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Development of high protein supplements containing synbiotics for athletes

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ABSTRACT

Background: Although protein consumption can enhance physical performance and muscle mass in athletes, excessive protein intake may cause an imbalance in gut microbiota (dysbiosis), negatively affecting the gastrointestinal tract. Probiotics and prebiotics have been shown to positively affect athletes' gut health.

Objective: Therefore, this research aimed to develop and evaluate a high-protein supplement containing a synbiotic formulation for athletes, focusing on nutritional value, microbiological analysis, sensory evaluation, and packaging design. In addition, the product was evaluated for probiotic survival in the gastrointestinal tract and its impact on gut microbiota in a fecal batch fermentation system.

Methods: Appropriate probiotic strains were selected to formulate the product according to the Thai RDI guidelines. Standard nutritional value and microbial analysis methods were applied to evaluate the product. The product prototype was designed to be appealing, and the final product was evaluated through sensory analysis. The fecal batch fermentation (*in vitro*) was used to study changes in the composition and proportion of gut microorganisms, utilizing next-generation sequencing (NGS) technology to sequence nucleotides. In addition, gut metabolites, including short-chain fatty acids (SCFAs), were also analyzed to further assess the product's impact on gut health.

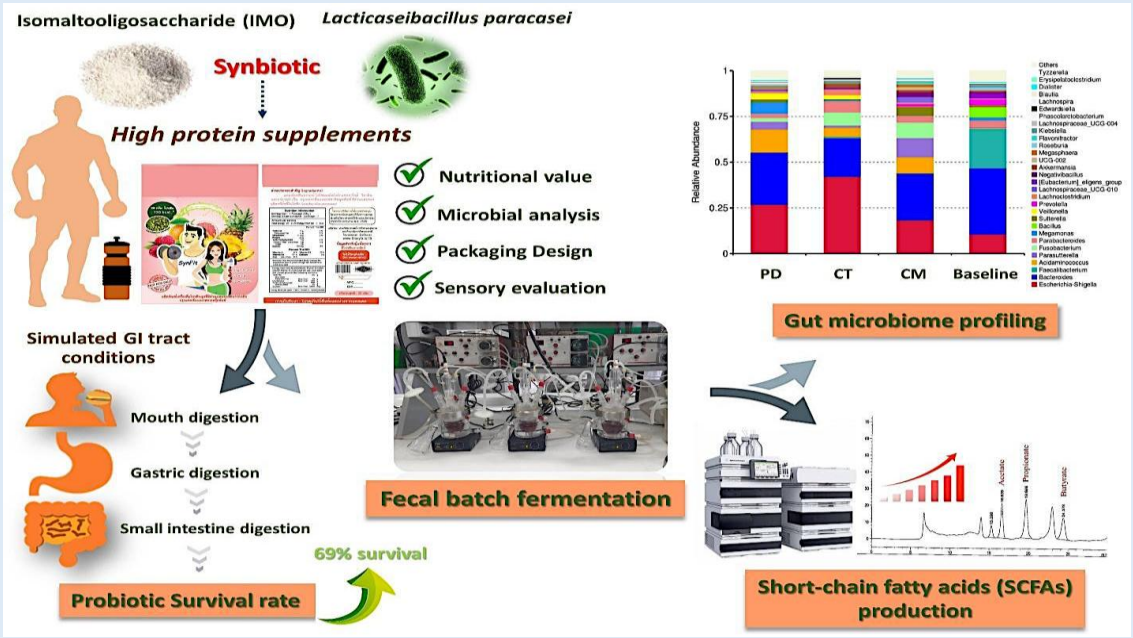
Results: *Lactobacillus paracasei* was selected for the product formulation due to its efficient growth in a prebiotic isomaltooligosaccharides (IMO) medium. The nutritional value of the product shows that it contains protein, carbohydrates, and fat in a ratio of 79:16:1, providing a total energy of 120 Kcal per 35g serving. Additionally, the microbial quality of the product met the standards set by the Food and Drug Administration of Thailand. The packaging was designed as an attractive plastic sachet with a nutrition label. The product was well-received, with the sensory

evaluation showing positive results. The probiotic survival rate in simulated gastrointestinal conditions was approximately 69%. Gut microbiome profiling demonstrated favorable results after 24 hours of fermentation of the developed product, highlighting the growth of beneficial bacteria, particularly those that produce SCFAs (*Acidaminococcus*, *Megamonas*, and *Parabacteroides*), along with the suppression of pathogens, especially *Escherichia-Shigella*. The concentration of SCFAs, including acetic acid, propionic acid, and butyric acid, significantly increased after fermenting the developed product for 24 hours, with concentrations of 70.50±2.25, 38.60±1.20, and 22.22±0.85 mM, respectively.

Conclusion: The research shows that the high-protein supplement with a synbiotic formulation tailored for athletes meets established quality standards and is well-received for its prototype and flavor. Beyond providing athletes with a substantial protein boost, this supplement could offer various health advantages by promoting the growth of beneficial gut bacteria and aiding in the production of their associated metabolites. As a result, it can be concluded that this cutting-edge synbiotic protein supplement supports both gut health and athletic performance.

Novelty of the Study: This study presents the first-ever development of a high-protein supplement formulated with a synbiotic approach, addressing both muscle recovery and gut microbiota balance in athletes. Unlike conventional protein supplements, this formulation integrates Lactobacillus paracasei and isomaltooligosaccharides (IMO) to enhance probiotic survival (69%) while promoting the growth of SCFA-producing beneficial bacteria and suppressing pathogens in a fecal batch fermentation system. By improving gut health, performance, and recovery, this synbiotic solution transforms sports nutrition and gives athletes a competitive advantage.

Keywords: functional food; bioactive compounds; athletes; synbiotic; batch culture; microbiome; short-chain fatty acid



Graphical Abstract: Development of high protein supplements containing synbiotics for athletes

INTRODUCTION

Adequate protein intake is essential, especially for those who play sports and exercise to build muscle [1]. Therefore, it is necessary to consume a sufficient amount of protein daily. As recommended by the World Health Organization (WHO), nutritional guidelines and protein requirements for adults are 0.83 grams per kilogram of body weight per day [2]. Meanwhile, the Thai Recommended Daily Intakes (Thai RDI) set by the Ministry of Public Health specifies a daily protein intake of 50 grams.

Studies on the effectiveness of adequate protein intake have shown that it can significantly enhance physical performance in exercise, muscle building, and body repair [2-3]. However, there is relatively little research on the advantages and disadvantages of consuming excessively high amounts of protein and its effects on other bodily functions [4]. Regular high daily protein intake can alter the balance of gut microbiota, which may have both beneficial and adverse effects on health [5]. Excess protein that the body cannot utilize undergoes fermentation in the large intestine, which increases the number of proteolytic and pathogenic bacteria. The growth of these harmful bacteria promotes their metabolic processes, leading to the production of several toxic substances, such as urea, indoxyl sulfate (IS), *p*-cresyl sulfate (PCS), ammonia, hydrogen, and histamine [6].

Furthermore, studies in animal models have found that beneficial gut bacteria (probiotics) can produce SCFAs, which affect muscle energy homeostasis, fat storage in muscles, and glucose and fat metabolism [7]. Probiotic bacteria may also directly impact athletes' physical performance by mechanisms such as reducing inflammation and modulating the immune system [8]. They may also help regulate energy metabolism, which is crucial for athletic performance [9].

For the aforementioned reasons, while high protein products can enhance physical performance and muscle mass, they may have long-term health consequences, such as an increased risk to gut health. Excessive consumption of protein can cause gut microbiota imbalance (dysbiosis), leading to an increase in proteolytic bacteria that negatively affect digestive and other bodily systems, which are all connected to gut microbiota. Therefore, using probiotics and prebiotics in athletes is expected to promote performance during competitions and long-term health benefits. Consequently, the availability of a wide variety of such products on the market would be highly valuable.

Thus, this research aims to develop a high-protein supplement containing synbiotics for athletes. Furthermore, the microbiological and sensory evaluations of the product were studied. Nutritional analysis was conducted to create nutritional labels and packaging designs. Finally, the finished product was evaluated for the survival rate of probiotics in the simulated gastrointestinal tract, and their effects on gut microbiota were studied in the fecal batch fermentation system.

MATERIALS and METHODS

This research has been approved by the Human Research Ethics Committee of Sirindhorn College of Public Health Yala under the ethics code SCPHYLIRB-2566/146.

Selection of probiotic strains: A review of literature and research on the effects of probiotics on health was conducted to select probiotic strains for developing a high-protein supplement with synbiotics for athletes. The selected strains must be approved for use according to the Ministry of Public Health's announcement on the use of probiotics in food in Thailand. This study selected four probiotic strains: *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and

Bifidobacterium animalis. IMO was selected for this study for the prebiotic source due to its ability to promote beneficial gut bacteria, resist digestion, and enable cost-effective production using affordable agricultural resources available in Thailand. These strains of probiotics were cultured in MRS broth with IMO as a prebiotic source using the pour-plate technique. The number of microbial colonies was counted and reported as CFU/mL to select the best-growing strains for further study.

Product formulation: Nutritional ingredients were purchased from certified suppliers. The main nutrient composition included commercial isolate whey protein (90% protein) and prebiotics (IMO). Commercially available food-grade encapsulated probiotic powders, vitamins, and minerals were used, along with flavoring agents. The product composition consisted of 75% protein and 25% carbohydrates (as IMO powder), along with a vitamin and mineral premix that provides 30% of the Thai Recommended Daily Intakes. The premix contains vitamin A, vitamin D, vitamin E, vitamin K, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, and vitamin C, as well as iron, calcium, phosphorus, magnesium, iodine, and potassium. The formulated product contains a single strain of encapsulated probiotics at a concentration of 10^8 CFU/g. It was prepared in powdered form and stored in a sterile amber container under refrigeration at 4°C prior to nutritional analysis.

Nutritional analysis and labeling: the finished product's nutritional composition was examined by SGS (Thailand) Co., Ltd., located in Songkhla, Thailand. The macronutrient analysis encompassed various components: total fat, saturated fat, cholesterol, protein, carbohydrates, energy, total sugars, and dietary fiber. These were tested using several methods, including AOAC (2019) protocols and in-house methods like GC/FID, HPLC-RI, and others. For micronutrients, the analysis focused on sodium, calcium, and iron, employing in-house procedures based on AOAC (2019) standards. Vitamins present in the product, such as vitamin A, B1, and B2, were measured according to specific in-house methods and references, including the Bulletin of the Department of Medical Sciences (1995) for vitamin A.

Packaging Design: The high protein supplement containing synbiotics for athletes was available in single-serving plastic sachets, each containing 35 grams (120 Kcal). The packaging included nutritional information, product brand, product property, ingredients, manufacturer details, and other essential product information, all presented with colorful designs to enhance its appeal.

Microbiological quality: Microbiological testing was carried out on the finished product. A 1 g sample was diluted in 9 mL of distilled water through serial dilutions. The pour plate technique was employed to count bacteria using plate count agar, while potato dextrose agar, supplemented with 0.01% chloramphenicol, was used to prevent bacterial growth during yeast counting. For mold enumeration, Sabouraud dextrose agar was utilized. The plates were incubated at 37°C for 48 hours to support bacterial growth and at 27°C for three days to foster the development of yeasts and molds. The microbiological evaluations followed these standards: Total Plate Count (FDA BMA, 2001), Salmonella spp. (FDA BMA, 2016), Staphylococcus aureus (FDA BMA, 2016), Listeria monocytogenes (ISO 11290-1: 2017), Bacillus cereus (FDA BAM Online, 2020), and Clostridium perfringens (FDA BMA, 2001).

Product sensory evaluation: Panelists evaluated the sensory attributes of the developed and commercial

products using a 9-point hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like). Fifty semi-trained panelists evaluated the sensory quality of the product in terms of color, smell, taste, appearance, texture, and overall acceptance. After the sensory evaluation, the developed product was compared to a commercial product.

The survival rate of probiotics in a simulated gastrointestinal tract: The viability of probiotics in the final product was assessed under simulated gastrointestinal (GI) conditions, following the procedure outlined by Keawyok et al. [11]. The encapsulated probiotics were placed in a tube with human saliva containing α-amylase (0.33 unit/mL) and incubated at 37°C. After 3 minutes, 1 mL samples were collected, and probiotic survival was evaluated using the pour-plate method on MRS agar. The remaining saliva solution was then combined with sterile simulated gastric fluid (pH 2.0) and 3 mg/mL pepsin (Sigma, USA) and incubated at 37°C. Samples were taken at 60 and 120 minutes to count viable cells. Next, the mixture was transferred to a tube containing sterile simulated intestinal fluid (pH 8.0), 3 mg/mL pancreatin, and 1% bile salt (Sigma, USA), and incubated at 37°C for 60 and 240 minutes. After incubation, samples were collected, and cells were counted using the drop-plate method on MRS agar with 0.5% CaCO3. The survival rate was determined by comparing the number of cells before and after the incubation period, as per the equation in Keawyok et al. [11].

$$Survivalrate(\%) = \frac{loglogCFU/mLatthesampling}{logCFU/mLattheinitial} \times 100$$

Preparation of fecal slurry: Fresh fecal samples were collected from volunteers for the fecal batch fermentation study. Purposive sampling was used to select participants based on specific criteria. The sample group includes 3 to 5 individual participants. This number is based on the fecal slurry preparation method [12-13]. The inclusion criteria for participants included those who exercise daily and reside within a 5-kilometer radius of Prince of Songkla University. Additionally, volunteers were male or female gender of age between 25 and 45 years old, with no history of chronic diseases or gastrointestinal surgery. They had not taken antimicrobial agents within the past 3 months and were not consuming fiber, prebiotics, probiotics, or synbiotics. Furthermore, participants were not currently restricting or modifying their diet.

Volunteers were excluded if they were current or former smokers, had a history of metabolic or gastrointestinal disorders, had consumed prebiotics, probiotics, synbiotics, or fiber within two weeks prior to participation, or had taken antibiotics within three months before fecal sample collection.

However, participants were to be excluded if they needed to take antimicrobial agents or undergo medical treatment, wished to withdraw from the study, or experienced illness during the fecal sample collection period. Additional exclusions included requiring antimicrobial agents or consuming fiber, prebiotics, probiotics, synbiotics, yogurt, or other fermented dairy products during the collection period.

On the scheduled date for sample collection, fresh feces will be gathered, diluted (1:10) in buffer containing 0.1 M phosphate-buffered saline (PBS) solution at pH 7.2, and homogenized for 2 minutes using a Seward Stomacher 400 Circulator (UK). The mixture was then filtered through stomacher bags to produce a fecal slurry, which was used directly as the inoculum for fecal batch culture fermentation.

Fecal Batch Fermentation: The in vitro fecal batch fermentation was carried out under anaerobic conditions to monitor the growth of fecal bacteria during the

fermentation of a high-protein synbiotic supplement for athletes (PD), a commercial product (CM) and a control basal medium (CT). The fermentation took place in a 320 mL, water-jacketed glass vessel set at 37°C, where a pre-reduced basal medium was added. To maintain the anaerobic environment, nitrogen gas (N2) was introduced, and after that, the fecal slurry and study samples were added. The pH level was controlled using an automated pH controller to ensure it stayed within the range of 6.8 ± 0.2 during fermentation.

The basal medium contained the following components per liter: 2 g peptone water, 2 g yeast extract, 0.1 g NaCl, 0.04 g K2HPO4, 0.04 g KH2PO4, 0.01 g MgSO4.7H2O, 0.01 g CaCl2.6H2O, 2 g NaHCO3, 0.005 g haemin, 0.5 g L-cysteine HCl, 0.5 g bile salts, 2 mL Tween 80, 10 mL vitamin K, and 4 mL of 0.025% (w/v) resazurin solution. The mixture was adjusted to pH 7.0 using 1 mol/L HCl and kept under anaerobic conditions at 37°C [13].

A 10% (w/v) fecal slurry was prepared by blending pre-reduced 0.1 mol/L phosphate buffer (pH 7.0) with the fecal sample in a stomacher for 2 minutes. The soluble fraction of the slurry was then added to achieve a final concentration of 1% (w/v) [14]. The experimental setups included:

- Vessel 1 (CT): fecal slurry with basal medium
- Vessel 2 (PD): fecal slurry with basal medium and the experimental product
- Vessel 3 (CM): fecal slurry with basal medium and the commercial product

Each fermentation vessel was continuously stirred using a magnetic stirrer and kept at 37°C in a circulating water bath. The pH was automatically maintained at 6.8 ± 0.2 using a pH controller. Samples (5 mL) were taken from each vessel at the start and after 24 hours of incubation to evaluate short-chain fatty acids (SCFAs) [15] and to perform gut microbiome analysis.

Gut microbiome analysis: Microbial community analysis was performed using 16S rRNA gene sequencing (NGS) to assess the diversity and composition of bacteria in the samples. First, 375 µl of each sample was collected during fermentation and mixed with 1,125 mL of 4% (w/v) paraformaldehyde solution (pH 7.2) to preserve the bacterial cells. The mixture was stored at 4°C for at least 4 hours to allow for fixation. The samples were washed twice with filtered PBS and centrifuged at 13,000 ×g for 10 minutes. The cell pellets were then resuspended in a combination of 150 µl sterile PBS and 150 µl 95% ethanol and stored at -20°C for a minimum of one hour or up to three months [16].

DNA extraction was assessed for quality and concentration using Nanodrop spectrophotometry, with further confirmation through gel electrophoresis. The extracted DNA underwent 16S rRNA gene sequencing, focusing on the V3-V4 regions. Data processing and analysis were conducted using QIIME2 (Version QIIME2-202006) for bioinformatics. The sequence data were transformed into amplicon sequence variants (ASVs), which were represented in bar plots to show the relative abundance of taxa at the phylum and genus levels.

For accurate data analysis, raw sequences were filtered and processed, removing any sequences with low abundance (less than five reads) using DADA2, a tool within QIIME2 designed for noise reduction [17]. The diversity and composition of the gut microbiota were evaluated through Principal Coordinates Analysis (PCoA), utilizing Bray-Curtis distance metrics to explore differences in bacterial community structures across the samples. The relative abundance of bacterial species was depicted in bar graphs, with different colors representing the proportions of each microbial group present in the samples.

Analysis of short-chain fatty acids: Samples collected from the fecal batch fermentation were examined for short-chain fatty acids (SCFAs) using high-performance liquid chromatography (HPLC) with a UV detector [14]. To prepare the samples, 2 mL of each was centrifuged at 17,000 ×g for 15 minutes to remove any solid particles. The supernatant was then filtered through a 0.2 µm nylon membrane to eliminate remaining impurities. The SCFAs were separated using an Aminex HPX-87H ion-exclusion column (BIO-RAD, USA), with a 7.8 mm diameter and 300 mm length, set to a temperature of 50°C. A UV detector, tuned to 215 nm, was used to detect the SCFA peaks. Concentrations of SCFAs in the samples were determined by comparing the area of each sample's chromatographic peaks to those of the known standards. Chemstation software (CHEM32 version, USA) was used for data processing. Standard solutions of acetic acid, propionic acid, and butyric acid were prepared at concentrations of 10, 20, 40, 80, 100, and 250 mM and used to generate external calibration curves for quantifying the SCFAs in the experimental samples [14].

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Statistical analysis: The data are expressed as the mean ± standard deviation from three independent trials. Statistical analysis was carried out using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to identify significant differences, with a significance threshold set at $p < 0.05$. All statistical computations were performed using SPSS software (version 18).

RESULTS AND DISCUSSION

Selection of suitable probiotic strains for prebiotics: The bioactive compounds in functional foods offer advantages beyond basic nutrition, providing health benefits and disease prevention. In sports nutrition, these compounds help reduce oxidative stress, enhance recovery, and support immune function. They promote overall health and address the specific needs of athletes by boosting endurance and strength, improving athletic performance, and accelerating recovery [18]. Bioactive compounds derived from plants, mushrooms, and animals serve as the foundation of functional foods. Polysaccharides, widely found in nature, and their indigestible derivatives, such as fiber, are crucial in sustaining a healthy symbiotic relationship between humans and gut microbiota [19]. This study used isomaltooligosaccharides (IMO) as a prebiotic ingredient to develop the product. Probiotic strains capable of utilizing IMO for growth were selected. The four probiotic strains used in the study consisted of *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Bifidobacterium longum*, and *Bifidobacterium animalis*. The growth of probiotic strains at the initial stage and after 24 hours of incubation, with IMO as the carbon source, is shown in Table 1. Initially, the count of *Lactobacillus rhamnosus* was 8.17 ± 0.10 log CFU/mL. which increased slightly to 8.23 ± 0.03 log CFU/mL after 24 hours. *Lactobacillus paracasei* exhibited significant growth, with its initial count of 8.24 ± 0.12 log CFU/mL increasing to 9.45 ± 0.09 log CFU/mL. Similarly, the initial counts of *Bifidobacterium longum* and *Bifidobacterium animalis* were 8.14 ± 0.06 and 8.09 ± 0.04 log CFU/mL, respectively. After 24 hours, their counts increased to 8.76 ± 0.03 and 9.12 ± 0.10 log CFU/mL, respectively.

The results demonstrated that all four probiotic strains could grow in IMO-enriched media. Among them, *Lactobacillus paracasei* was selected for product development due to its favorable growth and superior microbial count compared to the other strains ($p<0.05$). Prebiotics, such as IMO, are carbohydrates that support the growth of beneficial bacteria [20], while probiotics are live microorganisms that promote gut health and offer various health benefits when consumed. The combination of prebiotics and probiotics, known as

synbiotics, aims to enhance the survival and colonization of beneficial microbes in the gut by providing both live microorganisms (probiotics) and the substrates (prebiotics) that support their growth and activity. This study aimed to develop a high-protein supplement containing *Lactobacillus paracasei* and IMO to support gut health for athletes.

Current evidence suggests that the balance of gut microbiota and exercise can influence physical performance by enhancing training adaptation, reducing physiological stress during recovery, and improving mood and mental resilience after intense exercise [21]. Studies have explored various ways probiotics can benefit sports performance, including reducing oxidative stress and inflammatory responses, boosting athletic performance, strengthening the immune system, and preventing upper respiratory tract infections [22]. Moreover, several studies highlight the relationship between gut microbiota diversity and exercise in managing obesity. A balanced microbiome helps regulate metabolism, weight, and inflammation, whereas dysbiosis is associated with obesity. Research suggests that probiotics, particularly those from the genus *Lactobacillus*, may assist in preventing obesity. However, additional studies are required to better understand the underlying mechanisms and enhance intervention strategies [23]. Therefore, combining prebiotics and probiotics offers an attractive solution for enhancing athletic performance and overall well-being.

Table 1. The growth of probiotic strains at the initial stage and after 24 hours of incubation in broth, where the carbon source was replaced with isomaltooligosaccharide.

Probiotic strains	**The bacterial counts**	
	Initial of incubation (log CFU/mL)	**24 hours of incubation** (log CFU/mL)
Lactobacillus rhamnosus	8.17 ± 0.10^{Ba}	8.23 ± 0.03^{Ad}
Lactobacillus paracasei	8.24 ± 0.12^{Ba}	9.45 ± 0.09^{Aa}
Bifidobacterium longum	8.14 ± 0.06^{Ba}	8.76 ± 0.03^{Ac}
Bifidobacterium animalis	8.09 ± 0.04^{Ba}	9.12 ± 0.10^{Ab}

*Capital letters are used to describe differences in microbial count in each row. Lowercase letters are used to describe differences in microbial count in each column. Different letters mean connote significant difference ($p<0.05$).

Nutrition analysis and nutrition labeling: The high-protein supplement containing synbiotic for athletes was found to contain 75.59% protein, 15.30% carbohydrates, 0.96% fat, and 8.15% other ingredients, with an approximate macronutrient distribution ratio of 79:16:1 for protein, carbohydrates, and fat, respectively. The product provides a total of 372 Kcal per 100 g. For a serving size of 35 g, it delivers 120 Kcal, with 26 g of protein, 0.33 g of total fat, 0.08 g of saturated fat, 0.54 mg of cholesterol, 5.35 g of carbohydrates, 0.18 g of total sugars, and 0.19 g of dietary fiber. The mineral and vitamin content per serving includes 150.85 mg of sodium (Na), 19.95 mg of calcium (Ca), 0.07 mg of iron (Fe), 0.36 mg of vitamin B1 (thiamine), and 0.81 mg of vitamin B2 (riboflavin).

The nutritional labeling was prepared by comparing the product's nutrient content with the Thai Recommended Daily Intakes (Thai RDI) for the population aged 6 years and older, based on a daily energy intake of 2,000 kilocalories. The percentage of vitamins and

minerals per serving of the product aligns with the Thai RDI recommendations: sodium at 8%, calcium at 2%, and vitamins B1 and B2 at 25% and 50%, respectively.

The dietary fiber analysis of the product showed it contains 0.55 g per 100 g of product or 0.19 g per serving. Our previous studies have shown that isomaltooligosaccharides are approximately 65% resistant to digestion by enzymes in the gastrointestinal tract [10]. Current dietary fiber intake recommendations in Europe and the United States are between 30–35 grams per day for men and 25–32 grams per day for women [24]. The Thai RDI recommends a daily dietary fiber intake of 25 grams for individuals aged 6 years and older. It is well established that dietary fiber and prebiotics offer numerous health benefits, particularly for gastrointestinal health. Maintaining good gastrointestinal health is crucial for enhancing athletic performance, as it supports optimal nutrient absorption, energy levels, and overall well-being, which contribute to better endurance and recovery. However, dietary fiber is commonly obtained from other food sources as well.

Microbiological quality of product: The finished product was evaluated for its microbiological quality. The results indicated that the levels of yeast and mold, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Clostridium perfringens* were either very low or not detected in the product during this study. Therefore, the results of the microbiological tests were found to comply with Thai FDA regulations regarding the microbial quality of powdered products. However, the total plate count exceeded the permissible limits set by Thai FDA regulations. This elevated microbial count was likely due to the inclusion of the probiotic strain *Lactobacillus paracasei* in the product.

Design and development of the product prototype: Medical research indicates that the protein needs of athletes can vary daily based on factors such as age, type of sport, intensity of training, and other individual variables. However, Sports Nutrition Association recommendations suggest that athletes consume between 1.4 and 2.0 g of protein per kilogram of body weight per day [25]. As a general guideline, the recommended protein intake is 1–2 scoops (25–50 grams) per day. In this study, the developed product is designed with a recommended serving size of 35 g (26 g of protein) to be dissolved in 250 mL of water. The total energy per serving is 120 Kcal or about 0.5 kcal/mL. The product prototype was flavored with a fruit punch flavor to make it more appealing to consumers.

Moreover, the product was packaged in plastic sachets, with a prototype featuring nutritional labeling. The nutritional information on the product label includes the proportions of macronutrients, vitamins, and minerals in relation to the recommended daily intake (Thai RDI). Details on the front and back of the product packaging include the brand, nutritional information, ingredients, manufacturer, product features, and other essential information. The packaging highlights the product's high protein content, low sugar, and low fat, while also emphasizing the inclusion of probiotics for added health benefits. Furthermore, the design of the packaging is user-friendly, convenient, and visually appealing, making it attractive and easy for consumers to handle.

Product sensory evaluation: The finished product, a high-protein supplement containing synbiotics for athletes, was evaluated as sensory quality by fifty semi-trained panelists. The evaluation covered various attributes of the product, including appearance, color, thickness, aroma, taste, sweetness, saltiness, bitterness/astringency, mouthfeel, aftertaste, and overall preference, using a 9-point hedonic scale. The prototype product received favorable scores across all

categories: appearance (7.82 ± 1.35), color (8.03 ± 1.44), thickness (7.15 ± 1.52), aroma (7.02 ± 1.67), taste (6.85 ± 1.77), sweetness (6.76 ± 1.77), saltiness (6.65 ± 1.98), bitterness/astringency (6.68 ± 1.85), mouthfeel (6.62 ± 2.13), aftertaste (6.71 ± 2.11), and overall preference (7.03 ± 1.76), as shown in Table 2. Certain attributes, particularly appearance, color, aroma, and taste, scored significantly higher than those of the control and commercial products ($p<0.05$). This indicates that the synbiotic formulation improves the nutritional value and enhances the sensory acceptability of the supplement.

These findings are consistent with previous studies that shows how the incorporation of functional ingredients, such as probiotics and prebiotics (synbiotics), can positively influence both the nutritional and sensory properties of functional foods, making them more appealing to consumers. Additionally, sensory attributes play a critical role in the consumer acceptance of functional foods, especially for products targeting specific populations, such as athletes, where both performance benefits and palatability are important [26]. Therefore, the positive sensory scores in this study underscore the potential for synbiotic-based products to meet both health and taste preferences in the athletic supplement market.

Table 2. Sensory scores of the developed high-protein drink containing synbiotics for athletes.

Attributes	Samples		
	Control	Commercial	Developed product
Appearance	7.06±1.071^{b}	7.12±1.29^{b}	7.82±1.35^{a}
Color	7.15±1.077^{b}	7.21±1.38^{b}	8.03±1.44^{a}
Thickness	7.00±1.279^{a}	7.12±1.66^{a}	7.15±1.52^{a}
Aroma	6.15±1.743^{b}	6.65±1.31^{b}	7.02±1.67^{a}
Taste	5.91±1.975^{b}	6.53±1.71^{b}	6.85±1.77^{a}
Sweetness	6.29±1.679^{a}	6.62±1.74^{a}	6.76±1.77^{a}
Saltiness	6.32±1.870^{a}	6.47±1.83^{a}	6.65±1.98^{a}
Bitterness/Astringency	6.41±1.811^{a}	6.65±1.72^{a}	6.68±1.85^{a}
Mouthfeel	6.41±1.794^{a}	6.32±1.98^{a}	6.62±2.13^{a}
Aftertaste	6.35±1.998^{a}	6.41±1.98^{a}	6.71±2.11^{a}
Overall preference	6.50±1.813^{a}	6.71±1.73^{a}	7.03±1.76^{a}

*Different lowercase letters above the numbers in each row indicate significant differences between samples ($p<0.05$)

Survival rates of probiotics in simulated gastrointestinal conditions: Probiotics' ability to survive digestive conditions is their necessary characteristic. Therefore, evaluating the survival of probiotics in the gastrointestinal system and their ability to reach and ferment in the large intestine can confirm their probiotic properties. This study demonstrated that the encapsulated probiotic in the developed product is resistant to enzymatic and acidic digestion under simulated gastrointestinal conditions, including simulated saliva, gastric fluid, and intestinal fluid.

The average survival rates of probiotics under simulated gastrointestinal conditions are shown in Table 3. The initial count of the probiotic was 6.87 ± 0.05 log CFU/mL. After 3 minutes of incubation in artificial saliva with α-amylase (pH 7.5), the bacterial count was 6.67 ± 0.19 log CFU/mL, indicating a survival rate of 97.05%. Under simulated gastric conditions (pH 2), the bacterial counts after 60 minutes of incubation were 4.95 ± 0.14 log CFU/mL and 4.85 ± 0.08 log CFU/mL after 120 minutes, reflecting survival rates of 72.02% and 70.60%, respectively. Finally, the survival rates of the encapsulated probiotic in the developed product under simulated intestinal conditions were 4.81 ± 0.10 log CFU/mL and 4.74 ± 0.07 log CFU/mL at 60 minutes and 240 minutes of incubation, respectively. Consequently, the survival rates of the microorganisms decreased to 69.96% and 69.06%, respectively, when their survival was calculated after simulated intestinal digestion. The results indicate that using food-grade commercial probiotics encapsulated through a spray-drying technique supported their survival and enabled them to reach the large intestine, where they can provide health benefits. This finding is consistent with previous research by Priya et al. [27], which demonstrated that encapsulation techniques can enhance the survival rate of *Lactobacillus acidophilus*, which generally has a low survival rate through the human digestive system.

Table 3. Average survival rates of probiotic (*Lactobacillus paracasei*) in simulated gastrointestinal conditions.

Gastrointestinal condition	Incubation period (min)	Probiotic survival	
		(log CFU/mL)	Survival rate (%)
Mouth: Artificial saliva containing α-amylase	0	6.87±0.05	100
	3	6.67±0.19	97.05
Stomach: Simulated gastric fluid with pepsin	60	4.95±0.14	72.02
	120	4.85±0.09	70.60
Small intestine: Simulated intestinal fluid with pancreatin	60	4.81±0.10	69.96
	240	4.74±0.07	69.06

Gut microbiome composition: A high-protein supplement containing synbiotics for athletes was evaluated for its effectiveness in promoting the growth of beneficial microorganisms in the gut using a fecal batch fermentation system. Principal coordinates analysis (PCoA) using Bray-Curtis distance was applied to compare the bacterial community structure differences in the gut microbiota across samples. The results showed clear differences in the gut microbial community structure between the samples before fermentation (Baseline) and after 24 hours of fermentation for the control (CT), developed product (PD), and commercial product (CM) groups. However, the samples fermented for 24 hours with the developed product and commercial product exhibited similarities, as shown in Figure 1. These results indicate that both samples share similar gut microbial communities, likely due to the similarities in their main components, particularly the type of protein

present in the product, which is whey protein isolate. The presence of whey protein isolates in both samples may contribute to the alignment of their microbial profiles by providing similar nutrient profiles that support the growth of comparable bacterial species in the gut. [17].

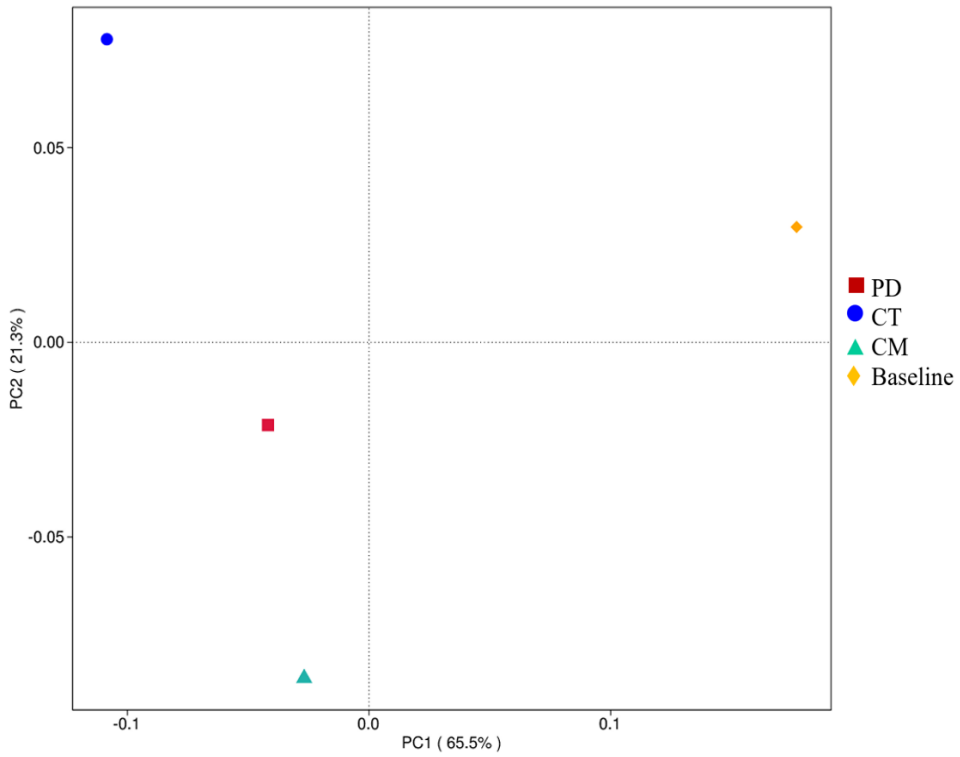

Figure 1. Principal coordinates analysis (PCoA) based on the Bray-Curtis dissimilarity distance matrix of microbial communities between samples. The yellow trapezoid represents the samples before fermentation (Baseline). The blue circle, green triangle, and red square represent the samples after 24 hours of fermentation for the developed product (PD), the control (CT), and the commercial product (CM), respectively.

In addition, the effects of a high-protein supplement containing synbiotics for athletes on the gut microbiome profile at the phyla level are shown in Figure 2. The distribution of the primary phyla of microorganisms includes *Proteobacteria*, *Bacteroidota*, and *Firmicutes*. Notably, the proportion of gut microbiome in *Proteobacteria* increased approximately 3-4 times in CT (45.67%), PD (33.55%), and CM (34.07%) after 24 hours of fermentation, compared to the baseline (13.64%). In contrast, the ratio of *Bacteroidota* was 43.24% at baseline. However, after 24 hours of fermentation, the *Bacteroidota* ratio decreased to 31.11%, 27.36%, and 37.92% for CT, PD, and CM, respectively. Moreover, the results showed that the ratio of Firmicutes at baseline, CT, PD, and CM was 40.58%, 17.10%, 32.23%, and 24.59%, respectively. It is notable that the proportion of Firmicutes in PD was approximately twice as high compared to CT. In addition, other phyla in the top 10 in this analysis include *Verrucomicrobiota*, *Actinobacteria*, *Desulfobacterota*, *Cyanobacteria*, and *Actinobacteriota*.

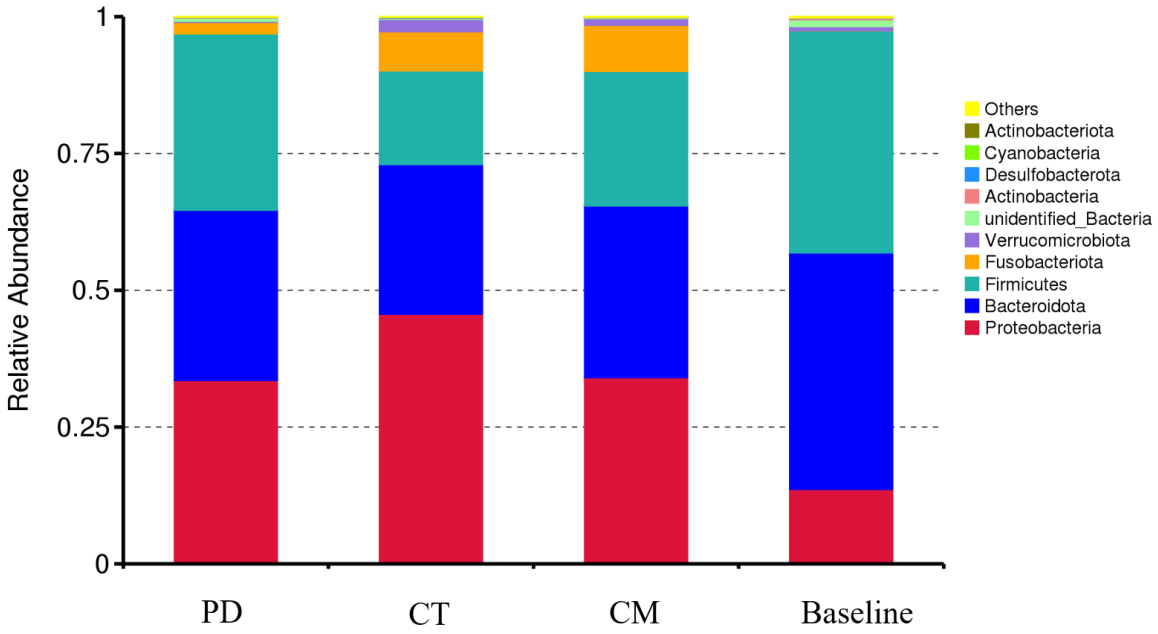

Figure 2. The relative abundance of gut microbiome at the top 10 phyla levels was analyzed for samples before fermentation (Baseline) and after 24 hours of fermentation in a fecal batch fermentation system, including the developed product (PD), the control (CT), and the commercial product (CM).

The top 30 genera of gut microbiota in the feces of subjects at baseline and after fermentation for 24 hours with the developed product, control, and commercial product are shown in Figure 3. The results showed that before fermentation (baseline), the top 3 genera consisted of *Bacteroides*, *Faecalibacterium*, and *Escherichia-Shigella*, accounting for 36.04%, 21.54%, and 10.66%, respectively. However, after fermentation with PD, CT, and CM, the top 3 abundant genera were *Escherichia-Shigella*, *Bacteroides*, and *Acidaminococcus*. It is noteworthy that *Escherichia-Shigella* accounted for 42.22% of CT, approximately 2–3 times higher than the proportions found in PD (26.92%) and CM (18.39%). It is well known that *Escherichia-Shigella* refers to a taxonomic grouping including the genera *Escherichia-Shigella*, both members of the family *Enterobacteriaceae*. *Escherichia-Shigella* is typically associated with negative health effects, particularly when pathogenic strains of these bacteria infect humans [28]. At baseline, the proportion of *Bacteroides* was 36.04%. However, after 24 hours of fermentation, the ratio decreased to 28.39%, 20.90%, and 25.42% for the PD, CT, and CM groups, respectively.

It is interesting that the proportion of *Acidaminococcus* increased after 24 hours of fermentation, accounting for 12.51%, 4.95%, and 8.78% in the PD, CT, and CM groups, respectively. Notably, the increase in *Acidaminococcus* in the PD group was approximately 20 times higher compared to the baseline (0.05%). *Acidaminococcus* is a genus of gut microbiome recognized for its role in the fermentation of amino acids and the production of short-chain fatty acids (SCFA), essential for gut health [29]. In the context of athletes, SCFAs produced by the gut microbiome, including *Acidaminococcus* sp., may help optimize gut health and performance. These compounds can serve as an energy source for intestinal cells and may enhance recovery by modulating inflammation and promoting a healthy gut microbiome balance [30].

Moreover, during the fermentation of PD, the modulation of gut microbiota showed an increase in the proportion of beneficial bacteria populations, including *Parasutterella*, *Parabacteroides*, *Megamonas*, *Sutterella*, *Veillonella*, etc. Conversely, the levels of harmful bacteria, notably *Escherichia-Shigella* and *Fusobacterium*, were elevated in CT, which did not include any synbiotic ingredients for fermentation. A gut microbiota dominated by the *Escherichia-Shigella* genera is associated with low concentrations of short-chain fatty acids (SCFAs) and an increase in metabolic pathways linked to pathogenicity, as well as the production of substances related to endotoxemia [31]. While *Fusobacterium* is a natural component of the human microbiota, its overgrowth or presence under certain conditions can lead to significant health problems. In particular, *Fusobacterium nucleatum* has been linked to colorectal cancer by promoting tumor growth and suppressing immune responses [32]. Elevated levels of *Fusobacterium* are also observed in inflammatory bowel diseases (IBD), where they exacerbate the symptoms of Crohn's disease and ulcerative colitis [33].

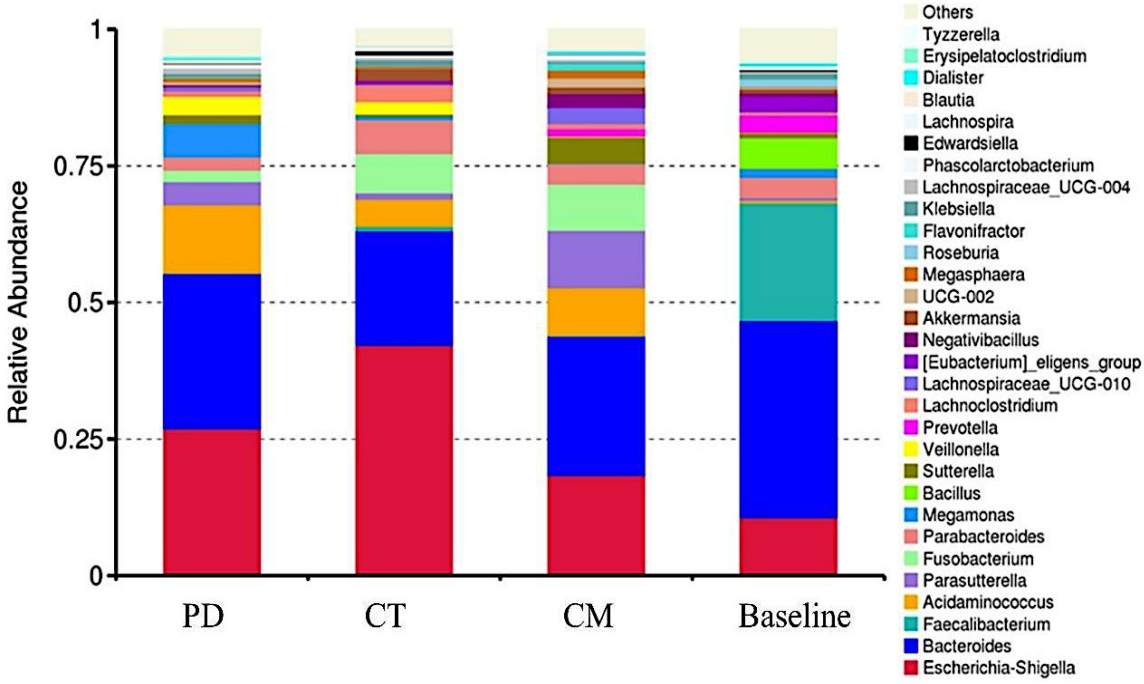

Figure 3. The top 30 genera of gut microbiota in the feces of subjects at baseline and after 24 hours of fermentation with the developed product (PD), control (CT), and commercial product (CM).

These findings indicate that a high-protein supplement containing synbiotics for athletes has the potential to support the growth of beneficial microorganisms, potentially contributing to the maintenance of a balanced gut microbiota. The balance of gut microbiota significantly influences athletic performance, recovery, and overall health by modulating energy levels, reducing fatigue, and enhancing immune function. Research indicates that a healthy and diverse gut microbiome can reduce systemic inflammation, support energy metabolism, and improve nutrient absorption, all of which are critical for training, rehabilitation, and optimal performance. For instance, a balanced microbiota helps in the production of short-

chain fatty acids (SCFAs), which have anti-inflammatory properties and play a role in energy production and recovery. Furthermore, the gut-brain axis also affects mental stress, which is essential for athletes under competitive pressure [34].

Additionally, imbalances in gut microbiota can be associated with issues related to the frequency of upper respiratory tract infections (URTI) and gastrointestinal (GI) tract infections, as well as changes in intestinal permeability [35]. Moreover, increased intestinal permeability, along with disruptions in mucus thickness and bacterial motility, is often observed and may potentially lead to gastrointestinal symptoms. During intense training or exercise, blood flow to the gastrointestinal tract can decrease by 60-70% when reaching 70% of maximum oxygen consumption (VO_2 max), potentially impacting intestinal blood circulation. This reduction in blood flow can impact intestinal function, potentially causing disruptions in gut barrier integrity and increasing intestinal permeability. Such changes can contribute to gastrointestinal symptoms like bloating, diarrhea, and abdominal discomfort, commonly experienced by athletes during or after strenuous exercise [36].

Prebiotics, probiotics, and synbiotics supplementation have been explored as a potential solution to alleviate GI issues commonly observed in athletes, particularly during periods of overtraining. Probiotics are believed to improve the gut microbiota, maintain gut barrier function, and reduce inflammation, which can help mitigate these symptoms. Some studies indicate that probiotic supplementation may be effective in preventing or reducing the severity of GI issues in endurance athletes [22, 37]. Therefore, balancing gut microbiota diversity to enhance athletic performance and health is of significant interest.

Short-chain fatty acids: Table 4 shows the concentrations of acetic acid, propionic acid, and butyric acid produced by gut microorganisms in the fecal batch fermentation system at baseline (0 hours) and after 24 hours of fermentation for the control, developed product, and commercial product. At the initial sampling or baseline, the concentrations of acetic acid and butyric acid were 1.485±0.07 mM and 0.61±0.01 mM, respectively. However, the concentration of propionic acid was not detected in this sampling, which may be due to the limitations of the measurement technique (limit of detection; LOD = 0.13 mM).

After 24 hours of fermentation, the acetic acid concentration significantly increased ($p<0.05$) to 70.50±2.25 mM and 21.01±0.88 mM for the developed and commercial products, respectively, while the control group, which did not receive any product samples, showed an acetic acid concentration of 6.31±0.38 mM. For propionic acid, the concentrations of the control, developed product, and commercial product were 2.64±0.16 mM, 38.60±1.20 mM, and 13.85±0.85 mM, respectively.

Additionally, the analysis of butyric acid concentration in the same experiment showed a similar trend, with a significant increase in short-chain fatty acids as fermentation time progressed. The highest concentration of butyric acid was observed in the developed product samples, measured at 22.22±0.58 mM after 24 hours of fermentation, while the control and commercial product samples had concentrations of 1.854±0.045 mM and 5.33±0.37 mM, respectively.

This result indicates that a high-protein supplement containing synbiotics for athletes can significantly increase SCFA concentrations by gut microorganisms in the fecal batch fermentation system, compared to the control group and commercial products.

Table 4. The concentrations of short-chain fatty acids (SCFAs) produced by gut microorganisms in the fecal batch fermentation system at baseline (0 hours) and after 24 hours of fermentation for the control, developed product, and commercial product.

Incubation time (hour)	Sample	SCFAs (mM)		
		Acetic acid	Propionic acid	Butyric acid
0	Baseline	1.48±0.07^{d}	ND	0.61±0.01^{d}
24	Control	6.31±0.38^{c}	2.64±0.16^{c}	1.85±0.04^{c}
	Developed product	70.50±2.25^{a}	38.60±1.20^{a}	22.22±0.85^{a}
	Commercial product	21.01±0.88^{b}	13.80±0.85^{b}	5.33±0.37^{b}

Different lowercase letters above the numbers in each column indicate a significant difference between samples and incubation time ($p<0.05$).

The main short-chain fatty acids (SCFA), including acetic acid, propionic acid, and butyric acid, are known to offer several health benefits. These acids support energy production in colon cells, protect against pathogenic microbial changes in the intestinal mucosa, enhance immunity and anti-inflammation, and inhibit tumor development in mammalian cells [38]. Typically, the ratio of free fatty acids produced during fermentation in the human colon is approximately 60:20:20 for acetic acid, propionic acid, and butyric acid, respectively. However, this ratio can vary depending on factors such as the type and abundance of bacterial populations, diet, and gut transit time, all of which influence SCFA production [39].

Recent studies have shown that the type of sport and exercise can influence the composition of gut microbiota in humans, which in turn is linked to changes in the synthesis of short-chain fatty acids (SCFAs) Recent studies have shown that the type of sport and exercise can influence the composition of gut microbiota in humans, which in turn is linked to changes in the synthesis of short-chain fatty acids (SCFAs) [40]. In addition, the dietary patterns of athletes, particularly those focusing on protein and carbohydrate intake to meet energy needs while reducing fiber consumption, can impact the balance of gut microbiota and directly affect SCFA production. Interestingly, long-term protein supplementation in athletes, at levels higher than the recommended daily intake, may also affect gut microbiota and influence SCFA production. A previous study found that the population of the *Bifidobacterium longum* strain, which produces acetic acid, significantly decreased compared to baseline levels after a 10-week period of protein supplementation (19.8 grams per day) [4]. Even within a 24-hour period, changes in diet composition and proportions can influence gut microbiota, thereby impacting both health and athletic performance. These findings highlight the crucial role of nutrition, especially in relation to exercise, in shaping the gut microbiota and regulating SCFA levels in athletes.

Additionally, IMO supplementation has been studied in several clinical trials, and it found that 30 days of IMO supplementation increased defecation frequency and stool weight in healthy individuals. Meanwhile, participants who received 10 g/day of IMO for 4 weeks increased defecation frequency and elevated levels of acetate and propionate in their feces [41]. Physiologically, acetate serves as an energy source for skeletal muscles, potentially extending exercise duration [42]. Moreover, inflammation is a natural response to exercise; chronic inflammation can impair recovery and performance [43]. SCFAs, particularly butyrate and propionate, have anti-inflammatory effects, and by regulating the inflammatory response, they may aid in faster recovery and reduce muscle soreness.

Furthermore, illness, injury, gastrointestinal infections, or fatigue, which may affect dietary intake in athletes, could also be factors influencing SCFA production in the gut. However, a deeper understanding and clinical research to explain the effects of SCFAs on the energy system are still of interest and may help elucidate mechanisms for enhancing athletic performance in the future.

Scientific Innovation and Practical Implications: This research introduces a novel high-protein synbiotic supplement designed specifically for athletes that effectively balances protein intake with gut microbiota health—a critical factor often overlooked in conventional sports nutrition. By incorporating *Lactobacillus paracasei* and isomaltooligosaccharides (IMO) as a prebiotic-probiotic combination, the formulation not only maintains a high protein content (79% of macronutrient composition) but also improves gut microbial composition by significantly enhancing SCFA production (acetic, propionic, and butyric acids) while suppressing pathogenic bacteria (*Escherichia-Shigella*).

The fecal batch fermentation model and next-generation sequencing (NGS) analysis provide unique, scientifically validated insights into the gut-modulating effects of this supplement, which distinguishes it from standard high-protein products that may disrupt gut microbiota. These scientific advancements demonstrate the product's ability to improve gut health through targeted microbial modulation, ensuring both muscle growth and overall digestive wellness. The product's practical formulation, sensory acceptance, and adherence to food safety regulations further highlight its practicality. As a result, it is a promising and economically viable innovation in functional sports nutrition, offering athletes a comprehensive solution that combines cutting-edge research with practical application.

In summary, by combining the latest findings in gut microbiota research with conventional protein supplementation, this high-protein synbiotic product represents a paradigm shift in sports nutrition. Athletes now have a scientifically proven alternative for improving their performance, recovery, and long-term health—one that goes beyond muscle-building. This product addresses the gut as a key element of holistic sports nutrition and ultimately provides athletes with a competitive edge in their training and competitions.

CONCLUSIONS

Based on the results of this study, *Lactobacillus paracasei* is a probiotic strain selected as suitable for isomaltooligosaccharides (IMO), and it was used as a key component in the high protein supplement containing synbiotics for athletes. Moreover, the developed product prototype, formulated according to the Thai Recommended Daily Intakes (Thai RDI), provides 120 kcal per 35 g serving and includes 26 g of protein. Additionally, the microbial quality of the product complied with the standards set by the Food and Drug Administration of Thailand. The packaging was designed as attractive plastic sachets featuring a nutrition label. The product acceptance evaluation showed satisfactory scores for appearance, color, consistency, smell, taste, sweetness, saltiness, bitterness/astringency, mouthfeel, aftertaste, and overall preference. The probiotic survival rate in simulated gastrointestinal conditions was approximately 69%. Gut microbiome profiling revealed positive outcomes after 24 hours of fermentation of the developed product, emphasizing the growth of beneficial bacteria, particularly SCFA-producing strains such as *Acidaminococcus*, *Megamonas*, and *Parabacteroides*, while also inhibiting pathogens, notably *Escherichia-Shigella*. Moreover, the analysis of short-chain fatty acids (SCFAs), including acetic, propionic, and butyric acids, showed a significant increase in their concentrations

after 24 hours of fermentation, surpassing those in both the control and commercial products.

The novelty of this study lies in the development of a high-protein supplement for athletes that incorporates a synbiotic formulation, uniquely combining *Lactobacillus paracasei* with IMO. Unlike conventional protein supplements, this product not only meets nutritional standards but also enhances gut microbiota health by promoting the growth of beneficial SCFA-producing bacteria while inhibiting pathogens. Additionally, the product demonstrates superior probiotic survival (69%) under simulated gastrointestinal conditions and significantly increases SCFA production compared to commercial alternatives. This dual functionality offering both nutritional and gut health benefits the supplement as a novel approach to supporting energy metabolism, inflammation reduction, recovery, and immune function for athletes.

Abbreviations: IMO: isomaltooligosaccharide, SCFAs: short-chain fatty acids, Thai RDI: Thai Recommended Daily Intakes, Kcal: kilocalorie, CFU: colony forming unit, GI: gastrointestinal, URTI: upper respiratory tract infections, VO_2 max: maximum volume of oxygen consumption, IBD: inflammatory bowel diseases.

Author Contributions: K.K. and S.J. conceived the concept and collected the materials. K.K. and S.J. wrote the manuscript and edited the article. Both authors read and approved the final version of the manuscript.

Competing Interests: The authors explicate that there are no conflicts of interest.

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REFERENCES

1. Amawi A, AlKasasbeh W, Jaradat M, Almasri A, Alobaidi S, Hammad AA, Bishtawi T *et al*: Athletes' nutritional demands: A narrative review of nutritional requirements. Front Nutr 2024, 10:1331854. DOI: https://doi.org/10.3389/fnut.2023.1331854
2. Zhao S, Zhang H, Xu Y, Li J, Du S, Ning Z: The effect of protein intake on athletic performance: a systematic review and meta-analysis. Front Nutr 2024, 11:1455728. DOI: https://doi.org/10.3389/fnut.2024.1455728
3. López Torres O, Fernández-Elías VE: Training and nutrition for performance: males, females, and gender differences. Nutrients 2024, 16:3979. DOI: http://doi.org/10.3390/nu16233979
4. Moreno-Pérez D, Bressa C, Bailén M, Hamed-Bousdar S, Naclerio F, Carmona M, Pérez M *et al*: Effect of a Protein Supplement on the Gut Microbiota of Endurance Athletes: A Randomized, Controlled, Double-Blind Pilot Study. Nutrients 2018, 10(3):337. DOI: https://doi.org/10.3390/nu10030337
5. Humińska-Lisowska K, Zielińska K, Mieszkowski J, Michałowska-Sawczyn M, Cięszczyk P, Łabaj PP, Wasąg B *et al*: Microbiome features associated with performance measures in athletic and non-athletic individuals: A case-control study. PLoS ONE 2024, 19(2):e0297858. DOI: https://doi.org/10.1371/journal.pone.0297858
6. Jackson R, Yao T, Bulut N, Cantu-Jungles TM, Hamaker BR: Protein combined with certain dietary fibers increases butyrate production in gut microbiota fermentation. Food Funct 2024, 15:3186. DOI: https://doi.org/10.1039/d3fo04187e
7. Zhang L, Zhang R, Li L: Effects of probiotic supplementation on exercise and the underlying mechanisms. Foods 2023, 12:1787. DOI: https://doi.org/10.3390/foods12091787
8. Aykut MN, Erdoğan EN, Çelik MN, Gürbüz M: An updated view of the effect of probiotic supplement on sports performance: A detailed review. Curr Nutr Rep 2024, 13:251–263. DOI: https://doi.org/10.1007/s13668-024-00527-x
9. Di Dio M, Calella P, Pelullo CP, Liguori F, Di Onofrio V, Gallè F, Liguori G: Effects of probiotic supplementation on sports performance and performance-related features in athletes: A systematic review. Int J Environ Res Public Health 2023, 20:2226. DOI: https://doi.org/10.3390/ijerph20032226

10. Keawyok K., Waree W, Jodnak S: Prebiotic properties of isomaltooligosaccharides from cassava as a potential ingredient in high-protein drinks for athletes. Bioact Compd Health Dis 2023, 6(3):38-55. DOI: https://doi.org/10.31989/bchd.v6i3.1063
11. Keawyok K, Sirinupong N, Wichienchot S: Nutritionally complete formula fortified with isomalto-oligosaccharide for hemodialysis patients. Funct Foods Health Dis 2020, 7(10):290-304. DOI: https://doi.org/10.31989/ffhd.v10i7.716
12. Plongbunjong V, Graidist P, Knudsen KEB, Wichienchot S: Starch-based carbohydrates display the bifidogenic and butyrogenic properties in pH-controlled faecal fermentation. Int J Food Sci Technol 2017, 52:2647-2653. DOI: https://doi.org/10.1111/ijfs.13553
13. Pansai N, Wungsintaweekul J, Wichienchot S: The effects of *Mitragyna speciosa* extracts on intestinal microbiota and their metabolites in *in vitro* fecal fermentation. J Sci Food Agric 2024, 104(14):8500–8510. DOI: https://doi.org/10.1002/jsfa.13677
14. Wichienchot S, Keawyok K: Effect of nutritionally complete formula on gut microbiota and their metabolite in fecal batch fermentation system. Funct Foods Health Dis 2021, 11(12):641-658. DOI: https://www.doi.org/10.31989/ffhd.v11i12.841
15. Chuaykarn N, Tampanna N, Wangkulangkul P, Wanitsuwan W, Yolsuriyanwong K, Wichienchot S: Comparative effectiveness of indigestible carbohydrates and plant polyphenols on the gut microbiota profile and metabolite alterations of obese patients. Bioact Carbohydr Diet Fibre 2024, 32:100443. DOI: https://doi.org/10.1016/j.bcdf.2024.100443
16. Anisong N, Siripongvutikorn S, Puttarak P, Wichienchot S: Fecal fermentation and gut microbiota modulation of dietary fibre and polyphenols from *Gnetum gnemon* Linn. leaves. Bioact Carbohydr Diet Fibre 2023, 30:100380. DOI: https://doi.org/10.1016/j.bcdf.2023.100380
17. Tampanna N, Chansuwan W, Wichienchot S. Effect of plant-based mung bean products on digestibility and gut microbiome profiling using in vitro fecal fermentation. Plant Foods Hum Nutr 2024, 79(2):460-467. DOI: https://doi.org/10.1007/s11130-024-01176-9
18. Martirosyan DM, Stratton S: Quantum and tempus theories of functional food science in practice. Funct Food Sci 2023, 3(5):55–62. DOI: https://doi.org/10.31989/ffs.v3i5.1122
19. Martirosyan DM, Lampert T, Lee M: A comprehensive review on the role of food bioactive compounds in functional food science. Funct Food Sci 2022, 3(2):64-79. DOI: https://doi.org/10.31989/ffs.v2i3.906
20. Obayomi OV, Olaniran AF, Owa SO: Unveiling the role of functional foods with emphasis on prebiotics and probiotics in human health: A review. J Funct Foods 2024, 119:106337. DOI: https://doi.org/10.1016/j.jff.2024.106337
21. Patel BK, Patel KH, Lee CN, Moochhala S: Intestinal microbiota interventions to enhance athletic performance—a review. Int J Mol Sci 2024, 25:10076. DOI: https://doi.org/10.3390/ijms251810076
22. Díaz-Jiménez J, Sánchez-Sánchez E, Ordoñez FJ, Rosety I, Díaz AJ, Rosety-Rodriguez M, Rosety MÁ, *et al*: Impact of probiotics on the performance of endurance athletes: A systematic review. IJERPH 2021, 18:11576. DOI: https://doi.org/10.3390/ijerph182111576
23. Williams K, Oo T, Martirosyan DM: Exploring the effectiveness of lactobacillus probiotics in weight management: A literature review. Funct Food Sci 2023, 3(5):42-54. DOI: https://doi.org/10.31989/ffs.v3i5.1115
24. Schönenberger KA, Mühlebach S, Huwiler VV, Stanga Z, Reber E, Pestoni G, Faeh D: Dietary fiber intake and its association with ultraprocessed food consumption in the general population of Switzerland: analysis of a population-based, cross-sectional national nutrition survey. BMJ Nutr Prev Health 2024, 0:e000727. DOI: https://doi.org/10.1136/bmjnph-2023-000727
25. Alhakbany MA, Alzamil HA, Alnazzawi E, Alhenaki G, Alzahrani R, Almughaiseeb A, 2022Al-Hazzaa HM: Knowledge, attitudes, and use of protein supplements among Saudi adults: gender differences. Healthcare 2022, 10:394. DOI: https://doi.org/10.3390/healthcare10020394
26. Gomez-Quintero DF, Kok CR, Hutkins R: The Future of Synbiotics: Rational Formulation and Design. Front Microbiol 2022, 13:919725. DOI: https://doi.org/10.3389/fmicb.2022.919725.
27. Priya AJ, Vijiayalakshmi SP, Raichur AM: Enhanced survival of probiotic *Lactobacillus acidophilus* by encapsulation with nanostructured polyelectrolyte layers through layer-by-layer approach. J Agric Food Chem. 2011, 59: 11838-11845. DOI: https://doi.org/10.1021/jf203378s
28. Ud-Din A, Wahid S: Relationship among Shigella spp. and enteroinvasive Escherichia coli (EIEC) and their differentiation. Braz J Microbiol 2014, 45(4):1131-1138. DOI: https://doi.org/10.1590/s1517-83822014000400002

29. Martin-Gallausiaux C, Marinelli L, Blottière HM, Larraufie P, Lapaque N: SCFA: mechanisms and functional importance in the gut. In Proceedings of the Nutrition Society Winter Meeting: 2-4 December 2019; London, UK. Proc Nutr Soc 2021, 80:37-49. DOI: https://doi.org/10.1017/S0029665120006916
30. Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kaitsas F, Lener E *et al*: Short-chain fatty-acid-producing bacteria: Key components of the human gut microbiota. Nutrients 2023, 15(9):2211. DOI: https://doi.org/10.3390/nu15092211
31. Baltazar-Díaz TA, González-Hernández LA, Aldana-Ledesma JM, Peña-Rodríguez M, Vega-Magaña AN, Zepeda-Morales ASM, López-Roa RI *et al*: Escherichia/Shigella, SCFAs, and metabolic pathways-the triad that orchestrates intestinal dysbiosis in patients with decompensated alcoholic cirrhosis from Western Mexico. Microorganisms 2022, 10(6):1231. DOI: https://doi.org/10.3390/microorganisms10061231
32. Dadgar-Zankbar L, Elahi Z, Shariati A, Khaledi A, Razavi S, Khoshbayan A: Exploring the role of Fusobacterium nucleatum in colorectal cancer: implications for tumor proliferation and chemoresistance. Cell Commun Signal 2024, 22:547. DOI: https://doi.org/10.1186/s12964-024-01909-y
33. Wang Z, Li B, Bao L, Chen Y, Yang J, Xu F, Shi S *et al*: *Fusobacterium nucleatum* aggravates intestinal barrier impairment and colitis through IL-8 induced neutrophil chemotaxis by activating epithelial cells. J Inflamm Res 2024, 17:8407-8420. DOI: https://doi.org/10.2147/JIR.S470376
34. Fu P, Wang C, Zheng S, Qiao L, Gao W, Gong L: Connection of pre-competition anxiety with gut microbiota and metabolites in wrestlers with varying sports performances based on brain-gut axis theory. BMC Microbiol 2024, 24:147. DOI: https://doi.org/10.1186/s12866-024-03279-4
35. Inczefi O, Bacsur P, Resál T, Keresztes C, Molnár T: The influence of nutrition on intestinal permeability and the microbiome in health and disease. Front Nutr 2022, 9:718710. DOI: https://doi.org/10.3389/fnut.2022.718710
36. Ribeiro FM, Petriz B, Marques G, Kamilla LH, Franco OL: Is there an exercise-intensity threshold capable of avoiding the leaky gut? Front Nutr 2021, 8:627289. DOI: https://doi.org/10.3389/fnut.2021.627289
37. Łagowska K, Bajerska J, Kamiński S, Del Bo C: Effects of probiotics supplementation on gastrointestinal symptoms in athletes: A systematic review of randomized controlled trials. Nutrients 2022, 14(13):2645. DOI https://doi.org/10.3390/nu14132645
38. Bermingham KM, Stensrud S, Asnicar F, Valdes AM, Franks PW, Wolf J, Hadjigeorgiou G *et al*: Exploring the relationship between social jetlag with gut microbial composition, diet, and cardiometabolic health, in the ZOE PREDICT 1 cohort. Eur J Nutr 2023, 62:3135-3147. DOI: https://doi.org/10.1007/s00394-023-03204-x
39. Nguyen HT, Hongsrichan N, Intuyod K, Pinlaor P, Yingklang M, Chaidee A, Sengthong C *et al*: Investigation of gut microbiota and short-chain fatty acids in Strongyloides stercoralis-infected patients in a rural community. Biosci Microbiota Food Health 2022, 41(3):121-129. DOI: https://doi.org/10.12938/bmfh.2021-054
40. Monda V, Villano I, Messina A, Valenzano A, Esposito T, Moscatelli F, Viggiano A *et al*: Exercise modifies the gut microbiota with positive health effects. Oxid Med Cell Longev 2017, 2017:3831972. DOI: https://doi.org/10.1155/2017/3831972
41. Mustika D, Xu W, Suzuki T: Current research on the role of isomaltooligosaccharides in gastrointestinal health and metabolic diseases. Prev Nutr Food Sci 2024, 29(2):93-105. DOI: https://doi.org/10.3746/pnf.2024.29.2.93
42. Kobayashi S, Morino K, Okamoto T, Tanaka M, Ida S, Ohashi N, Murata K *et al*: Acetate derived from the intestinal tract has a critical role in maintaining skeletal muscle mass and strength in mice. Physiol Rep 2024, 12:e16047. DOI: https://doi.org/10.14814/phy2.16047
43. Cerqueira É, Marinho DA, Neiva HP, Lourenço O: Inflammatory effects of high and moderate intensity exercise-A systematic review. Front Physiol 2020, 10:1550. DOI: https://doi.org/10.3389/fphys.2019.01550