In Vitro Gastrointestinal Tolerance and Survival of Probiotics in a Novel Avocado-Palm Sugar Synbiotic Ice Cream Matrix

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***Abstract*—This research used a simulated human gastrointestinal system to evaluate the biological viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* within a functional synbiotic ice cream. The formulation replaced standard dairy ingredients with avocado pulp, unrefined palm sugar, and chicory root inulin. Probiotic survival rates were tracked across a sequential 240-minute *in vitro* digestion model, which included an initial oral phase, a 120-minute gastric phase (simulated gastric juice with pepsin at pH 2.0 and 3.0), and a 120-minute intestinal phase (simulated intestinal fluid with pancreatin and oxgall bile salts at pH 7.5). The baseline formulation without prebiotics $\left((T_{\{1\}}\right)$ showed poor resistance to gastric transit, with *B. bifidum* cell counts dropping significantly from (8.52) to \\$(4.95)((p < 0.05))$ due to low stomach acid stress. However, adding (4.0%) chicory root inulin $\left((T_{\{3\})}\right)$ significantly improved survival rates, maintaining protective cell counts of \\(7.95\\text{ log CFU/g}) for *L. acidophilus* and (7.58) for *B. bifidum* at the end of the gastric phase. By the end of the 240-minute digestion cycle, the complete synbiotic formulation $\left((T_{\{3\})}\right)$ kept viable cell populations well above the minimum therapeutic threshold $\left((\backslash ge\ 10^{7\backslash text\{\frac{CFU}{g}\})}\right)$, whereas the unprotected control populations collapsed. This study demonstrates that the combination of avocado monounsaturated fatty acids and chicory inulin forms an effective protective barrier that shields probiotic bacteria during digestion, ensuring they reach the lower intestine in therapeutically active quantities.**

***Index Terms*—In Vitro Digestion, Probiotic Viability, Gastric Tolerance, Bile Resistance, Chicory Inulin, Shielding Mechanism.**

I. INTRODUCTION

To deliver genuine health benefits, probiotic foods must ensure that helpful microorganisms survive processing, storage, and human digestion. Regulatory bodies state that functional foods must contain a minimum of viable probiotic cells per gram at the time of consumption to positively influence gut health. However, passing through the human gastrointestinal tract (GIT) presents severe biochemical challenges, including harsh stomach acids (pH 1.5 − 3.0), digestive enzymes like pepsin, and fat-dissolving bile salts in the small intestine.

$[Mouth: Mechanical\ Shear]$—

$> [Stomach: HCl \,\&\, Pepsin\ (pH\ 2.0)]$—

$> [Intestine: Bile\ Salts\ (pH\ 7.5)]$

$|$ $|$

$Cell\ Denaturing\ Obstacle$ $Membrane\ Dissolution\ Obstacle$

Traditional ice cream matrices can provide modest protection for probiotics because their milk fat content and freezing temperatures help insulate the bacterial cells. However, replacing dairy ingredients with plant-based alternatives completely shifts the physical and chemical environment, which can alter how well these bacteria survive digestion.

This study examines a novel formulation where milk fat is replaced by lipid-dense avocado pulp and sucrose is substituted with unrefined palm sugar. Avocado pulp provides healthy monounsaturated fatty acids (such as oleic acid), while palm sugar offers minerals and low-glycemic carbohydrates.

To improve both the structural integrity and the biological survival rates of this formulation, chicory root inulin was introduced as a prebiotic agent. While previous studies have evaluated probiotic survival in standard dairy systems, this research uses an *in vitro* gastrointestinal model to investigate how a combined avocado, palm sugar, and chicory inulin matrix protects *L. acidophilus* and *B. bifidum* during digestion.

II. REVIEW OF LITERATURE

Probiotic bacteria face severe physical and chemical stresses as they pass through the human digestive tract. In the stomach, hydrochloric acid lowers the pH, causing hydronium ions to flood the bacterial cells. This acid influx denatures internal proteins, disrupts vital enzyme activities, and damages bacterial DNA, often leading to cell death. When the remaining cells enter the small intestine, they encounter bile salts, which act as natural detergents that can dissolve the lipid membranes of the bacteria, causing cell breakdown.

Previous research shows that the physical layout of the food matrix plays a key role in shielding bacteria from these digestive stresses. Avocado pulp contains a natural emulsion of long-chain fatty acids that can coat the probiotic cells, creating a hydrophobic layer that slows down the penetration of stomach acids.

Concurrently, chicory root inulin functions as a key component of this protective system. As a long-chain fructan carbohydrate, inulin does not dissolve in stomach acid or get broken down by human digestive enzymes. Instead, it forms a thick micro-gel matrix around the probiotics, creating a physical barrier that restricts the movement of acids and digestive fluids.

This structural shield slows down the penetration of destructive ions, allowing the bacteria to maintain their internal osmotic balance and survive transit into the lower intestine.

III. MATERIALS AND METHODS

3.1 Synthesis of Experimental Frozen Matrices

The formulations evaluated included:

- Treatment ($T_{\{0\}}$) (Control): Traditional dairy ice cream base with probiotics.
- Treatment ($T_{\{1\}}$): Avocado pulp + unrefined palm sugar base ((0%) chicory inulin).
- Treatment ($T_{\{3\}}$): Avocado pulp + unrefined palm sugar base + (4.0%) chicory root inulin.

All variations were inoculated with equal populations ((1.0%)) of *Lactobacillus acidophilus* (ATCC 4356) and *Bifidobacterium bifidum* (ATCC 29521) prior to freezing, yielding a baseline count of approximately (8.50).

3.2 In Vitro Gastrointestinal Simulation Model:

Gastrointestinal transit was simulated sequentially at (37) using the standardized international INFOOGEST static protocol:

- Oral Phase: A (10) sample was mixed with (10) of Simulated Salivary Fluid (SSF) containing ((75)) and agitated for 2 minutes at pH 7.0.
- Gastric Phase: The oral mix was adjusted to pH 2.0 using (1) and mixed with Simulated Gastric Fluid (SGF) containing pepsin ((2000)). The mixture was incubated in an orbital shaking incubator at 95 RPM for 120 minutes.
- Intestinal Phase: The gastric mix was adjusted to pH 7.5 using (1) and mixed with Simulated Intestinal Fluid (SIF) containing pancreatin ((100)) and (1.0%) oxgall bile salts, then incubated under shaking conditions for another 120 minutes.

3.3 Probiotic Enumeration Matrix

Samples ((1)) were collected at checkpoints 0, 120, and 240 minutes. Serial dilutions were prepared using sterile (0.1%) peptone water. *L. acidophilus* was enumerated using modified MRS agar incubated anaerobically at ($37^{\{C\}}$) for 48 hours. *B. bifidum* was selectively grown on MRS agar supplemented with (0.05%) L-cysteine hydrochloride and (10%) propionic acid to ensure anaerobic specificity. Survival rates were calculated using log transformations:

IV. RESULTS AND DISCUSSION

4.1 Survival Dynamics During Simulated Gastric Transit

The survival rates of both probiotic strains during gastric and intestinal simulation are detailed in Table 1.

Table 1: Probiotic Survival Curves (Log CFU/g) Across Digestion Phases

The unprotected formulation $\left((T_{\{1\}})\right)$ showed poor resistance to gastric transit. *Bifidobacterium bifidum* counts fell drastically from an initial baseline of (8.52) to (4.95) after 120 minutes of exposure to stomach acid, falling well below the therapeutic threshold. This sharp drop confirms that *Bifidobacterium* strains are highly sensitive to acidic environments when unprotected by prebiotics.

In contrast, the prebiotic-fortified formulation $((T_3)$ maintained exceptionally high survival rates, with *L. acidophilus* at (7.95) and *B. bifidum* at (7.58). The (4.0%) chicory root inulin created a thick, protective gel matrix around the bacteria, physically slowing down the infiltration of destructive hydronium ions and shielding the bacterial cells from acid damage.

4.2 Tolerance to Intestinal Bile Stresses:

Entering the intestinal phase (120 to 240 minutes) introduced significant bile salt stress. In the control $\left((T_{\{0\}})\right) and\ unfortified\ \left((T_{\{1\}}\right)$ treatments, bacterial populations continued to decline, with *B. bifidum* dropping to $(4.32)\ and\ (4.05)$ respectively, indicating widespread breakdown of the cell membranes.

However, formulation $(T_{\{3\}})$ demonstrated robust resistance, preserving viable populations of (7.62) for *L. acidophilus* and (7.15) for *B. bifidum*. This sustained viability occurs because the inulin polymer network can physically trap and bind bile salt molecules, preventing them from interacting directly with and dissolving the lipid membranes of the probiotics.

Additionally, the natural unrefined sugars and minerals in palm sugar provide essential osmotic support, helping the cells maintain their integrity during these environmental shifts. These findings demonstrate that the combination of avocado pulp lipids and chicory root inulin forms an effective biological shield, ensuring the probiotics survive transit to the lower intestine in active, therapeutic quantities.

V. CONCLUSION

This *in vitro* digestion study confirms that a functional synbiotic ice cream formulated with avocado pulp and unrefined palm sugar can successfully protect live probiotics through the human digestive tract when reinforced with \(4.0\%\) chicory root inulin. The inulin gel framework works alongside the natural fats in avocado to create a robust physical barrier that shields *L. acidophilus* and *B. bifidum* from harsh stomach acids and destructive bile salts. By maintaining survival counts well above the critical therapeutic threshold (\(\ge 10^7\text{ CFU/g}\)) throughout the entire digestion process, this optimized plant-substituted matrix offers a highly effective manufacturing pathway for delivering active, health-promoting probiotics to the human gut.

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