

Article

A Low-Water-Activity Five-Strain Synbiotic Matrix for Gut Microbiota Support: Formulation Performance and Human Evidence

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Abstract: Delivery is the first practical constraint in any oral probiotic study. We examined a BALIMONT five-strain probiotic-prebiotic powder containing *Limosilactobacillus fermentum*, *Levilactobacillus brevis*, *Lactobacillus gasseri*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* together with antioxidant nutrients, plant/fruit polyphenols and a prebiotic carrier. The representative 3.0-g sachet contained approximately 2.0×10^{10} CFU at preparation. With staged premixing and low-temperature, low-humidity post-blending, water activity was 0.21 and moisture was 4.2%. After 12 months, viable-cell retention was 70.4% and polyphenol retention was 84.6%; 61.7% of viable cells remained after sequential simulated gastric and intestinal digestion. We compared these formulation findings with human studies of probiotic mixtures, inulin-type fructans and synbiotic interventions. The clinical literature shows reproducible changes in selected microbial taxa and, in some populations, microbial-metabolite, immune or functional endpoints. The formulation data therefore define a stable starting dose for direct microbiome testing, while the human studies identify the outcomes and responder variables that are most useful to measure.

Keywords: probiotics; synbiotic; gut microbiota; inulin; fructooligosaccharides; BALIMONT; viable-cell stability; healthy aging.

Introduction

For a dry synbiotic powder, loss of viability can occur well before ingestion. Moisture uptake, prolonged contact with acidic or redox-active ingredients and poorly controlled blending conditions can all change the viable dose that eventually reaches the gut. The formulation used here was built around that problem: antioxidant/polyphenol ingredients and probiotic cells were handled in separate premixes, followed by a short low-shear final blend and moisture-barrier packaging.

Human studies offer several distinct comparators rather than one uniform probiotic effect. Spaiser et al. observed shifts in fecal bacteria and cytokine profiles with an *L. gasseri*-containing mixture in older adults [1]. Macfarlane et al. reported increased bifidobacteria and butyrate, with lower Proteobacteria and TNF- α , after a *B. longum*-inulin synbiotic [2]. In a general adult population, Tremblay et al. found dose-related recovery of supplemented strains with relatively modest community-wide change [3]. Inulin-type fructans produced specific microbiota shifts in the controlled study by Vandeputte et al. [4], and De Giani et al. found intervention-dependent microbiota and immune-marker responses when inulin-based prebiotics were given alone or with probiotics [5]. In older adults with prefrailty or frailty, Yang et al. linked an inulin-oligofructose intervention with both

microbial/metabolic changes and improvement in frailty-related measures [6]. These studies set useful biological benchmarks without treating their strains or doses as interchangeable with the BALIMONT matrix.

Materials and Methods

Formulation and physicochemical performance framework

Each 3.0-g unit contained the five-strain blend, antioxidant nutrients, 300 mg of plant/fruit polyphenol material and a predominantly prebiotic carrier fraction. Added viable cells were distributed at 24%, 18%, 22%, 18% and 18% among *L. fermentum*, *L. brevis*, *L. gasseri*, *P. pentosaceus* and *P. acidilactici*, respectively. The antioxidant/polyphenol premix and the probiotic premix were prepared separately at 15-25°C and relative humidity ≤30%. Final post-blending was performed at 5-25 rpm for 8-20 min with material temperature ≤25°C, followed by sieving and moisture-barrier packaging. Water activity, moisture, 12-month viable-cell and polyphenol retention, and viable-cell survival after 2 h gastric plus 4 h intestinal simulation were recorded.

Component	Representative amount	Functional/formulation role
Five-strain probiotic blend	≈650 mg; 2.0×10 ¹⁰ CFU added	<i>L. fermentum</i> 24%; <i>L. brevis</i> 18%; <i>L. gasseri</i> 22%; <i>P. pentosaceus</i> 18%; <i>P. acidilactici</i> 18%
Vitamin C	80 mg	Antioxidant nutrient
Microencapsulated vitamin E	15 mg α-TE	Antioxidant nutrient
Selenium	40 µg elemental	Selenium yeast or sodium selenite premix
Zinc	8 mg elemental	Zinc glycinate example
Plant/fruit polyphenol component	300 mg	Blueberry/pomegranate/apple polyphenol example
Prebiotic carrier	≈1,900–1,950 mg	FOS/resistant dextrin/inulin/gum arabic; q.s. to 3.0 g

Human evidence identification and synthesis

Human studies were selected when they measured microbial composition, microbial metabolites, strain recovery, immune-related signals or prebiotic/synbiotic effects. Trial findings were retained at the level actually tested. Differences in strain identity, dose, age, baseline microbiota and intervention duration were treated as study variables, not smoothed into a single class effect.

Results

Long-term viability and polyphenol preservation

The staged matrix had Aw 0.21 and 4.2% moisture. Twelve-month viable-cell retention was 70.4%, and polyphenol retention was 84.6%. The direct single-step blend retained 49.1% of viable cells and 72.8% of polyphenols. Viable-cell retention was 44.5% after blending at 45% RH, 54.2% with ordinary polyethylene packaging and 50.6% after probiotic precontact with vitamin C/polyphenols. The size of these separations makes process control a measurable part of dose preservation rather than a minor manufacturing detail.

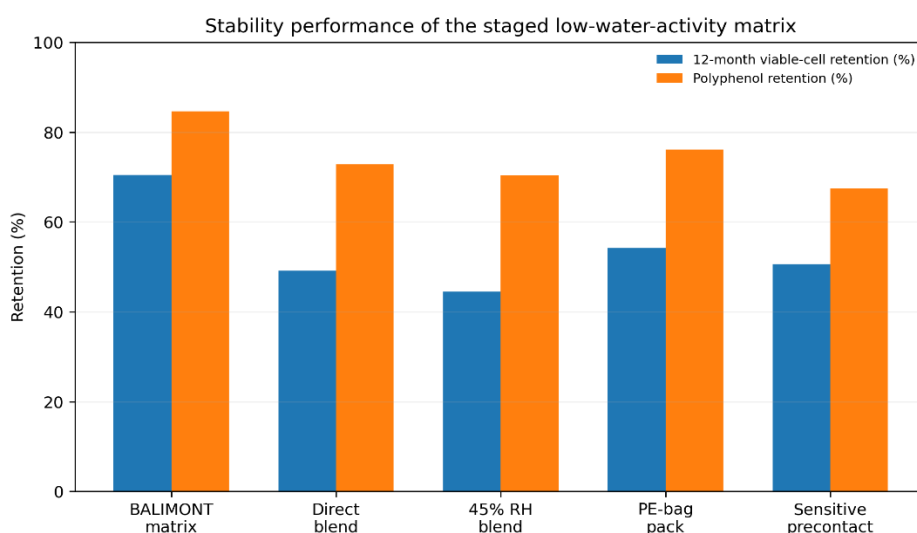


Figure 1. Twelve-month viable-cell retention and polyphenol retention under the evaluated formulation/process conditions. Values are direct measured endpoints; no inferential error bars were available.

Simulated gastrointestinal survival

After sequential simulated gastric and intestinal exposure, 61.7% of viable cells remained in the representative matrix. Direct blending retained 49.1%, and prior contact with sensitive components retained 50.6%. The assay does not measure colonization; it shows how much of the stored viable-cell payload remained available after a defined gastrointestinal stress sequence.

Human evidence for microbiota modulation

Study	Population/design	Intervention	Microbiota-relevant finding
Spaiser et al. [1]	Older adults; randomized DB placebo crossover	<i>L. gasseri</i> + <i>Bifidobacterium</i> mixture	Increase in fecal lactobacilli/bifidobacteria and reduction in <i>E. coli</i> ; cytokine profile also shifted
Macfarlane et al. [2]	43 older volunteers; 4-wk crossover	<i>B. longum</i> + inulin-based prebiotic	<i>Bifidobacteria</i> +1.4 log; <i>Proteobacteria</i> -1.0 log; butyrate increased; TNF- α reduced
Tremblay et al. [3]	General adults; 28 d DB placebo RCT	5 or 25 billion CFU multistrain probiotic	Well tolerated; dose-related recovery/persistence of supplemented strains; modest community-level changes
Vandeputte et al. [4]	Controlled human intervention	Inulin-type fructans	Specific microbiota shifts, including bifidogenic responses and associations with stool characteristics
De Giani et al. [5]	Healthy adults; DB placebo RCT	Inulin-based prebiotics $\pm$ probiotics	Microbiota and selected immune-marker responses varied by intervention

Discussion

The formulation results and the clinical literature address different parts of the same exposure chain. The present data concern preservation of the administered microbial/polyphenol payload, whereas the studies summarized in Table 2 show what selected probiotic or prebiotic exposures can do once delivered. More recent work extends that context. Kim et al. linked probiotic supplementation with changes in cognition, mood and gut microbiota in community-dwelling older adults [7]. A second

multicenter study from the same research program found sex-dependent microbial and peripheral immune-cell responses [8]. Lazou-Ahren et al. reported a modest anti-inflammatory signal in adults over 70 years [9], while Eastwood et al. examined acute and chronic cognitive/mood responses to a multistrain probiotic in healthy older adults [10]. The endpoint pattern is therefore broader than taxonomic abundance alone and argues for measuring microbial recovery together with function and host response.

The carrier fraction deserves the same attention as the strain list. Birkeland et al. found a clear bifidogenic response to inulin-type fructans in type 2 diabetes, with fecal SCFA behavior that did not simply mirror taxonomic change [11]. Li et al. later showed that inulin and fructooligosaccharides produced distinct microbiota and metabolic responses across normal-weight and overweight/obese adults [12]. Strain-level work also shows why species names are not enough: *L. plantarum* Dad-13 changed gut-microbiota and intestinal-health measures in overweight adults [13], whereas *L. plantarum* ECGC 13110402 produced lipid responses with limited whole-community microbiome change [14]. At a broader dietary level, the NU-AGE intervention linked a Mediterranean-diet-associated microbiome shift with lower frailty and improved health status in older Europeans [15]. For BALIMONT, these studies support a study design that records the delivered viable dose, the carrier composition and the host phenotype in the same protocol.

Study Considerations and Future Directions

A direct next-stage study can keep the formulation questions simple. Study batches should be released against Aw, moisture, viable-cell count and polyphenol-retention specifications before dosing. Stool sampling at baseline, an early time point and the end of intervention can track strain recovery and community structure; targeted SCFAs and other fermentation products can be measured alongside barrier-related markers and gastrointestinal symptoms. Age, habitual fiber intake and baseline microbiota are practical stratification variables because the published trials show that each can influence the observed response.

Conclusion

The BALIMONT five-strain matrix retained 70.4% of viable cells and 84.6% of polyphenols after 12 months, and 61.7% of viable cells remained after simulated gastrointestinal exposure. These formulation data give the probiotic-prebiotic system a defined delivery profile. Human trials of related probiotics and prebiotics show measurable, but strain-, substrate- and host-dependent, microbiota and functional responses. A formulation-specific human study can now test those responses against a controlled viable dose instead of relying on ingredient identity alone.

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