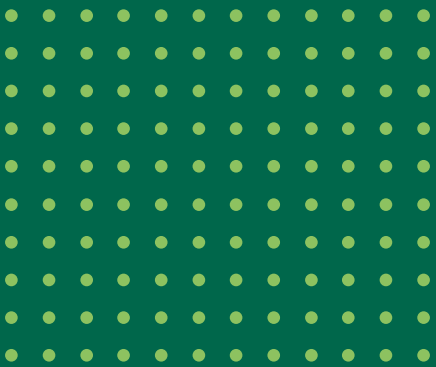




Csat[®]

MICROBIOME **STUDY**

Unveiling the microbiome-mediated potential of a carob-based formulation (Csat[®]): human fecal fermentation, SCFA production, and potential connections with satiety pathways.



PHARM[△]ACTIVE
BIOTECH PRODUCTS

What is Csat®?

Csat® is standardized to ≥21.5% Sliminoids®, a proprietary complex of carob bioactives including galactomannans, inositols, and gallic acid, which together support satiety, metabolic balance, and antioxidant protection. These compounds act synergistically and may help modulate weight ³⁻⁵, improve insulin sensitivity ⁶, and reduce oxidative stress ⁷.



In 2022, 43% of adults aged 18 and over were classified as overweight, and 16% were classified as obese¹. The global weight loss dietary supplements market is estimated to bring in revenue of **US\$ 37.18 billion** by the end of 2026². **15%** of U.S. adults have taken food supplements for weight management in the last 12 months².

Satiety & Lipid Control

Csat® contains galactomannan, which has been studied for its interaction with mechanisms associated with satiety signaling by forming gels that trap water and lipids, thereby slowing gastric emptying. Additionally, it retains bile salts, which aids in reducing blood cholesterol levels. Csat® also influences other metabolic pathways, including the **reuptake of LDL** and the inhibition of HMG-CoA reductase activity³⁻⁵.

Insulin Sensitivity & Antioxidant Support

Csat® contains **inositols**, which have been studied for their role in glucose metabolism, promoting the reuptake of glucose from the blood through GLUT4 transporters⁶.

Additionally, Csat® includes **gallic acid**, which exhibits antioxidant activity in experimental models, a hallmark of metabolic syndrome, by neutralizing reactive oxygen species⁷.

(1) CRN: "Thescience behind the supplements"; (2) World Health Organization (2022): 1747-1759.; (3) Giuntini E.B., et al. Foods 2022; 11: 3934. (14) Bakr A.F., et al. ACS Omega 2023; 8: 24680–24694. (5) Antonowski T., et al. Nutrients 2019; 11: 2314. (6) Antonowski T, Osowski A, Lahuta L, Górecki R, Rynkiewicz A, Wojtkiewicz J. Health-Promoting Properties of Selected Cyclitols for Metabolic Syndrome and Diabetes. Nutrients. 2019, 11, 2314. (7) Masenga, S.K.; Kabwe, L.S.; Chakulya, M.; Kirabo, A. Mechanisms of Oxidative Stress in Metabolic Syndrome. Int. J. Mol. Sci. 2023, 24, 7898. <https://doi.org/10.3390/ijms24097898>.

Microbiome Study

This study was designed to evaluate whether Csat®, a standardized carob-based formulation, can modulate human gut microbiota activity and enhance short-chain fatty acid (SCFA) production in vitro. SCFAs generated through microbial fermentation of dietary fibers are linked to metabolic regulation and appetite signaling. While Csat® has previously demonstrated satiety and metabolic benefits in animal models, its effects on human-derived microbial communities had not been assessed. The objective was therefore to determine whether Csat®, compared with its individual carob fractions, could promote coordinated microbial and functional shifts associated with sustained SCFA production.

Human fecal microbiota were fermented anaerobically with Csat®, its individual carob fractions, or control medium and analyzed over 48 hours. Compared with the control, Csat® induced the strongest response, producing the highest levels of acetate, propionate, and butyrate while preserving microbial balance. Taxonomic and functional shifts indicated coordinated carbohydrate degradation and cross-feeding activity, supporting a microbiota-mediated mechanism aligned with previous in vivo findings.

Microbiome

Modulation of the gut microbiota has emerged as a promising strategy to support metabolic health and appetite regulation, particularly in the context of the growing prevalence of obesity and metabolic syndrome (MetS). Short-chain fatty acids (SCFAs), produced through microbial fermentation of non-digestible carbohydrates, play a central role in gut–brain communication, enteroendocrine signaling, and energy homeostasis. However, their metabolic impact is context-dependent, highlighting the need for standardized and mechanistically characterized nutritional interventions.

Csat®, a standardized carob-based formulation rich in galactomannans, inositols, and gallic acid, has previously demonstrated satiety and metabolic benefits in preclinical models, including improvements in body weight, insulin sensitivity, and inflammatory markers. Nevertheless, its direct influence on human gut microbiota and SCFA production had not been investigated. To address this gap, the present study employed an in vitro fermentation model using human fecal microbiota to evaluate microbial composition, predicted functional capacity, and SCFA output following exposure to Csat® compared with its individual carob fractions. This integrated approach provides mechanistic insight linking prior in vivo efficacy with human-relevant microbiota modulation and supports the rationale for future clinical evaluation.

A closer look at SCFAs

Acetate, propionate and butyrate are central metabolites produced when gut bacteria utilize non-digestible nutrients. These compounds have been associated in the scientific literature with several physiological pathways, including signaling through FFAR2 and FFAR3 receptors and the modulation of hormones such as GLP-1 and PYY. The relationship between SCFAs and host metabolism is complex and context-dependent. For this reason, mechanistic models are valuable tools for clarifying upstream microbial and metabolic effects before moving into clinical research.

What was evaluated?

In this study, researchers incubated human gut microbiota under anaerobic conditions and compared Csat® with a control medium and two individual carob fractions (a galactomannan-rich seed fraction and a pulp extract).

They assessed:

- Changes in microbial composition (full-length 16S rRNA sequencing)
- SCFA concentrations over time (measured by GC-FID)
- Predicted functional capacity using bioinformatic tools (PICRUST2, COG and CAZy databases)

What Changed with Csat®*

After 24 hours, Csat® showed higher SCFA production (Figure 1) compared to control:

- Acetate: 71.5 mM vs 51.8 mM
- Propionate: 52.8 mM vs 33.7 mM
- Butyrate: 48.6 mM vs 34.3 mM

Importantly, while the galactomannan-rich fraction (GM) and the pulp extract (CPE) each induced fermentation responses, Csat® consistently generated the highest and most sustained levels of all three major SCFAs, particularly at 48 hours.

GM showed strong early activation of carbohydrate-degrading potential, whereas CPE induced a broader but more transient microbial response. In contrast, Csat® integrated these complementary effects, maintaining targeted enrichment of *Bacteroides* species while supporting butyrate-associated genera such as *Anaerostipes* and *Blautia*. Concurrently, reduced isovalerate levels under Csat® suggested a metabolic shift away from proteolytic fermentation and toward sustained carbohydrate-driven metabolism.



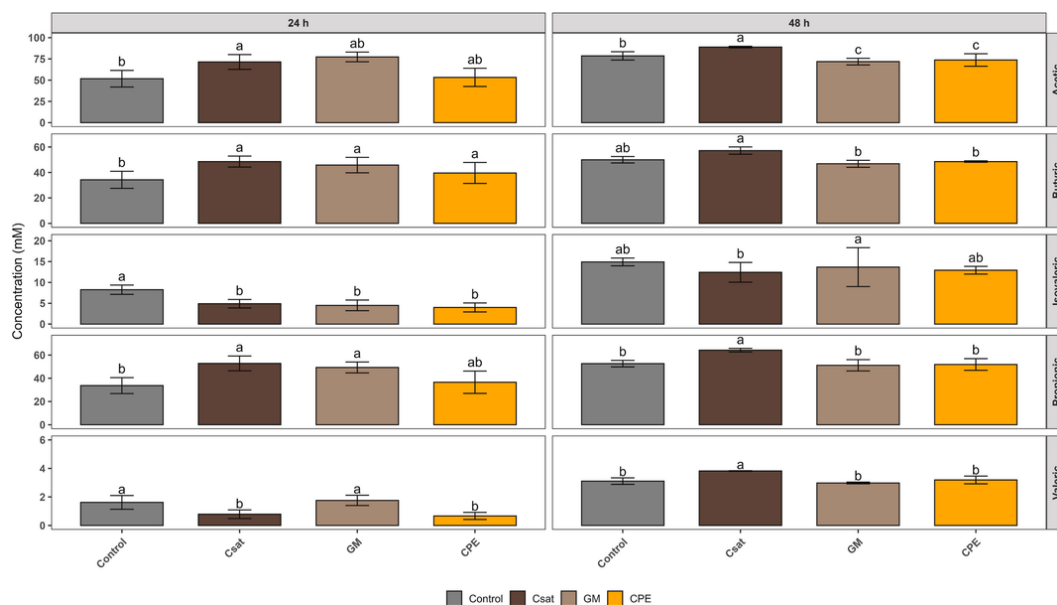


Figure 1. Short-chain fatty acid (SCFA) concentrations (mM) in control and treatment groups (Csat®, GM, and CPE) after 24 h and 48 h of fermentation. Data are shown as mean ± SD (n = 3). Statistical differences were assessed by one-way ANOVA followed by Tukey's post hoc test (p < 0.05). Different letters above bars indicate significant differences among groups within each time point.

Correlation analyses (Figure 2) further indicated coordinated activity between primary degraders and secondary fermenters under Csat®, consistent with cross-feeding dynamics known to sustain SCFA generation.

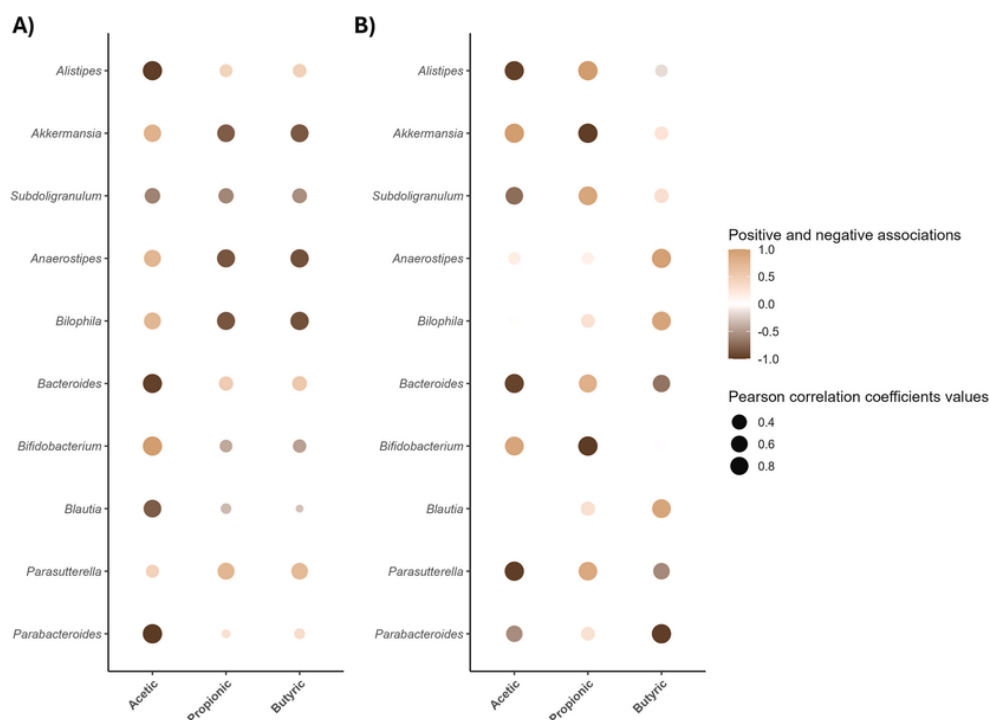


Figure 2. Pearson's correlation between relative abundance of the 10 most abundant bacterial genera and short-chain fatty acids (SCFAs) during fecal fermentation of Csat®. (A) Correlations at 24 h and (B) at 48 h of fermentation. Circle size represents the absolute value of the correlation coefficient, and color scale indicates the direction and strength of the association (brown = positive, beige = negative). Only significant associations (p < 0.05) are shown.

Functional predictions revealed a temporally coherent pattern. The GM fraction showed early enrichment of glycosidase-related signatures consistent with rapid extracellular depolymerization of complex carbohydrates. In contrast, Csat® exhibited a later increase in predicted sugar transport systems, particularly ATP-binding cassette (ABC) transporters, suggesting enhanced uptake and intracellular processing of fermentation products over time.

This integrated functional profile, combining hydrolytic activation and sustained substrate transport, was not observed to the same extent with the isolated fractions. It provides a mechanistic explanation for the higher and more sustained SCFA production under Csat® fermentation.

While these functional inferences are based on predictive bioinformatic tools rather than direct enzyme activity measurements, they are consistent with the metabolite output and taxonomic shifts observed.

Taken together, these findings support the biological plausibility of a microbiota-mediated pathway in which coordinated carbohydrate depolymerization, cross-feeding dynamics, and efficient substrate utilization converge to enhance SCFA production, a process mechanistically linked in the broader literature to satiety-related signaling pathways.

Why this matters

The microbiome field is moving beyond generalized claims of microbiota modulation toward a mechanistic understanding of how specific, standardized ingredients reshape microbial metabolism.

In this context, Csat® stands apart. Unlike isolated carob fractions, Csat® is a fully characterized and standardized formulation integrating multiple bioactive components under controlled manufacturing conditions. This compositional precision enables reproducible functional outcomes, as illustrated in the integrated fermentation model shown in Figure 3.



By combining complementary structural fractions of carob, Csat® promotes coordinated carbohydrate depolymerization, substrate uptake, and sustained short-chain fatty acid production. This integrated microbial response links directly to biological pathways involved in:

- Satiety signaling through the gut–brain axis
- Enteroendocrine hormone modulation (GLP-1, PYY)
- Energy metabolism and substrate partitioning
- Systemic metabolic regulation

Quantifying metabolite output, identifying taxonomic shifts, and predicting functional capacity provides a mechanistic bridge between ingredient composition and physiological relevance.

In this way, the study does not simply demonstrate microbiota changes; it supports a rational, evidence-based framework for developing metabolically active ingredients designed to interact with the gut–brain–metabolic axis.

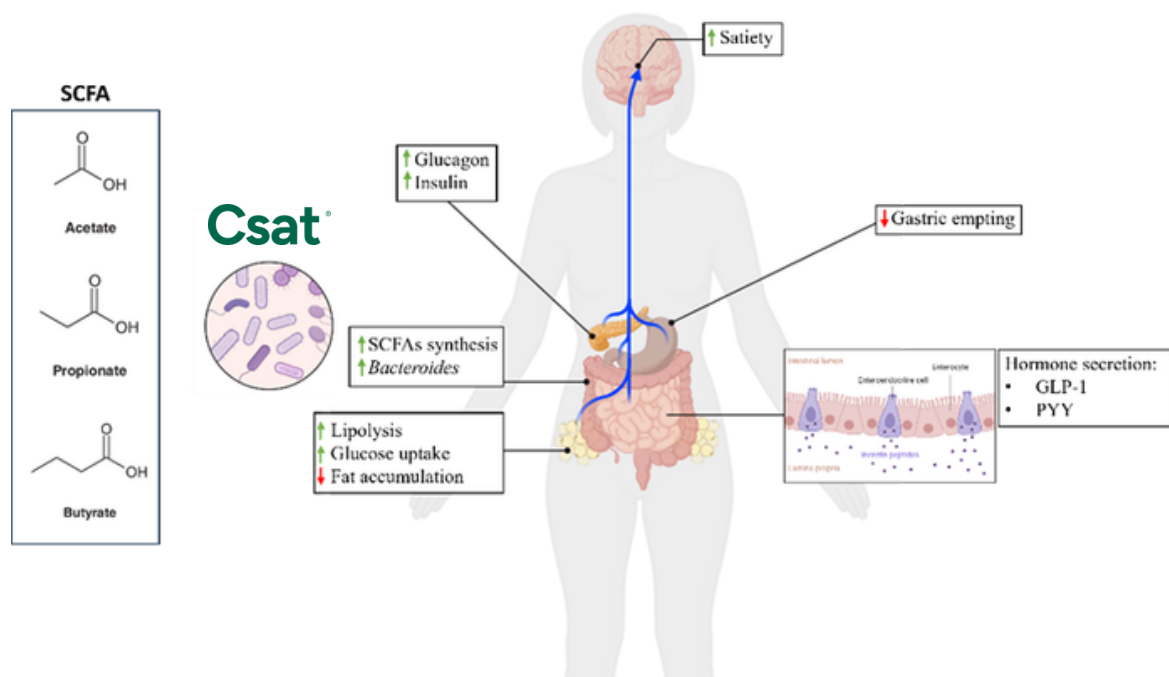


Figure 3. Conceptual model summarizing the microbiota-mediated mechanisms associated with Csat® fermentation, integrating SCFA production, taxonomic shifts, enteroendocrine signaling, and systemic metabolic regulation.

**These results describe microbiota-mediated mechanisms observed under controlled in vitro conditions and do not establish physiological effects in humans. Clinical validation is necessary to confirm translational relevance.*

HEADQUARTERS AND MANUFACTURING PLANT

Ave. de Dr. Severo Ochoa, 37, Local 4J
29108 Alcobendas, Madrid (Spain)
+34 91 112 38 48

QC AND R&D LABORATORIES

Calle Faraday, 7
28049, Madrid (Spain)

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