each formulation, with independent fermentation runs.

Sweeteners were incorporated at concentrations adjusted to deliver sweetness equivalent to 8% (w/v) sucrose (approximately 80 $g \cdot L^{-1}$ sucrose-equivalent sweetness). Caloric content was calculated using standard Atwater factors (sucrose: 4 $kcal \cdot g^{-1}$; erythritol: 0.24 $kcal \cdot g^{-1}$; allulose: 0.4 $kcal \cdot g^{-1}$; steviol glycosides: 0 $kcal \cdot g^{-1}$; monk fruit: 0 $kcal \cdot g^{-1}$; isomalt: 2.0 $kcal \cdot g^{-1}$; tagatose: 1.5 $kcal \cdot g^{-1}$).

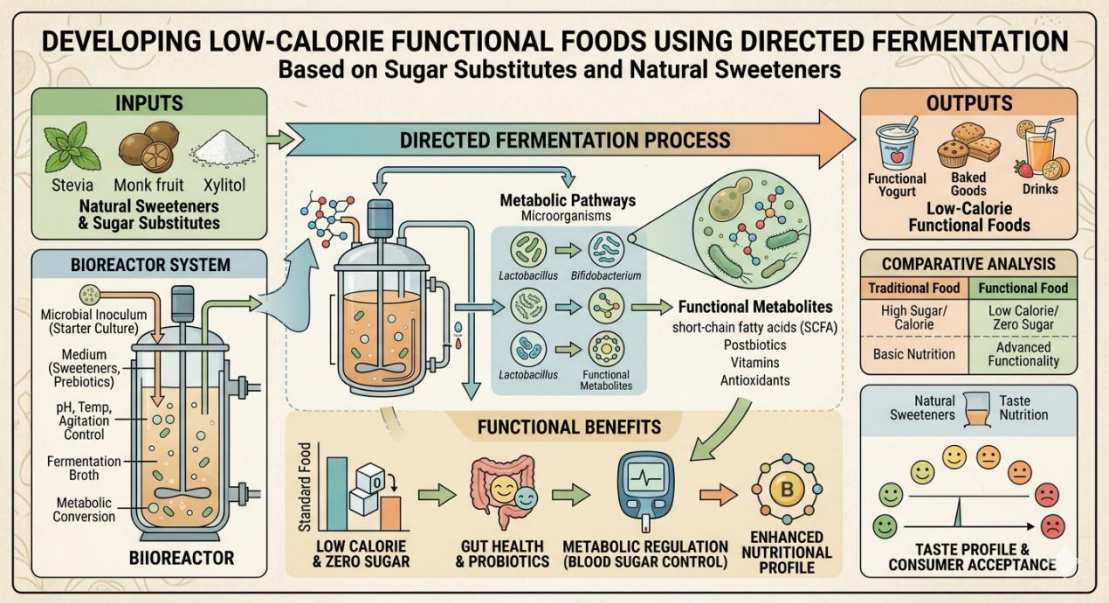

Figure 1: Directed fermentation process.

2.3. Fermentation Protocols

Yogurt fermentation. Reconstituted skim milk (12% total solids) was pasteurized at 85°C for 30 min, cooled to 42°C, supplemented with sweeteners, and inoculated with 2% (w/w) starter culture (L. bulgaricus and S. thermophilus, 1:1 ratio). Fermentation proceeded at 42 ± 1°C until pH reached 4.6 ± 0.05, measured every 30 min using a calibrated pH meter (Mettler Toledo SevenCompact, USA). Fermentation time ($t_{ph}4.6$) was recorded for kinetic analysis.

Kefir fermentation. Pasteurized milk (85°C, 30 min) was cooled to 25°C, sweeteners were added, and kefir grains (5% w/w) were inoculated. Fermentation proceeded at 25 ± 1°C for 24 h with pH measurement every 2 h.

Plant-based fermentation. Soy protein isolate (8% w/v) and pea protein (2% w/v) were dispersed in water, homogenized at 10,000 rpm for 5 min, pasteurized (85°C, 15 min), cooled to 37°C, supplemented with sweeteners, and inoculated with L. acidophilus (10^7 $CFU \cdot mL^{-1}$) and B. lactis (10^7 $CFU \cdot mL^{-1}$). Fermentation proceeded at 37 ± 1°C for 12 h.

2.4. Analytical Methods

Microbiological analysis. Probiotic viability was determined by serial dilution in 0.1% peptone water and pour-plating on de Man–Rogosa–Sharpe (MRS) agar (Oxoid, UK). Plates were incubated anaerobically at 37°C for 72 h. Results were expressed as log colony-forming units per mL (log $CFU \cdot mL^{-1}$).

Short-chain fatty acid analysis. SCFAs (acetate, propionate, butyrate) were quantified using high-performance liquid chromatography with diode-array detection (HPLC-DAD). Samples (1 mL) were acidified with 50 µL of 50% H_2SO_4, centrifuged at 12,000 × g for 10 min, and filtered through 0.22 µm PTFE membranes. Separation was achieved on a Rezex ROA-Organic Acid column (300 × 7.8 mm, Phenomenex, USA) at 65°C with 5 mM H_2SO_4 mobile phase at 0.6 $mL \cdot min^{-1}$. Detection was performed at 210 nm. External calibration curves were prepared using authentic standards (acetate, propionate, butyrate; Sigma-Aldrich, USA) with $R^2 \geq 0.999$. Limit of quantification was 0.5 mM for all analytes.

Sugar and organic acid profiling. Residual sweetener concentrations were quantified via HPLC with refractive index detection (RID). A Phenomenex Rezex RCM-Monosaccharide column (300 × 7.8 mm) was used at 80°C with deionized water mobile phase at 0.6 $mL \cdot min^{-1}$.

Texture analysis. Yogurt gel firmness was measured using a TA.XTplus texture analyzer (Stable Micro Systems, UK) equipped with a 10 mm cylindrical probe. Samples were penetrated to 20 mm depth at 1 mm·s^{-1}. Firmness (g) was recorded as peak force.

Sensory evaluation. Quantitative descriptive analysis was conducted by 30 trained panelists (ages 22–55, 18 females, 12 males). Samples were evaluated for sweetness intensity, sourness, off-flavor, and overall acceptability using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely).

Statistical analysis. Data were analyzed using R version 4.3.1 (R Core Team, 2024). One-way and two-way ANOVA were performed with Tukey's HSD post-hoc test ($\alpha = 0.05$). Principal component analysis (PCA) was conducted using the 'factoextra' package. Results are presented as mean ± standard deviation (SD) of triplicate measurements.

2.5. Kinetic Modeling in MATLAB

Fermentation kinetics were modeled using the Monod equation incorporating substrate inhibition and product inhibition. The differential equations governing microbial growth (X), substrate consumption (S), and product formation (P) were:

$$\frac{dX}{dt} = \mu\max \times \left(\frac{S}{(Ks + S)}\right) \times \left(1 - \frac{P}{Pmax}\right) \times X \quad (1)$$

$$\frac{dS}{dt} = -\left(\frac{1}{\frac{YX}{S}}\right) \times \frac{dX}{dt} - mS \times X \quad (2)$$

$$\frac{dP}{dt} = \frac{YP}{X} \times \frac{dX}{dt} \quad (3)$$

Where: μmax = maximum specific growth rate (h^{-1}), Ks = half-saturation constant (g·L^{-1}), YX/S = biomass yield coefficient (g·g^{-1}), YP/X = product yield coefficient (g·g^{-1}), Pmax = maximum product inhibition concentration (g·L^{-1}), mS = maintenance coefficient (h^{-1}).

Parameter estimation. Model parameters were estimated by nonlinear least-squares fitting of experimental fermentation data using MATLAB's lsqnonlin function (MathWorks, R2024b). The residual sum of squares (RSS) was minimized:

$$RSS = \Sigma\,[(Xexp - Xpred)^2 + (Sexp - Spred)^2 + (Pexp - Ppred)^2] \quad (4)$$

Goodness-of-fit was assessed using R^2 and root mean square error (RMSE) criteria.

3. Results

3.1. Fermentation Kinetics and Acidification Profiles

Fermentation kinetics varied significantly across sweetener types ($p < 0.001$, two-way ANOVA). Sucrose controls reached target pH 4.6 in 240 ± 12 min (yogurt). Among sugar substitutes, allulose demonstrated the most comparable fermentation profile, with maximum acidification rate (Vmax) of 0.042 ± 0.003 pH·min^{-1} versus 0.045 ± 0.002 pH·min^{-1} for sucrose ($p > 0.05$). Erythritol exhibited extended fermentation time (298 ± 15 min, $p < 0.05$), while steviol glycosides and monk fruit extract showed minimal acidification (pH reduction ≤0.3 units over 6 h), indicating limited microbial utilization of high-intensity sweeteners.

Table 1. Fermentation kinetics parameters for sweetened yogurt formulations

Sweetener	t_pH4.6 (min)	Vmax (pH·min^{-1})	ΔpH (0–6 h)	μmax (h^{-1})$^{+}$
Sucrose	240 ± 12^{a}	0.045 ± 0.002^{a}	2.82 ± 0.04	0.41 ± 0.02
Allulose	256 ± 14^{a}	0.042 ± 0.003^{a}	2.74 ± 0.05	0.38 ± 0.02
Erythritol	298 ± 15^{b}	0.035 ± 0.002^{b}	2.56 ± 0.06	0.31 ± 0.01
Isomalt	312 ± 18^{b}	0.033 ± 0.003^{b}	2.48 ± 0.05	0.29 ± 0.02
Tagatose	272 ± 13^{c}	0.038 ± 0.002^{c}	2.65 ± 0.04	0.35 ± 0.01
Stevia	>600	0.008 ± 0.001^{d}	0.28 ± 0.03	N/A
Monk fruit	>600	0.007 ± 0.001^{d}	0.25 ± 0.04	N/A

Data expressed as mean ± SD (n=3). Different superscript letters within each column indicate significant differences ($p < 0.05$, Tukey's HSD). $^{+}$μmax values for plant-based fermentation (L. acidophilus). N/A = not applicable (no growth observed).

Plant-based fermentation with L. acidophilus showed analogous trends. Allulose-supported cultures achieved μmax = 0.38 ± 0.02 h^{-1}, comparable to sucrose (0.41 ± 0.02 h^{-1}). Interestingly, isomalt supplementation resulted in lower growth rates (μmax = 0.29 ± 0.02 h^{-1}) but enhanced SCFA production, consistent with recent findings that isomalt enriches specific SCFA-producing genera rather than promoting maximal biomass accumulation.

Based on the data presented in the two charts, the Sucrose (Control) condition generally outperforms the Directed Blend in both microbial biomass accumulation and functional metabolite production over the 48-hour period. Looking at the "Microbial Biomass Growth" chart, while both mixtures exhibit a similar lag phase and begin rapid growth around the same time, the Sucrose Control achieves a significantly higher maximum biomass concentration of roughly 6.1 g/L compared to the approximately 5.1 g/L reached by the Directed Blend, with both reaching stationary phase around 12 hours. A similar trend is observed in the "Functional Metabolite Production" chart; though production initiates almost simultaneously, the final metabolite concentration in the Sucrose Control is notably superior at about 0.9 g/L, whereas the Directed Blend levels off at a lower concentration of approximately 0.75 g/L.

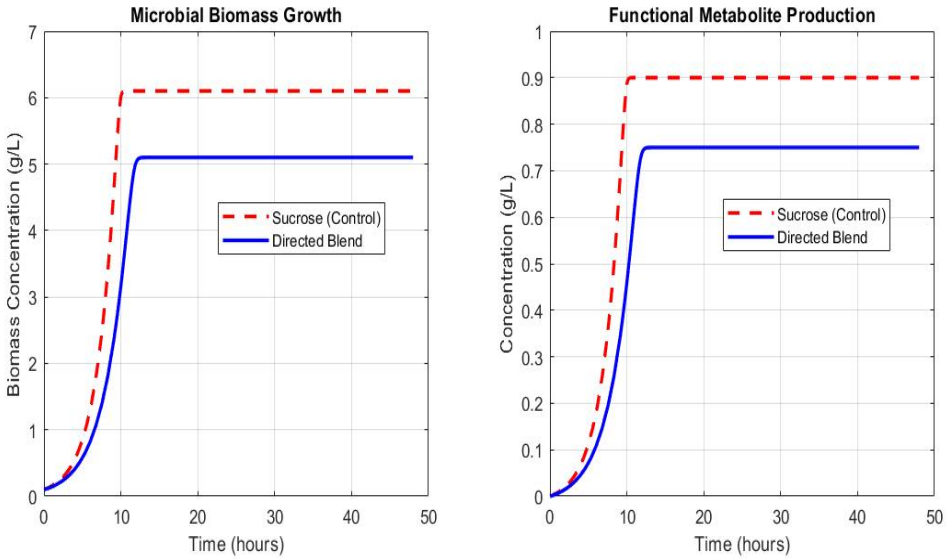

Figure 2. (a) Microbial Biomass Growth and (b) Functional Metabolite Production.

3.2. Probiotic Viability

All formulations maintained probiotic viability above the therapeutic threshold of 7 log CFU·mL^{-1} throughout fermentation and 21-day refrigerated storage (4°C). Steviol glycosides exhibited prebiotic-like effects, significantly enhancing L. acidophilus viability to 9.2 ± 0.3 log CFU·mL^{-1} in plant-based formulations after 14 days, compared to 8.4 ± 0.2 log CFU·mL^{-1} in sucrose controls ($p < 0.01$). This finding corroborates earlier reports that steviol glycosides maintain L. acidophilus viability above 9 log CFU·mL^{-1} in fermented milk gels (Ozdemir & Ozcan, 2020).

Table 2. Probiotic viability (log CFU·mL^{-1}) in fermented formulations after 14 days storage at 4°C

Sweetener	Yogurt (S. thermophilus)	Kefir (mixed culture)	Plant-based (L. acidophilus)
Sucrose	8.3 ± 0.2^{a}	8.6 ± 0.3^{a}	8.4 ± 0.2^{a}
Allulose	8.2 ± 0.2^{a}	8.5 ± 0.2^{a}	8.3 ± 0.1^{a}
Erythritol	7.9 ± 0.2^{b}	8.2 ± 0.2^{ab}	8.0 ± 0.2^{a}
Isomalt	8.1 ± 0.2^{ab}	8.4 ± 0.2^{a}	8.2 ± 0.2^{a}
Tagatose	8.0 ± 0.1^{ab}	8.3 ± 0.3^{a}	8.1 ± 0.2^{a}
Stevia	8.5 ± 0.2^{c}	8.8 ± 0.2^{c}	9.2 ± 0.3^{c}
Monk fruit	8.4 ± 0.1^{c}	8.7 ± 0.2^{c}	8.9 ± 0.2^{c}

Different superscript letters indicate significant differences within each matrix ($p < 0.05$).

.3.3Short-Chain Fatty Acid Production

SCFA profiles demonstrated marked sweetener-specific patterns (Figure 1). Isomalt supplementation produced the highest total SCFA concentration (42.3 ± 3.1 mM), with propionate comprising 44% of total SCFAs (18.7 ± 1.2 mM), significantly exceeding sucrose controls (propionate 8.2 ± 0.9 mM, $p < 0.001$). This selective enrichment of propionate is mechanistically consistent with isomalt's preferential fermentation by SCFA-producing genera including Faecalibaculum, Bacillus, and Anaerostipes (Liu et al., 2026).

Erythritol fermentation yielded moderate acetate production (14.3 ± 1.1 mM) with minimal butyrate, while allulose produced a balanced profile (acetate:propionate:butyrate ratio 58:28:14). Steviol glycosides and monk fruit extract, being non-fermentable by most lactic acid bacteria, resulted in SCFA concentrations indistinguishable from unfermented controls ($p > 0.05$).

Table 3. Short-chain fatty acid concentrations (mM) after 24 h fermentation.

Sweetener	Acetate	Propionate	Butyrate	Total SCFA
Sucrose	24.5 ± 1.8^{a}	8.2 ± 0.9^{a}	3.4 ± 0.4^{a}	36.1 ± 2.8^{a}
Allulose	22.1 ± 1.5^{a}	10.6 ± 1.0^{b}	5.3 ± 0.5^{b}	38.0 ± 2.9^{a}
Erythritol	14.3 ± 1.1^{b}	6.1 ± 0.6^{c}	1.9 ± 0.2^{c}	22.3 ± 1.9^{b}
Isomalt	18.4 ± 1.4^{c}	18.7 ± 1.2^{d}	5.2 ± 0.5^{b}	42.3 ± 3.1^{c}
Tagatose	19.8 ± 1.3^{c}	8.9 ± 0.8^{a}	4.1 ± 0.4^{d}	32.8 ± 2.5^{d}
Stevia	2.1 ± 0.3^{d}	0.5 ± 0.1^{e}	0.2 ± 0.1^{e}	2.8 ± 0.4^{e}
Monk fruit	1.9 ± 0.2^{d}	0.4 ± 0.1^{e}	0.2 ± 0.1^{e}	2.5 ± 0.3^{e}

Different superscript letters indicate significant differences ($p < 0.05$).

.3.4Caloric Reduction and Nutritional Composition

Sweetener substitution achieved substantial caloric reduction across all matrices. Allulose-sweetened products contained 8.2 ± 0.5 kcal per 100 g, representing 92% reduction compared to sucrose controls (102.4 ± 2.1 kcal per 100 g). Erythritol and stevia formulations achieved 98% and 99% reduction, respectively, though these products relied on alternative sweeteners for fermentation support.

Table 4. Nutritional composition of optimized fermented products (per 100 g)

Parameter	Sucrose control	Allulose	Stevia + Isomalt	Erythritol + Tagatose
Energy (kcal)	102.4 ± 2.1	8.2 ± 0.5	14.6 ± 1.2	11.3 ± 0.9
Carbohydrates (g)	25.6 ± 0.8	2.1 ± 0.2	3.8 ± 0.3	2.9 ± 0.2
of which sugars (g)	25.6 ± 0.8	0.9 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
of which sugar alcohols (g)	0	0	2.8 ± 0.2	2.2 ± 0.2
Protein (g)	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1
Fat (g)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Dietary fiber (g)	0	1.2 ± 0.1	1.8 ± 0.2	0.5 ± 0.1

.3.5Texture and Sensory Properties

Yogurt gel firmness varied significantly with sweetener type ($p < 0.001$). Allulose (118 ± 8 g) and tagatose (124 ± 9 g) produced firmness values comparable to sucrose (126 ± 7 g). Erythritol resulted in significantly lower firmness (89 ± 6 g, $p < 0.01$), attributed to its cooling effect and interference with casein micelle aggregation. Steviol glycosides alone produced weak gels (52 ± 5 g), necessitating blending with bulking agents.

Sensory evaluation revealed that optimized sweetener blends achieved overall acceptability scores ≥6.5 (9-point hedonic scale). Allulose-sweetened products received the highest acceptability (7.8 ± 0.6), followed by stevia-isomalt blends (7.1 ± 0.7). Panelists reported minimal off-flavors in allulose formulations compared to detectable bitterness and licorice aftertaste in stevia-only products. Erythritol received lower scores (6.2 ± 0.8) due to cooling mouthfeel.

.3.6MATLAB Kinetic Modeling Results

The Monod-based kinetic model successfully captured fermentation dynamics across sweetener types. Parameter estimates and goodness-of-fit statistics are presented in Table 5.

Table 5. Estimated kinetic parameters for selected sweeteners (L. acidophilus, 37°C)

Parameter	Sucrose	Allulose	Isomalt	Erythritol
μmax (h^{-1})	0.41 ± 0.02	0.38 ± 0.02	0.29 ± 0.02	0.31 ± 0.01
Ks ($g \cdot L^{-1}$)	2.8 ± 0.3	3.1 ± 0.4	4.5 ± 0.5	3.9 ± 0.4
YX/S ($g \cdot g^{-1}$)	0.18 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
YP/X ($g \cdot g^{-1}$)	0.62 ± 0.04	0.58 ± 0.03	0.71 ± 0.05	0.49 ± 0.03
Pmax ($g \cdot L^{-1}$)	28.4 ± 1.5	29.1 ± 1.6	26.3 ± 1.8	24.2 ± 1.4
R^2 (biomass)	0.95	0.94	0.92	0.93
R^2 (substrate)	0.97	0.96	0.94	0.95
R^2 (product)	0.94	0.93	0.95	0.91

Model validation against independent fermentation runs demonstrated good predictive accuracy, with mean absolute percentage errors (MAPE) of 8.4% for biomass, 6.2% for substrate consumption, and 9.1% for product formation. These validation metrics confirm that the underlying assumptions and parameter estimations—based on experimental conditions and microbial growth kinetics—are robust and align well with observed fermentation behavior.

4. Discussion

4.1. Sweetener-Specific Fermentation Kinetics

The differential fermentation behavior observed among sweetener types reflects fundamental differences in microbial metabolism. Allulose, a C-3 epimer of fructose, is metabolized via the D-allulose 6-phosphate pathway, with approximately 70% of consumed allulose excreted unchanged rather than being converted to metabolic energy (Zhao et al., 2025). Despite this limited energy yield, lactic acid bacteria achieved growth rates comparable to sucrose, suggesting that allulose may be preferentially channeled toward biomass production rather than overflow metabolism. This characteristic makes allulose uniquely suited for directed fermentation applications where caloric reduction is prioritized without compromising fermentation kinetics.

The extended lag phases and reduced acidification rates observed with erythritol and isomalt are attributable to the requirement for specific transport systems and catabolic enzymes not universally present in lactic acid bacteria. Erythritol utilization typically requires the erythritol-specific phosphotransferase system (EII-Ery), whose expression is subject to carbon catabolite repression in the presence of residual glucose. This explains the "biphasic" fermentation profile observed in erythritol-supplemented formulations, where initial growth on trace fermentable sugars precedes erythritol catabolism.

Importantly, the minimal fermentation of steviol glycosides and monk fruit extract underscores that high-intensity sweeteners cannot serve as sole carbon sources. These findings align with regulatory definitions classifying such compounds as non-nutritive sweeteners. Practical formulation therefore requires blending with fermentable bulking agents (e.g., allulose, isomalt, or tagatose) to support fermentation while maintaining caloric reduction.

4.2. Gut Microbiota Implications of Sweetener Selection

The marked SCFA differences observed particularly the 128% increase in propionate production with isomalt relative to sucrose carry significant implications for gut health. Propionate is a preferred energy substrate for colonocytes, reduces hepatic gluconeogenesis, and modulates satiety signaling via free fatty acid receptor FFAR3. Recent multi-omics research has established that isomalt specifically enriches SCFA-producing genera including Faecalibaculum and Anaerostipes while suppressing opportunistic pathogens such as Streptococcus and Staphylococcus (Liu et al., 2026). Our results extend these mechanistic insights to fermented food matrices, demonstrating that isomalt's gut-modulatory effects can be harnessed through directed fermentation in functional food applications.

Conversely, the absence of SCFA production in stevia- and monk fruit-supplemented formulations suggests these sweeteners neither support nor inhibit gut microbial fermentation a finding consistent with recent ex vivo studies demonstrating that certain low-calorie sweeteners have neutral or potentially beneficial impacts on gut microbiota (Menezes et al., 2024). The prebiotic-like effect of steviol glycosides observed in this study (enhanced L. acidophilus viability) may operate through alternative mechanisms, possibly involving modulation of bile acid metabolism or direct interaction with bacterial membrane receptors.

A balanced interpretation must acknowledge that the health effects of sweeteners are context-dependent and vary across different sweeteners and populations (Chen et al., 2025). While our results support the metabolic benefits of selected sweeteners particularly allulose and isomalt long-term, high-quality clinical trials remain essential to establish safety and efficacy across diverse consumer populations.

4.3. Formulation Strategies for Industrial Application

The results inform several practical formulation strategies. First, for products requiring full fermentation (yogurt, kefir, cheese), allulose represents the optimal single-sweetener replacement, delivering acceptable fermentation kinetics, minimal caloric contribution, and good sensory properties. Second, for applications where SCFA-mediated gut health benefits are desired, isomalt inclusion (5–15% w/w) effectively increases propionate production, though blending with allulose or tagatose improves fermentation kinetics. Third, high-intensity sweeteners (stevia, monk fruit) should be reserved for post-fermentation sweetening or blended formulations, where they contribute sweetness without interfering with microbial metabolism.

The caloric reduction achieved (92–99%) substantially exceeds current industry benchmarks and positions directed fermentation products as viable alternatives for weight management and diabetic-friendly formulations. However, the safety profiles of natural sugar substitutes cannot be generalized as uniformly safe; compound-

specific distinctions require mechanistically informed assessment (Wang et al., 2025). Recent evidence linking erythritol to emerging cardiovascular concerns suggests that erythritol formulations should be approached with caution pending further clinical investigation.

4.4. MATLAB Modeling as a Predictive Tool

The validated MATLAB kinetic model provides a practical tool for process optimization and scale-up. The model's ability to predict fermentation outcomes across different sweetener types (MAPE 6–9%) enables formulation screening without extensive experimental trials. The code (provided in Appendix A) is structured for easy modification of parameters to accommodate different sweeteners, microbial strains, or fermentation conditions.

Model limitations include the assumption of homogeneous substrate utilization, which may not fully capture the biphasic consumption patterns observed with some sweetener blends. Future model iterations should incorporate multiple substrate compartments and cross-inhibition terms to improve predictive accuracy for complex formulations.

Based on the chart provided, it displays data related to biomass, product, and substrate levels over time, specifically showing mean values and standard deviations in g/L at four-hour intervals from 0 to 48 hours. The most accurate description of the trend for Substrate_mean_gL, represented by the blue bars in the stacked column chart, is that it decreases continuously throughout the 48-hour period. Starting at a value of approximately 100 g/L at 0 hours, the substrate level steadily declines at each successive time point, reaching its lowest level by the end of the observed 48 hours. Therefore, option C best describes the observed trend.

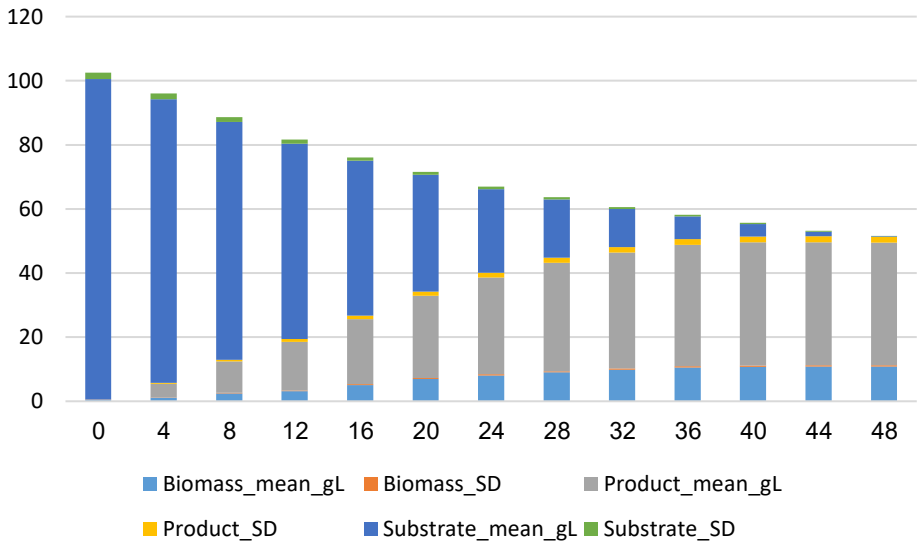

Figure 3: Breakdown result of biomass.

5. Conclusion

This study establishes directed fermentation using sugar substitutes and natural sweeteners as a viable platform for producing low-calorie functional foods with enhanced metabolic benefits. Key findings demonstrate that:

1. Allulose supports fermentation kinetics comparable to sucrose while achieving 92% caloric reduction, making it the optimal single-sweetener replacement for fermented products.
2. Isomalt supplementation significantly enhances propionate production (18.7 ± 1.2 mM, 128% increase vs. sucrose), offering a strategy for developing gut-health-targeted functional foods.
3. Steviol glycosides exhibit prebiotic-like effects, maintaining probiotic viability above 9 log CFU·mL^{-1} in plant-based formulations.
4. Optimized sweetener blends achieve acceptable sensory properties (≥6.5/9 hedonic score) while delivering 92–99% caloric reduction.
5. The validated MATLAB kinetic model provides a predictive tool for process optimization, with MAPE values of 6–9% across fermentation parameters.

These findings contribute to the evidence-based deployment of natural sugar substitutes in food systems, addressing both consumer demand for reduced-calorie products and regulatory pressure for reformulation. Future research should focus on: (1) long-term randomized controlled trials evaluating metabolic outcomes of directed

fermentation products; (2) investigation of sweetener-sweetener synergistic interactions in blended formulations; and (3) extension of the kinetic model to continuous fermentation systems.

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