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Optimization and Clinical Assessment of Nutritional Coffee Incorporating Fermented Lotus Leaves and Selected Herbal Bioactive Compounds

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Abstract

Background and Objective: Obesity is an increasing public health issue that needs practical and scientifically supported nutritional interventions. This study aimed to formulate functional coffees enriched with fermented lotus leaves (*Nelumbo nucifera*) using *Bacillus subtilis* to enhance polyphenol concentration and lipase enzyme activity. Additional components included breadfruit leaves (*Artocarpus altilis*), lotus seeds, notoginseng flowers (*Panax notoginseng*), caterpillar fungi (*Cordyceps militaris*), and collagen, selected for their complementary effects on metabolic functions, immune supports, sensory attributes and market feasibility.

Material and Methods: The multiple component formulation was optimized using mixture design integrated with response surface methodology. Efficacy was assessed through a 6-m randomized controlled trial involving 127 overweight adults. The trial used a double-blind placebo-controlled design to ensure reliability and minimize bias. Additionally, a consumer acceptance survey involving 800 participants was carried out to assess repurchase intention and product perception.

Results and Conclusion: Fermentation (10^7 CFU.g^{-1}, 35 °C, 65.00-70.00% relative humidity, 72 h) led to a 2.5-fold increase in polyphenol content and doubled lipase enzyme activity. In the clinical trial, participants consuming the nutritional coffee showed average weight increases of 1.40 kg, decreases in low-density lipoprotein cholesterol C by approximately 10.00 mg.dl^{-1} and increases in high-density lipoprotein cholesterol by nearly 3.00 mg.dl^{-1}. These improvements were statistically significant ($p < 0.05$) and were not associated with serious adverse effects. The consumer survey indicated a 65.00% repurchase intention, suggesting promising market potential. Although the study included limitations such as those of sample size, dropout rate and intervention time, the findings demonstrated metabolic benefits and industrial feasibility. This study provides a solid foundation for the development and commercialization of functional coffee targeting weight management and cardiovascular support. These findings provide valuable insights for researchers and industries worldwide interested in developing innovative functional beverages aimed at managing obesity and improving cardiovascular health.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Obesity is a significant global health challenge closely linked to metabolic disorders, highlighting the critical need for nutritional interventions derived from biotechnology. In particular, microbial fermentation combined with advanced optimization techniques such as response surface methodology (RSM) includes significant promises for developing effective herbal-based functional beverages to support weight management. In Asia, particularly in developing countries such as Vietnam, obesity rates have increased, significantly increasing the risk of type 2 diabetes and lipid metabolism disorders [1-3].

Coffee, well-known for its chlorogenic acid and polyphenolic compounds, has shown promising effects on lipid metabolism and fat accumulation prevention. However, its naturally high caffeine content may increase blood pressure in susceptible individuals; thus, posing certain safety concerns. To maximize coffee metabolic benefits and lessen such risks, blending coffee with bioactive Asian medicinal herbs has been suggested [4]. Specifically, this formulation moderates caffeine-linked safety concerns by incorporating herbal ingredients highlighted for their calming and antihypertensive effects. Fermented lotus leaves, breadfruit leaves, lotus seeds, *Panax notoginseng* flowers and *Cordyceps militaris* contain bioactive compounds such as polyphenols and flavonoids that help regulate blood pressure and moderate caffeine stimulating characteristics. Moreover, precise mixture design optimization ensures that caffeine content is in safe limits; thus, balancing the beneficial metabolic effects of coffee with improved safety profiles.

The synergistic interaction in chosen herbal bioactive compounds is primarily fueled by complementary mechanisms at enzymatic and metabolic levels. In details, polyphenols from fermented lotus leaves possess potent antioxidative functions, effectively inhibiting oxidative stress and inflammation pathways that complement obesity and dyslipidemia [5,6]. Flavonoids from breadfruit leaves augment antioxidative defenses, supporting overall antioxidant system strength [10,11]. Saponins derived from *P. notoginseng* accelerate lipid metabolism by promoting lipase activity to hydrolyze triglycerides and further oxidize fats [8]. Cordycepin from *C. militaris* further complements this process through modulating pathways of lipid biosynthesis and enhancing catabolism of lipids [12]. Chlorogenic acids in coffees include additional metabolic regulatory functions by enhancing glucose metabolism and insulin sensitivity, indirectly affecting lipid deposition and energy consumption [4]. In general, these bioactive compounds interact in multiple targeted biochemical pathways, leading to overall metabolic efficacy superior to single-herbal formulations. Such synergistic mechanism supports the rationale for combining them in the optimized nutritional coffee formulation.

Previous studies have primarily focused on short-term (6-8 w) assessments of single herbal ingredients or coffees, demonstrating individual metabolic benefits. For example, fermentation processes have shown significant increases in polyphenol bioavailability and enzymatic activities in herbal ingredients such as lotus leaves [5,6]. Solid-state fermentation using *Bacillus* (*B.*) *subtilis* has been reported to enhance polyphenol release and antioxidant activity in various plant materials [7]. Moreover, compounds such as notoginsenosides and saponins from *P. notoginseng* have been shown to improve lipid metabolism and protect against cardiometabolic disorders [8].

Nevertheless, comprehensive long-term studies investigating the synergistic interactions in multiple fermented herbal components within functional beverages, particularly regarding their sustained effects on weight management and cardiovascular health, are particularly limited [5-8]. This gap indicates that while individual bioactive components are recognized, the precise mechanisms underlying their combined effects in a functional beverage context are insufficiently understood. To address this gap, this study systematically optimizes the fermentation conditions of lotus leaves to substantially enhance bioactive polyphenols and lipase enzyme activity. These optimized herbal components are subsequently integrated into a nutritional coffee formulation. The hypothesis includes that combining fermented lotus leaves with selected herbal bioactive ingredients may yield enhanced metabolic and antioxidant effects, rather than those of individual ingredients alone.

This investigation uniquely integrates microbial fermentation optimization, rigorous clinical assessments and comprehensive consumer acceptance analyses. By systematically assessing synergistic interactions in multiple herbal bioactive compounds within fermented nutritional coffees, the study aimed not only to provide scientific validation but also to establish practical guidelines supporting the development and potential commerciali-zation of innovative functional beverages targeting improved metabolic health. Specific objectives included formulating and optimizing the beverage through microbial fermentation and mixture design, assessing clinical efficacy via a randomized controlled trial and assessing consumer acceptance and product feasibility through comprehensive surveys. Moreover, the study aimed to establish a scientific and practical foundation for the potential commercialization of a nutritional coffee product, addressing that full comercialization need further detailed safety assessments,

regulatory approvals and comprehensive large-scale production trials.

2. Materials and Methods

2.1. Coffee

Coffea canephora (Robusta) and *C. arabica* (Arabica) beans were collected from Krong Pac District, Dak Lak Province (12°38'N, 108°03'E), a region addressed for its basaltic soil and temperate climate in Vietnam. The beans were wet processed and sun-dried until they reached a moisture content of approximately 11.00%, as assessed using Kett PM-650 device and verified using AOAC 925.10 method [13]. To ensure consistency, the beans were stored at 20-25 °C with a relative humidity of nearly 60.00%. A Robusta:Arabica ratio of 70:30 (w/w) was chosen to balance aroma and boldness. For each roasting batch (5 kg), the beans were processed using Probat UG22 roaster at 190.00 °C ±2.00 for 12.00 min ±0.50, rapidly cooled down and yielded an Agtron color score of approximately 65. After roasting, the beans were finely ground to a particle size of ~150 µm, packed in aluminum bags with desiccants and stored under light-protected conditions at 25.00 °C ±2.00. For instant coffee production, a hot extraction was carried out using a coffee-to-water ratio of 1:10 (w/v) at 90-95 °C with continuous stirring for 30–60 min. Although the process parameters were initially set based on preliminary laboratory trials and prior publications, the mid-range conditions (90-95 °C, 30-60 min) were selected for subsequent experiments to balance extraction efficiency and bioactive compound stability. These settings aligned with standard industrial practices, ensuring scalability and reproducibility. The resulting extract was filtered, concentrated at 50.00 °C ±2 under a decreased pressure of approximately 0.08 MPa and spray-dried using Buchi B290 (inlet temperature of 160.00 °C ±2, feed rate of 5 $ml.min^{-1}$). The final product, with a moisture content less than 5.00%, was then packaged in multilayer aluminum bags and stored at 25.00 °C ±2.00.

2.2. Herbal Components

The herbal components consisted of *Nelumbo nucifera* (lotus leaves), *Artocarpus altilis* (breadfruit leaves), lotus seeds, *P. notoginseng* (notoginseng flowers) and *C. militaris* (caterpillar fungi), with hydrolyzed collagen (> 90% purity). All materials were supplied by Green Herbal Pharmaceutical, Vietnam (batch no. LN-SK-HT-CTH-202301) with certificates of analysis. Preliminary drying procedures were specifically tailored to each ingredient to balance energy efficiency with the preservation of bioactive compounds. Lotus and breadfruit leaves were dried at 45 °C for 48 h (achieving moisture levels < 8.00%), notoginseng flowers at 40 °C for 72 h, while lotus seeds were freeze-dried at -50 °C (4 Pa) to minimize thermal degradation. *Cordyceps militaris*, which was artificially cultivated, was dried at 50 °C for 24 h. Hydrolyzed collagen was prepared based on established protocols [8]. All dried materials were milled to a particle size of approximately 200 µm and stored at 4.00 °C ±1.00. Prior to formulation, microbiological analysis [14], polyphenol content assessment via the Folin-Ciocalteu method [15] and heavy metal analysis (Pb, Cd and Hg) were carried out using atomic absorption spectroscopy based on QCVN 8-2:2011/BYT [16]. The detection limits were 0.02 $mg.kg^{-1}$ for Pb, 0.01 $mg.kg^{-1}$ for Cd and 0.005 $mg.kg^{-1}$ for Hg, ensuring compliance with safety and quality standards. Hydrolyzed collagen and instant coffee were not subjected to the extraction, fiber removal and spray drying processes described later because their inherent low fiber content rendered such processing unnecessary. Instead, these ingredients were directly incorporated into the final formulation after appropriate quality control checks, ensuring that their bioactive characteristics were intact.

2.3. Chemicals and Equipment

Chemicals used in the study included gallic acid (≥ 98.00%; Sigma-Aldrich, USA) as the polyphenol standard, DPPH (2,2-diphenyl-1-picrylhydrazyl, ≥ 95%; Sigma-Aldrich, USA) for antioxidant assays, p-nitrophenyl palmitate (p-PNN) (≥ 98%; Sigma-Aldrich, USA) for lipase activity measurement, HPLC-grade methanol (Merck, Germany), 0.1 M phosphate buffer (pH 7.0) and double-distilled water. Key equipment included a Binder KBF climate chamber with ±0.5 °C, ±3% RH accuracy chamber (Binder, Germany), a Buchi B-290 spray dryer with a 0.70 mm nozzle (BUCHI Labortechnik, Switzerland), a Fritsch grinder (Fritsch, Germany), an IKA RCT basic stirrer (IKA, Germany), a Malvern Mastersizer 3000 particle size analyzer (Malvern Panalytical, UK) and a Probat UG22 coffee roaster (Probat-Werke, Germany). Polyphenol analysis was carried out using Folin-Ciocalteu method [15]. Furthermore, DPPH radical scavenging activity was assessed based on a previously described procedure [17]. Lipase enzyme activity was assessed following an established method [19]. Lipase enzyme activity was assessed based on the method described by Winkler and Stuckmann [19], with calibration curves achieving R^2 > 0.99. Lipase activity was assessed spectrophotometrically using p-NPP as substrate and 1 U of lipase activity was defined as the quantity of enzyme needed to release 1 µmol of p-nitrophenol per minute under assay conditions (at 37 °C, pH 7.0). Limit of detection and limit of quantification were assessed based on IUPAC guidelines [20] and all measurements were verified to include a relative standard deviation (RSD) leass than 5%.

2.4. Fermentation of Lotus Leaves

Dried lotus leaves (moisture < 8%) were inoculated uniformly with a food-grade strain of *B. subtilis* TH-VK422 (10^7 CFU.g^{-1}, CFU = colony-forming unit), classified as generally recognized as safe (GRAS) and selected based on preliminary screening for high lipase production and polyphenol bioconversion efficiency, that was supplied by the Laboratory of Biotechnology, Faculty of Biology and Environment, Ho Chi Minh City University of Industry and Trade (HUIT), Vietnam. The strain was kindly provided by Dr. Hoang-Dung Tran, who supervised its isolation and quality control. The *B. subtilis* was particularly chosen for fermenting lotus leaves because of specific advantages over other microbial fermentative agents such as fungi and yeasts. First, *B. subtilis* is GRAS, guaranteeing its use for functional food purposes [7]. Second, previous reports have demonstrated the superior enzymatic activity of *B. subtilis*, namely its robust lipase and protease production, which ensure better possible biotransformation and bioavailability of polyphenolic compounds [6,7].

In contrast to fungal fermentations, which generally need long incubation periods and can include risks linked to mycotoxin production, fermentation using *B. subtilis* can be carried out economically within short incubation times (48–72 h); thereby, guaranteeing practicability and safety [7,22]. Moreover, *B. subtilis* fermentation has been detected to effectively liberate bound polyphenols and other bioactive metabolites from plant matrices, significantly enhancing antioxidative and metabolic qualities of the fermented herbal products [6,7,21]. All of these characteristics strongly link to the use of *B. subtilis* as ideal microbial fermentation agent for functional enhancement of lotus leaves in this study. Fermentation was carried out using Binder KBF climate chamber and conditions optimized via Box-Behnken experimental design (Design-Expert v.11). The design assessed temperature (30–35 °C), relative humidity (60.00-70.00%) and time (48-72 h). All conditions were used in triplicate to ensure statistical reliability.

The use of triplicate replicates (n = 3) per condition is a standard practice in small-scale fermentation experiments and was reported sufficient to ensure statistical confidence and detect significant differences in polyphenol content, antioxidant activity and lipase activity. While a larger number of replicates improved power, a number of three was selected based on resource availability and methodological consistency with similar published studies. Following fermentation monitored by assessing polyphenol content, DPPH activity and lipase activity, the leaves were dried at 40 °C for 24 h to terminate microbial activity, milled and stored at 4.00 °C ±1.00. Optimal conditions were assessed using analysis of variance (p < 0.05) [8].

2.5. Extraction, Fiber Removal and Powder Production

The fibrous ingredients—including fermented lotus leaves, breadfruit leaves, lotus seeds, notoginseng flowers and *C. militaris*—were hot-water extracted at 80–90 °C using a 1:10 (w/v) ratio for 30–60 min with continuous stirring. These extraction parameters were selected based on preliminary trials that verified acceptable extraction yield and preservation of key bioactive compounds. A temperature range of 80–90°C was chosen following initial optimization trials investigating to achieve maximal extraction efficiency of polyphenolic compounds while minimizing losses in enzyme activity, particularly lipase. Increased temperatures significantly enhanced the solubility and diffusion of polyphenols and other bioactive plant-derived metabolites; thereby, improving overall yield [5,6].

Because lipase enzymes are temperature-sensitive, there is a risk of partial thermal inactivation. Preliminary trials demonstrated that lipase preserved approximately 80% of its original enzymatic activity at this temperature range, which seemed an acceptable compromise for the significant increase in polyphenol recovery. Extraction at temperatures less than 80°C led to inefficient compound recovery, while higher temperatures (> 90°C) caused significant enzyme degradation (> 30%) [8]. Therefore, a 80–90°C range was selected as optimal for balancing bioactive extraction with the preservation of lipase functionality, supporting the intended metabolic efficacy of the final product.

The resultant extracts were filtered through a 200-µm mesh and centrifuged at 5,000× g for 10 min to remove insoluble residues, targeting a residue level of < 0.05 g.100 ml^{-1}. Then, clarified supernatant was concentrated at 50.00 °C ±2.00 under a vacuum of approximately 0.08 MPa until the volume decreased to one-third of the original. The concentrated extract was spray-dried using Buchi B290 at inlet temperature of 160.00 °C ±2.00 and feed rate of 5 ml.min^{-1}. Although freeze-drying was addressed for its enzyme retention benefits, the higher associated costs led to the selection of spray drying [21]. The final powder, showing a moisture content less than 6%, was sealed in airtight packaging and stored at 4.00 °C ±1.00.

2.6. Mixture Design and RSM Optimization

A second-order mixture design was used to develop a formulation of seven independent variables (components), including (1) fermented lotus leaf extract powder, (2) breadfruit leaf powder, (3) lotus seed extract powder, (4) notoginseng flower extract powder, (5) *C. militaris* extract powder, (6) hydrolyzed collagen and (7) instant coffee. Fifteen formulations (including 12 edge points and three center points) were assessed in triplicate. The dependent variables (responses) monitored for each formulation included polyphenol content (mg GAE.g^{-1}; GAE = Gallic acid equivalent), DPPH radical scavenging activity (%), lipase activity (U.g^{-1}), sensory score (9-point scale) and dissolution time (s).

2.7. Pilot-scale Preparation of Optimized Coffee Blend

The optimal formulation identified in the RSM optimization was scaled up to a pilot production batch of approximately 100 kg. The scale-up process included (1) dry blending 100 kg of extract powders, instant coffee and collagen using 50-l ribbon mixer (batch capacity of ~20–25 kg per cycle) for 5–10 min; (2) dissolving the blended powder in 1,000 l of hot water (1:10 w/v) and stirring at 60 °C for 30 min using jacketed stainless-steel tank; (3) spray drying the solution using Buchi B-290 system (feed rate, 5 $ml.min^{-1}$; total process time, ~6–8 h; operating pressure, ~0.40 bar; inlet temperature, 160.00 °C ±2.00); and (4) packaging the resulting powder in multilayer aluminum bags (500 g per unit), ensuring a final moisture content less than 5.00% with storage at 25.00 °C ±2.00 and ~60.00% RH (AOAC 2019). Process parameters were continuously monitored to ensure batch uniformity and scalability.

2.8. Quality Control During Scale-up Production

To ensure consistency between laboratory-scale and pilot-scale batches, quality control parameters were systematically monitored through the scale-up process. Moisture content of the final powder was set at less than 5% and water activity was controlled at approximately 0.28 ±0.01 to prevent microbial growth. Spray drying conditions-including inlet temperature (160.00°C ±2.00), feed rate ($5ml.min^{-1}$) and outlet temperature (~85°C)-were monitored at regular intervals. Microbial safety was verified by total viable count, which was less than 10^3 $CFU.g^{-1}$ and heavy metal contents (Pb, Cd and Hg) were within the safety limits reported by QCVN 8-2:2011/BYT. Batch to batch uniformity was assessed by investigating key indicators, including polyphenol content, DPPH radical scavenging activity and lipase activity in three production batches. All powder batches were sealed in 500-g multilayer aluminum bags under low-humidity conditions and stored at 25.00 °C ±2.00 and approximately 60.00% RH. These measures ensured that the pilot-scale product included functional, sensory and safety characteristics of the optimized formulation.

2.9. Clinical Trial

A randomized, double-blind, placebo-controlled clinical trial was carried out over 6 m with 151 participants aged 25–55 y and a body mass index (BMI) ≥ 25.00 $kg.m^{-2}$. Participants were randomly assigned to intervention or control groups using block randomization (block size = 4), with clear inclusion and exclusion criteria to ensure study accuracy. The intervention group received the nutritional coffee product combined with standardized dietary and exercise counseling, while the control group received counseling only. Participants in the two groups included daily food diaries and physical activity logs, facilitating objective monitoring of compliance. Dietary adherence was assessed monthly by trained nutritionists through standardized food diaries, while exercise compliance-recommended at ≥ 30 $min.d^{-1}$ of moderate-intensity activity (e.g., brisk walking and cycling)—was self-managed but regularly reviewed and reinforced during monthly follow-up sessions. Compliance, adverse events and predefined outcome measures were continuously monitored, with data analysis carried out using repeated-measures ANOVA (significance set at $p < 0.05$).

2.10. Consumer Survey

A market survey was carried out over a 6-m time period with 1000 consumers aged 18-60 y. Using standardized questionnaire administered via convenience sampling, the survey collected information on product perception (e.g. aroma, taste, and repurchase intention), as well as demographic and consumption habit data. An anticipated dropout rate of approximately 20% was factored into the survey design.

2.11. Quality Control and Repeatability

All analytical measurements-including polyphenol content, DPPH radical scavenging activity, lipase enzyme activity, microbial analysis, sensory assessments, heavy metal analyses and optimization experiments-were carried out with at least three independent replications ($n \geq 3$). The RSD for each analytical method was set less than 5.00%; thereby, ensuring high reliability and reproducibility of the data.

2.12. General Workflow

Figure 1 presents an overview of the entire research workflow-from raw material selection through fermentation, extraction, pilot-scale production, clinical trial implementation and market assessment.

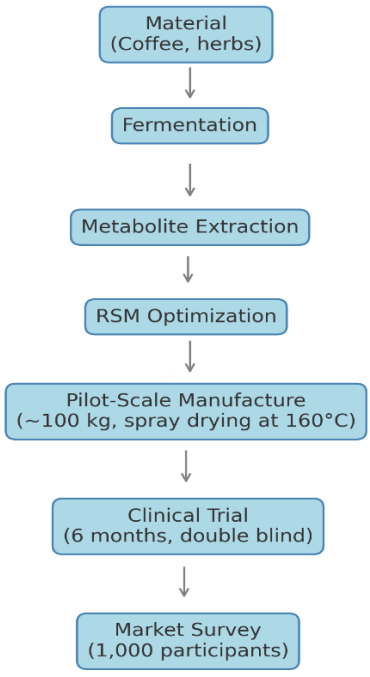


Figure 1. Graphical abstract summarizing the research workflow: starting from materials (coffee and herbs), followed by fermentation, extraction, RSM-based formulation optimization, pilot-scale manufacturing (~10 kg, spray drying at 160°C), a six-month double-blind clinical trial, and a 1,000-participant consumer market survey.

3. Results and Discussion

3.1. Quality and Characteristics of Raw Materials

Table 1 summarizes the assessed characteristics of the raw materials, including moisture content, polyphenol level, total viable aerobic microbial count (TVC) and heavy metal concentration (Pb, Cd and Hg) for the coffee blend (*C. canephora*:*C. arabica* 70:30), *N. nucifera* (lotus leaves), *A. altilis* (breadfruit leaves), lotus seeds, *P. notoginseng* (notoginseng flowers), *C. militaris* (caterpillar fungi) and hydrolyzed collagen. All raw materials were verified to comply with the microbiological and heavy metal safety criteria stipulated in QCVN 8-2:2011/BYT [16]. Specifically, TVC values were consistently less than 10^3 CFU.g^{-1} and the levels of Pb, Cd and Hg were less than detection limits. Each parameter was assessed in triplicate (n = 3) with an RSD less than 5%, except for the polyphenol assessments, which showed minor variability (±0.20–0.40 mg GAE g^{-1}; RSD < 10%). For example, coffee demonstrated a moisture content of approximately 11.00% ±0.02, while lotus leaves, breadfruit leaves, notoginseng flowers and *C. militaris* showed moisture levels in 7–8% range. Lotus seeds included a slightly lower moisture content (approximately 6–7%). Polyphenol content, assessed using Folin–Ciocalteu method [15], ranged 2.80-5.80 mg GAE.g^{-1}, coffee ranged 5.80 ±0.30 mg GAE.g^{-1}, *N. nucifera* ranged 4.90 ±0.40 mg GAE.g^{-1}, *A. altilis* ranged 3.70 ±0.30 mg GAE.g^{-1}, *P. notoginseng* flowers ranged 5.00 ±0.40 mg GAE.g^{-1} and lotus seeds ranged 2.80 ±0.20 mg GAE.g^{-1}. Compared to published literature, these polyphenol levels were moderate to relatively high. For example, previous studies reported that typical polyphenol contents for coffee beans ranged nearly 3.0-6.0 mg GAE.g^{-1} [29,30] and polyphenol levels in herbal materials such as lotus leaves and linked plant extracts typically ranged 2.0-7.0 mg GAE.g^{-1} [5,6,7]. Thus, the present values (e.g. 5.80 mg GAE.g^{-1} for coffee and 4.90 mg GAE.g^{-1} for lotus leaves) indicated strong potential for antioxidant activity and beneficial bioactive characteristics. Hydrolyzed collagen, with a moisture content of 4.10%, did not show detectable levels of polyphenols. These data not only verified that the raw materials included the necessary safety standards but also establish a robust baseline-particularly 4.90 mg GAE.g^{-1} value in *N. Nucifera*-for assessing subsequent fermentation effects. Additionally, the assessed lipase enzyme activities ranged 11.00-26.00 U.g^{-1}, where 1 U is reported as the quantity of enzyme needed to release 1 µmol of p-nitrophenol per minute at 37 °C, pH 7.0, using p-NPP as substrate.

3.2. Fermentation Outcomes

Fermentation parameters such as temperature, relative humidity and duration are well known to affect the biotransformation of bioactive compounds in medicinal plants by *B. subtilis* [7] [22]. As shown in Figure 2A, the raw (unfermented) lotus leaf powder included vibrant green color with a polyphenol content of approximately 4.90 mg GAE.g^{-1}, DPPH radical scavenging activity of nearly 60.00% and lipase activity of 15-20 U.g^{-1}. Following fermentation under optimized conditions, the powder color shifted to darker brownish-green (Figure 2B), suggesting an increase in bioactive compounds. Box-Behnken Design was used to systematically assess the effects of three key factors—temperature (30–35 °C), relative humidity (60–70%) and fermentation time (48–72 h)—on the response variables (polyphenol content, DPPH activity and lipase activity). Detailed results from 15 experimental runs (12 edge points and three center points), each carried out in triplicate (n = 3, RSD < 5%), are present in Table 2. The second-order ANOVA demonstrated a high level of statistical significance ($p < 0.001$) and an excellent model fit ($R^2 > 0.95$) with no significant lack-of-fit ($p = 0.21$). All three factors significantly affected the fermentation outcomes ($p < 0.01$) and significant interactions were observed between temperature and time ($p < 0.05$).

Table 1. Physicochemical, Microbiological, and Heavy Metal Parameters of Raw Materials (Mean ± SD, n = 3; TVC assessed following ISO 4833-1:2013; heavy metals evaluated according to QCVN 8-2:2011/BYT; polyphenol content determined using method [28]; "–" indicates below the detection limit.)

Raw Material	Moisture content (%)	Polyphenol (mg GAE.g^{-1})	TVC (CFU.g^{-1})	Pb (mg.kg^{-1})	Cd (mg.kg^{-1})	Hg (mg.kg^{-1})
Coffee (*Robusta arabica*)	11.00 ± 0.20	5.80 ± 0.30	<10^3	<0.05	<0.01	<0.01
Lotus leaves (*Nelumbo nucifera*)	7.50 ± 0.10	4.90 ± 0.40	<10^3	<0.05	<0.01	<0.01
Breadfruit leaves (*Artocarpus altilis*)	7.30 ± 0.20	3.70 ± 0.30	<10^3	<0.05	<0.01	<0.01
Lotus seeds	6.80 ± 0.20	2.80 ± 0.20	<10^3	<0.05	<0.01	<0.01
Notoginseng flowers (*Panax notoginseng*)	7.10 ± 0.20	5.00 ± 0.40	<10^3	<0.05	<0.01	<0.01
Caterpillar fungus (*Cordyceps militaris*)	7.20 ± 0.10	4.30 ± 0.30	<10^3	<0.05	<0.01	<0.01
Hydrolyzed collagen (>90% purity)	4.10 ± 0.10	–	<10^3	<0.05	<0.01	<0.01

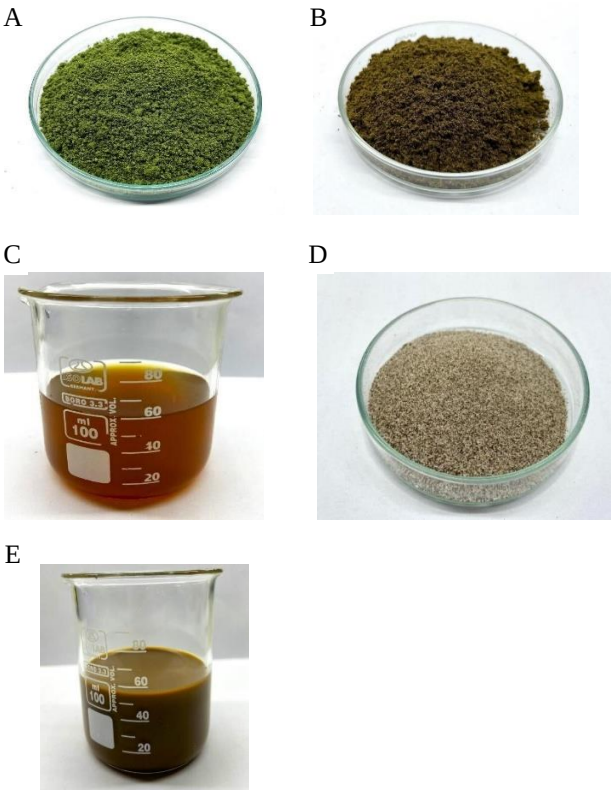


Figure 2. Representative images illustrating key stages in the production of the nutritional coffee product: (A) Unfermented lotus leaf powder exhibits a characteristic green color with a fine, loose texture, retaining its mild herbal aroma and preserving most natural bioactive compounds prior to fermentation; (B) Fermented lotus leaf powder processed with *Bacillus subtilis* transitions to a darker brownish-green shade compared to its unfermented counterpart, remaining loose and relatively uniform, with enhanced bioactive compounds (polyphenols, enzymes) and a distinctive aroma post-fermentation; (C) Lotus leaf extract obtained through hot-water extraction, displaying a yellow-brown hue and moderate clarity, assessed for fiber residues, polyphenol content, and bioactive compounds before concentration or spray drying; (D) Spray-dried nutritional coffee powder from a 100-kg pilot batch, characterized by fine particles (~120 µm in diameter) and a light brown-gray appearance, maintaining most bioactive compounds and desirable sensory attributes suitable for subsequent testing; (E) Reconstituted nutritional coffee beverage prepared by dissolving spray-dried powder in hot water (90–95 °C), exhibiting a deep brown color and fully dissolving within approximately 30 seconds without sediment, possessing a consistent flavor profile aligned with the product's intended sensory quality.

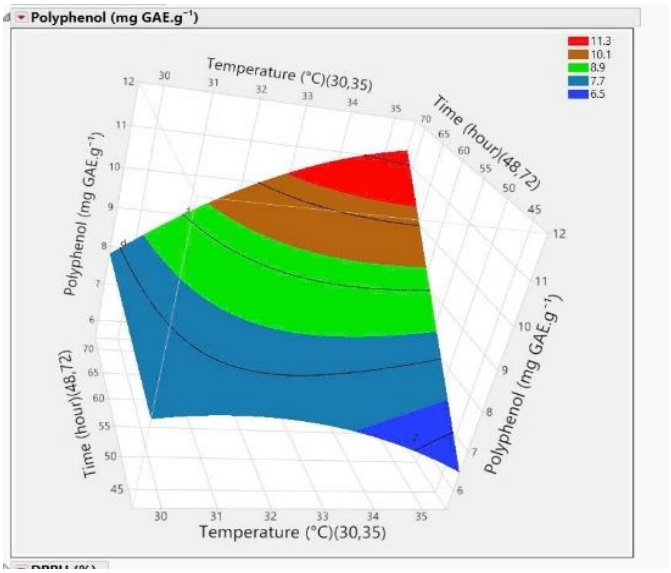
Table 2. Box-Behnken Experimental Matrix and Fermentation Results for Lotus Leaf (n = 3, RSD < 5%; polyphenol measured as mg GAE/g [28], DPPH (%) following BrandWilliams (1995) [30], lipase activity (U/g) according to Bradford/Winkler-Stuckmann [31]. Experimental levels: -1, 0, +1 correspond to 30, 32.5, 35°C; 60, 65, 70% RH; 48, 60, 72 hours.)

Run	Temperature (°C)	RH (%)	Time (h)	Polyphenol (mg GAE.g^{-1})	DPPH (%)	Lipase (U.g^{-1})
1	30 (–1)	60 (–1)	48 (–1)	7.20 ± 0.30	62.50 ± 2.10	20.80 ± 1.40
2	30 (–1)	70 (+1)	72 (+1)	9.80 ± 0.40	75.10 ± 2.30	33.70 ± 1.80
3	35 (+1)	60 (–1)	72 (+1)	11.90 ± 0.50	78.60 ± 2.50	39.60 ± 2.00
4	35 (+1)	70 (+1)	48 (–1)	9.50 ± 0.40	73.50 ± 2.10	31.90 ± 1.90
5	30 (–1)	65 (0)	60 (0)	8.20 ± 0.20	68.20 ± 2.00	25.40 ± 1.30
6	35 (+1)	65 (0)	60 (0)	9.20 ± 0.40	71.40 ± 2.20	28.60 ± 1.50
7	32.5 (0)	60 (–1)	48 (–1)	7.90 ± 0.30	65.60 ± 1.90	23.90 ± 1.40
8	32.5 (0)	70 (+1)	48 (–1)	9.80 ± 0.50	72.40 ± 2.40	30.40 ± 1.70
9	32.5 (0)	60 (–1)	72 (+1)	10.20 ± 0.40	76.80 ± 2.30	35.10 ± 2.10
10	32.5 (0)	70 (+1)	72 (+1)	11.50 ± 0.60	77.20 ± 2.20	37.80 ± 2.00
11*	30 (–1)	65 (0)	60 (0)	8.00 ± 0.30	67.50 ± 2.10	24.70 ± 1.20
12	35 (+1)	65 (0)	60 (0)	9.00 ± 0.50	71.20 ± 2.00	28.50 ± 1.80
13†	32.5 (0)	65 (0)	60 (0)	9.10 ± 0.40	70.90 ± 2.10	27.90 ± 1.30
14†	32.5 (0)	65 (0)	60 (0)	9.20 ± 0.40	70.70 ± 2.00	28.10 ± 1.50
15†	32.5 (0)	65 (0)	60 (0)	9.10 ± 0.30	71.10 ± 2.10	28.30 ± 1.60

(Run #11 is a replicate, † Three replicate runs at the center point: 32.5°C, 65% RH, 60 h.)

Figure 3A presents a three-dimensional (3-D) surface plot, showing that under optimal conditions—35 °C and 72 h—the polyphenol content reached approximately 12 mg GAE.g^{-1}. Complementary contour plots in Figure 3B indicate that DPPH radical scavenging activity increased to 78.00–80.00% under these conditions, while the 3-D plot in Figure 3C demonstrates that lipase activity peaked at approximately 38–40 U.g^{-1}. Validation of the three independent batches verified that these improvements were reproducible, with variation was 5.00%. In summary, fermentation under the optimized conditions of 35 °C, 65.00–70.00% RH and 72 h resulted in a nearly 2.50-fold increase in polyphenol content relative to the raw material, with significant enhancements in antioxidant capacity and lipase activity. These results provide a solid foundation for the subsequent extraction, fiber removal, drying and final formulation of the nutritional coffee products. This 2.5-fold increase in polyphenol content was well similar to or exceeded enhancements reported in previous studies involving microbial fermentation. For example, He et al. [5] reported nearly 1.5 to 2-fold increases in polyphenols through fermentation of lotus leaves, while Juan and Chou [6] reported up to 2-fold increases in polyphenols when fermenting black soybeans with *B. subtilis*. The observed enhancement in lipase activity (approximately doubled) was similar to or higher than those described in similar fermentation studies using *B. subtilis* on plant substrates, typically reporting increases 1.5 to 2-fold [7,22]. Therefore, these results indicated that the present optimized fermentation process was effective and competitive when benchmarked against the current literature.

A



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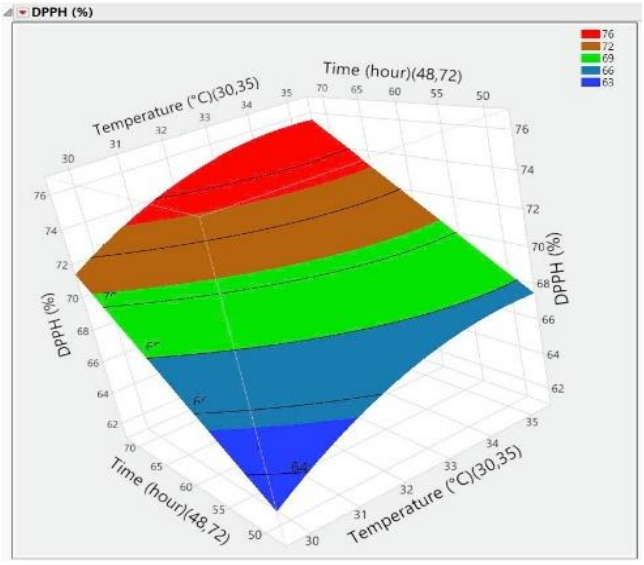

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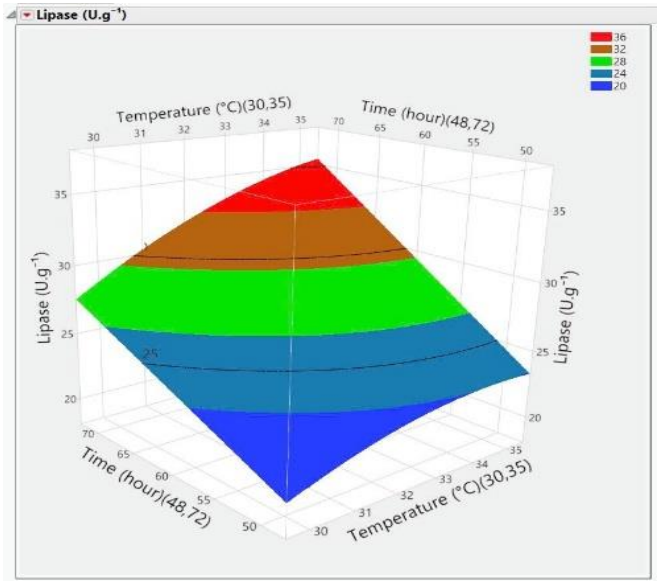


Figure 3. Three-dimensional surface plots illustrating the effects of fermentation temperature (30–35 °C) and duration (48–72 hours) on bioactive properties of lotus leaves fermented with Bacillus subtilis: (A) polyphenol content (mg GAE.g^{-1}), with color gradient from blue (7.30 mg GAE.g^{-1}) to red (11.30 mg GAE.g^{-1}) highlighting increased polyphenol concentration under optimized conditions (~35 °C, 72 h); (B) DPPH radical scavenging activity (%), with antioxidant capacity ranging from 63.00% (blue) to 76.00% (dark red), emphasizing enhancement at optimal fermentation conditions (~35 °C, 72 h); (C) lipase enzyme activity (U.g^{-1}), with enzymatic activity increasing from 19 U.g^{-1} (blue) to 35 U.g^{-1} (dark red), demonstrating optimal enzymatic production at approximately 35 °C and 72 h.

3.3. Extraction, Fiber Removal and Spray Drying of the Fermented Lotus Leaves

To achieve fiber-decreased extract powder enriched with bioactive compounds, the fermented lotus leaves were hot-water extraced, filtration-centrifuged, concentrated and spray dried. Although similar procedures were used to other fibrous medicinal ingredients (breadfruit leaves, lotus seeds, notoginseng flowers and *C. militaris*), detailed results were present only for the fermented lotus leaves because they showed significant changes in polyphenol content and lipase activity. The process was divided into two major stages as follows.

Filtration-centrifugation: The fermented lotus leaves were extracted using hot water at 80–90°C with 1:10 (w/v) ratio for 30–60 min under continuous stirring. The resulting extract, showing yellow-brown hue and moderate clarity (Figure 2C), was filtered through a 200-µm mesh and centrifuged at 5,000× g for 10 min to remove insoluble fibers. As detailed in Table 3, this step decreased the fiber residues from 1.30 ±0.20 to less than 0.05 g.100 ml^{-1} (approximately 96% increases, $p < 0.01$), while the polyphenol content decreased slightly by nearly 6% (10.80 ±0.30 to 10.20 ±0.30 mg GAE.g^{-1}, $p < 0.05$) and lipase activity showed a negligible increase of nearly 3.60% (39.20 ±1.50 to 37.80 ±1.30 U.g^{-1}, $p > 0.05$). After spray drying**,** the polyphenol content further decreased by approximately 11% (10.20 ±0.30 to 9.10 ±0.30 mg GAE.g^{-1}, $p < 0.01$) and the lipase activity decreased by nearly 17.00% (37.80 ±1.30 to 31.40 ±1.20 U.g^{-1}, $p > 0.05$), resulting in final powders with a moisture content of 5.60% ±0.20. The overall recovery yield—calculated as a ratio of the final spray-dried extract powder weight to dry weight of the initial fermented lotus leaves—was assessed as 20.50% ±1.20 (w/w). This final product demonstrated excellent solubility in hot water, making it well appropriate for incorporation into the final formulation (Section 3.4).

In summary, the combined processing steps allowed the fermented lotus leaves to preserve approximately 80% of their initial lipase activity and 84% of their polyphenol content, with an overall recovery yield of nearly 20.5% (w/w). These results have been included in Table 3, providing a comprehensive overview of the extraction efficiency. Compared to similar herbal extraction and drying processes reported in previous literature, this recovery yield of 20.5% could be reported as moderate within a typical expected range. For example, herbal extraction yields commonly range approximately 15-30%, depending on the specific plant material, extraction temperature and solvent ratio [6, 7, 21]. Given the current optimized extraction parameters aimed at balancing compound recovery with minimal bioactive degradation, yield of 20.5% indicated satisfactory process efficiency consistent with industry standards and published studies.

3.4. Mixture Design and RSM Results

To develop a multiple-component nutritional coffee formulation comprising seven ingredients— fermented lotus leaves, breadfruit leaves, lotus seeds, notoginseng flowers, caterpillar fungi, collagen and coffee (100% w/w)—second-order mixture design was used. The formulation content was established as follows: collagen ≤ 2%; caterpillar fungi, 0.30–0.50%; coffee ≥ 50%; fermented lotus leaves, 10.00–25.00%; notoginseng flowers, 3–5%; breadfruit leaves, 9.00–10.00% and lotus seeds, 6–8%. A ≤ 2% limit for collagen was established based on preliminary sensory tests and previous literature, which indicated that higher collagen concentrations could negatively affect sensory attributes by causing undesirable texture changes such as increased viscosity and off-flavors [31, 32]. Despite this concentration limit, collagen significantly contributed to the formulation by enhancing sensory characteristics, particularly mouthfeel smoothness and creaminess as well as providing additional nutritional benefits linked to joint and skin health [31, 33]. Thus, limiting collagen to ≤ 2% optimally balanced sensory acceptance with functional and nutritional contributions.

Table 3. Changes in fiber residue, polyphenol content, lipase activity, and moisture through filtration-centrifugation and spray drying of fermented lotus leaf extract (Mean ± SD, n = 3).

Processing Stage	Fiber Residue (g.100 mL^{-1})	Polyphenol (mg GAE.g^{-1})	Lipase Activity (U.g^{-1})	Moisture (%)	Recovery Yield (%)
Before Filtration–Centrifugation	1.30 ± 0.20	10.80 ± 0.30	39.20 ± 1.50	–	–
After Filtration–Centrifugation	<0.05	10.20 ± 0.30	37.80 ± 1.30	–	95.00 ± 2.00
After Spray Drying	–	9.10 ± 0.30	31.40 ± 1.20	5.60 ± 0.20	20.50 ± 1.20*

*Note: Recovery yield after spray drying is calculated from the weight of the final powder relative to the dry weight of initial fermented lotus leaves. Fiber residue showed ~96.00% reduction after filtration–centrifugation ($p < 0.01$). Polyphenol content had a ~6.00% loss after filtration–centrifugation ($p < 0.05$), and an additional ~11.00% loss after spray drying ($p < 0.01$). Lipase activity showed a non-significant ~3.60% reduction after filtration–centrifugation ($p > 0.05$), and an additional ~17.00% reduction after spray drying ($p > 0.05$)

The experimental design consisted of 15 formulations (12 edge points and three center points), each replicated three times (n = 3). The second-order ANOVA analysis demonstrated high statistical significance ($p < 0.05$) with a strong model fit ($R^2 > 0.95$) and no significant lack-of-fit ($p > 0.05$), verifying that the model was well fixed for the investigated range. Analysis of interactions in ingredients revealed several significant synergistic and antagonistic effects. Specifically, significant positive interactions ($p < 0.05$) were observed between fermented lotus leaves and coffee, enhancing polyphenol content and antioxidant capacity. Additionally, interactions between breadfruit leaves and lotus seeds showed positive effects on lipase enzyme activity. In contrast, a mild antagonistic effect was detected between collagen and coffee at collagen concentrations above 2%, negatively affecting sensory scores. These interactions emphasized complexity of the ingredient effects in multiple component formulations, highlighting the necessity of careful ingredient ratio optimization to achieve balanced sensory and functional attributes. Five key response parameters were monitored:

1. Polyphenol content (mg GAE.g^{-1});
2. DPPH radical scavenging activity (%);
3. Lipase enzyme activity (U.g^{-1}) with a target of achieving at least 70% of the initial value (~28.00 U.g^{-1}, associating to ~40.00 U.g^{-1});
4. Sensory score was assessed on a 9-point hedonic scale by a panel of 8–10 trained panelists (five females and five males, aged 25–45 y) from staff members and graduate students of the Faculty of Biology and Environment, Ho Chi Minh City University of Industry and Trade, Vietnam. Panelists were standard trained prior to assessment, focusing on sensory analysis methodologies, identification of key attributes and calibration exercises to ensure consistent scoring and interpretation of sensory descriptors;
5. Dissolution time (s).

A detailed summary of the mixture design outcomes is present in Table 4. Using a desirability function approach for multiple objective optimization, a near-optimal formulation was identified: approximately 20.00% of fermented lotus leaves, 9.90% of breadfruit leaves, 7.00% of lotus seeds, 3.70% of notoginseng flowers, 0.37% of caterpillar fungi, 1.70% of collagen and nearly 57.00–58.00% of coffee, which achieved a desirability index of ~0.95. Figures 4A and 4B illustrate ternary plots, analyzing effects of key formulation components on polyphenol content and DPPH radical scavenging activity, respectively. These visualizations verified increased proportion of the fermented lotus leaves and coffee positively affected antioxidant characteristics of the final product. To validate the RSM model, three pilot-scale production batches (approximately 1 kg each) were produced using the optimal formulation.

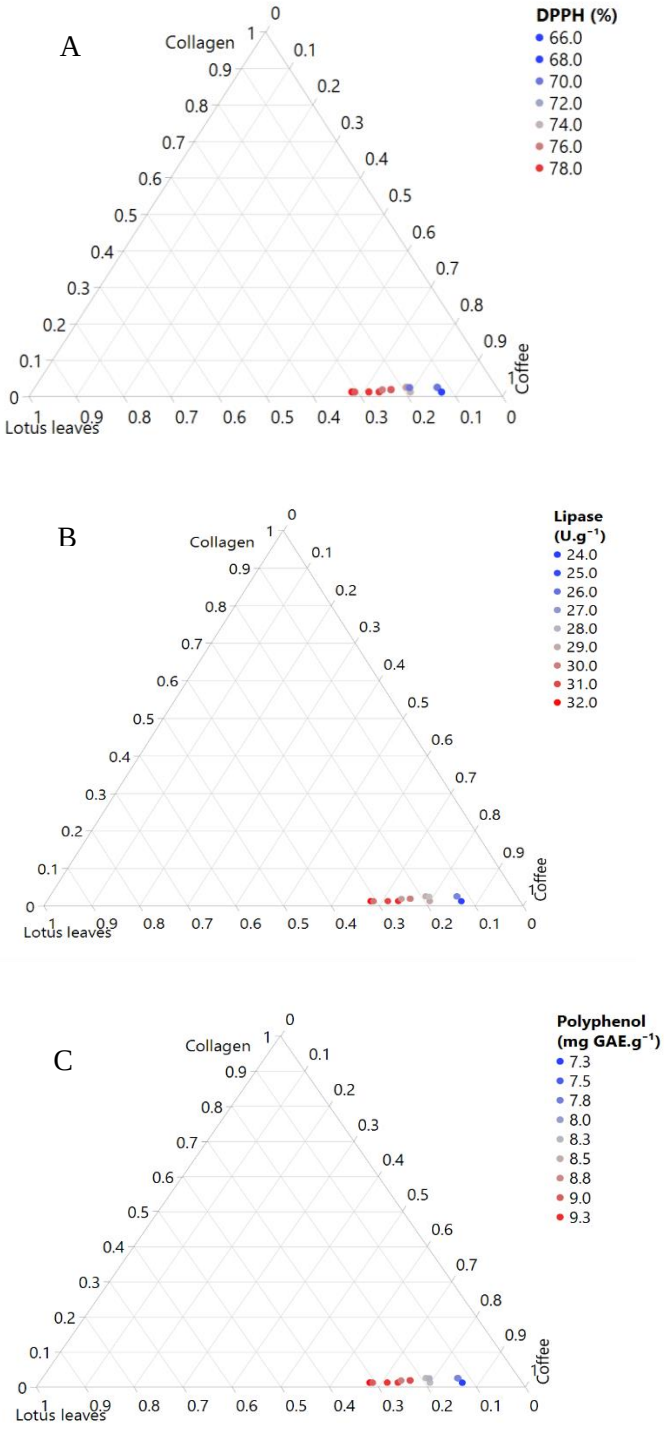


Figure 4. Ternary plots illustrating the effect of blending ratios among fermented lotus leaf, collagen, and coffee within a mixture design: (A) Effect on DPPH radical scavenging activity (%), with antioxidant activity ranging from 66.00% (blue) to 78.00% (red), indicating variations across blend formulations; (B) Effect on lipase enzyme activity (U.g^{-1}), with enzymatic activity varying from 24.00 U.g^{-1} (blue) to 32.00 U.g^{-1} (red), highlighting differe-nces based on component ratios; (C) Combined summary showing polyphenol content (mg GAE.g^{-1}, 7.30–9.30 mg), DPPH activity (66.00-78.00%), and lipase activity (24.00-32.00 U.g^{-1}), illustrating optimal blending ratios for maximizing bioactive properties in the nutritional coffee formulation.

Table 4. Mixture design matrix (RSM optimization) and validation results for nutritional coffee formulation (Mean ± SD).

Run	Lotus Leaf (%)	Breadfruit Leaf (%)	Lotus Seed (%)	Notoginseng Flower (%)	Cordyceps (%)	Collagen (%)	Coffee (%)	Polyphenols (mg GAE.g^{-1})	DPPH (%)	Lipase (U.g^{-1})	Sensory (/9)	Dissolution Time (s)	Predicted Polyphenols (mg GAE.g^{-1})	Experimental Polyphenols (mg GAE.g^{-1})	Deviation (%)
1	10.00	9.00	6.00	5.00	0.50	2.00	67.50	7.50 ± 0.20	70.00 ± 3.00	25.10 ± 0.80	6.50 ± 0.30	35.00 ± 3.00	–	–	–
2	25.00	9.00	6.00	3.00	0.30	1.00	55.70	9.00 ± 0.30	77.00 ± 2.00	30.20 ± 1.10	7.50 ± 0.40	32.00 ± 2.00	–	–	–
3	15.00	10.00	6.00	5.00	0.30	2.00	61.70	8.40 ± 0.30	75.00 ± 3.00	28.90 ± 1.20	6.80 ± 0.30	33.00 ± 2.00	–	–	–
4	10.00	9.50	7.00	3.00	0.40	2.00	68.10	7.80 ± 0.20	70.00 ± 2.00	26.50 ± 1.00	6.90 ± 0.40	37.00 ± 3.00	–	–	–
5	20.00	9.00	6.00	3.00	0.30	1.50	60.20	8.80 ± 0.40	76.00 ± 2.00	29.40 ± 1.30	7.20 ± 0.30	29.00 ± 2.00	–	–	–
6	18.00	9.50	7.00	3.50	0.40	1.50	60.10	9.10 ± 0.30	77.00 ± 3.00	30.50 ± 1.10	7.50 ± 0.40	28.00 ± 2.00	–	–	–
7	15.00	9.00	8.00	3.00	0.50	1.00	63.50	8.30 ± 0.30	74.00 ± 3.00	29.10 ± 1.10	6.70 ± 0.40	34.00 ± 2.00	–	–	–
8	22.00	9.00	6.00	5.00	0.30	1.00	56.70	9.20 ± 0.40	78.00 ± 3.00	31.10 ± 1.30	7.40 ± 0.50	32.00 ± 2.00	–	–	–
9	10.00	9.00	6.00	3.00	0.50	1.00	70.50	7.30 ± 0.30	68.00 ± 2.00	24.80 ± 0.90	6.40 ± 0.30	36.00 ± 2.00	–	–	–
10	25.00	9.50	6.00	4.00	0.30	1.00	54.20	9.40 ± 0.30	79.00 ± 3.00	32.00 ± 1.40	7.60 ± 0.40	31.00 ± 2.00	–	–	–
Validation	18.00	9.00	7.00	4.00	0.40	1.50	60.10	9.00 ± 0.30	76.00 ± 3.00	30.20 ± 1.10	7.40 ± 0.40	28.00 ± 2.00	9.50	9.30 ± 0.30	2.10

Note: Experimental values closely matched the model predictions, confirming the validity and reliability of the optimized RSM formulation.

Table 4 compares experimental assessments against the model predictions. The deviations between the predicted and experimental values were consistently less than 5.00% ($p > 0.05$). Specifically, the experimental results were as follows:

- Polyphenol content, ~9.30 mg GAE.g^{-1};
- DPPH radical scavenging activity, ~78.00%;
- Lipase enzyme activity, ~29.00 U.g^{-1} (~ 72.00% of the initial target);
- Sensory score, ~7.60/9; and
- Dissolution time, ~25 s.

Figure 4C presents a comparative analysis of the model-predicted with experimentally assessed values for key parameters, including polyphenol content, DPPH activity, lipase activity and dissolution time. The close alignment between the predicted and observed values verified accuracy and robustness of the RSM model in optimizing the formulation. Sensitivity assays adjusting the collagen and fermented lotus leaf content by ±2.00% verified that the model predictions were robust, with errors of 5.00%. Although the sensory assessment was based on a relatively small panel (8–10 participants), these findings strongly supported that the combination of mixture design and RSM was an effective strategy for optimizing a multiple-component nutritional coffee formulation. Further studies should focus on expanding the sensory panel and refining the model with additional 3D surface and contour plots, as well as undertaking large-scale clinical assessments to assess long-term product stability and efficacy.

3.5. Pilot Production Results

Following assessment of the optimal formulation (approximately 20.00% of fermented lotus leaves, 9.90% of breadfruit leaves, 7% of lotus seeds, 3.70% of notoginseng flowers, 0.37% of caterpillar fungus, 1.70% of collagen and the rest of coffee), a 100-kg pilot production batch was prepared to assess product stability and prepare the product-tentatively named "Nutrition Coffee Love World"- for clinical trials (Section 3.6). The product was packaged in 500-g aluminum bags under controlled conditions (25.00 °C ±2.00, RH ~60.00%). The multilayer aluminum packaging effectively contributed to product stability by providing superior barriers against moisture, oxygen and light—key factors that accelerate degradation of bioactive compounds such as polyphenols and enzymes. Specifically, aluminum layers significantly limited oxygen ingress and moisture vapor transmission; thereby, minimizing oxidative reactions and enzymatic degradations. Additionally, the opaque nature of aluminum packaging protects sensitive bioactive compounds from light-induced deterioration, collectively ensuring that the nutritional and functional qualities of the coffee products were stable through storage. Although full international certification has not been achieved, the product meets the criteria for clinical assessment. All ingredients used in this nutritional coffee formulation have carefully been selected and verified to comply fully with relevant regulatory standards, specifically meeting microbiological, heavy-metal and quality criteria by Vietnamese National Technical Regulations (QCVN 8-2:2011/BYT [16]). Furthermore, Certificates of Analysis (COA) provided by the supplier validated the quality, purity and safety of each herbal component and collagen, ensuring their appropriateness for clinical uses.

Spray drying was carried out using Buchi B-290 with inlet temperature of 160.00 °C ±2.00, feed rate of 8-10 ml.min^{-1} and processing capacity of 12-15 l.h^{-1}, yielding outlet temperature of 80-85 °C and total drying time of 2–3 h. Special attention was specified to the high-moisture phase during the initial 30-45 min to ensure that the outlet temperature did not exceed 90 °C; thereby, minimizing lipase degradation. Five production batches ($n = 5$) were produced and compared with a 1-kg batch to assess consistency. Figure 2D shows spray-dried nutritional coffee powders from the 100-kg pilot batch, characterized by fine particles (~120 µm) with light brown-gray color. Figure 2E demonstrates a reconstituted beverage prepared with hot water (90–95 °C), showing deep brown color and complete dissolution within 30 s without sedimentation.

The quality attributes of the 100-kg pilot product and the changes in key quality parameters within 3–6 m of storage are comprehensively summarized in Table 5. These attributes included moisture content, water activity, particle size (D50), microbial load, heavy metal content, dissolution time, polyphenol content, DPPH radical scavenging activity, lipase activity, sensory assessment score and their stability over storage. Specifically, polyphenol content decreased from 9.10 to 8.70 mg GAE.g^{-1} (~4% loss), lipase activity decreased from 28.50 to 27.00 U.g^{-1} (~5.00% loss), DPPH activity decreased from 77.00 to 75.00%, sensory scores slightly decreased from 7.60 to 7.40/9 and moisture content increased marginally from 4.80 to 5.00%. Moreover, ANOVA verified that these changes were 5.00% ($p > 0.05$) and microbial loads and heavy metal levels were stable, demonstrating product stability. Acceptance criteria for batch-to-batch variation and degradation were clearly set at ≤ 5% based on industrial standards and previous literature, indicating that variations in bioactive compound levels and enzyme activity within this range included minimal effects on overall product efficacy and quality. Specifically, maintaining polyphenol content and lipase enzyme activity variations less than 5% ensured consistent functional benefits, antioxidant capacity and sensory characteristics within the production batches, aligning with typical quality control standards for functional food products [21, 34]. Additionally, microbial analysis included

total aerobic mesophilic bacteria as general hygiene indicators and explicitly assessed for pathogenic microorganisms such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, molds and yeasts. All these microorganisms were enumerated less than detection limits, ensuring compliance with microbiological safety standards based on QCVN 8-2:2011/BYT [16]

A separate cost comparison between 1-kg and 100-kg batches is provided in Table 6, the 1-kg batch included a cost of approximately 13 USD.kg^{-1}, whereas the 100-kg batch achieved a cost of ~6 USD.kg^{-1}, underscoring a significant economy of scale. Figure 5A illustrates quality parameters over storage, while Figure 5B provides a comparison between the model-predicted and experimentally assessed values. Overall, the pilot production data verified that the product included stable quality and that scaling up to 100 kg significantly decreased production costs. Further studies address accelerated shelf-life testing, further scaling to ≥ 500 kg, expanding the sensory panel to > 30 participants and carrying out long-term *in vivo* trials to assess economic and technical viabilities of the formulation.

3.6. Clinical Trial Results

A randomized controlled trial was carried out within 6 m in adults aged 18–59 y with a BMI ranging 23-40 kg.m^{-2}, following the Asian criteria for overweight and obesity [24]. From 153 volunteers, two volunteers were excluded due to incomplete data, resulting in a final enrollment of 151 subjects, who were randomized into two groups. The intervention group (n = 78) consumed "Nutrition Coffee Love World" (1–2 sachets per day, each containing 18 g) alongside standardized counseling to decrease daily caloric intake by approximately 300–500 kcal and engage in at least 30 min of daily exercises. The control group (n = 73) received a similar dietary and exercise counseling, which was delivered using standardized protocol to ensure consistency in groups. At 6 m, 66 participants in the intervention group and 61 in the control group completed the study (n = 127), corresponding to an attrition rate of approximately 16%. Block randomization (block size = 4) was used with an expected attrition rate of 15.00% and the study was carried out based on the CONSORT guidelines [25]. All participants provided written informed consent and the study protocol was approved by the 7A Military Hospital Ethics Committee.

Table 7 presents the baseline characteristics of the two groups (n = 127 after 6 m). The groups were similar in age (p = 0.772), sex distribution (p = 0.864), BMI (p = 0.741), hypertension prevalence (p = 0.963), baseline glucose level (p = 0.801) and baseline LDL-C (p = 0.912). The detailed clinical outcomes, including changes in weight, BMI, waist circumference, body fat percentage (assessed by bioelectrical impedance analysis, BIA), lipid profile and glucose level from baseline (M0) to 6 m (M6) for the two groups, are comprehensively summarized in Table 8. The intervention group showed significant improvements of weight increase (-1.40 kg ±2.10, p = 0.032), BMI decrease (-0.50 ±0.90 kg.m^{-2}, p = 0.041), waist circumference increase (-1.00 cm ±3.50, p = 0.049) and body fat increase (-1.40% ±2.60, p = 0.046). Visceral fat significantly decreased by -8.30 cm² ±12.50 (p < 0.05). For lipid profiles, LDL-C significantly decreased by -12.20 ±15.80 mg.dL^{-1} (p < 0.01) and total cholesterol decreased significantly by -17.30 ±24.20 mg.dl^{-1} (p < 0.01). Changes in glucose, HDL-C and triglycerides were minor. The control group showed minimal, statistically non-significant changes in all parameters. Fasting glucose levels decreased slightly in the intervention group (-0.20 mmol.l^{-1}, p = 0.05) but increased marginally in the control group (+0.10 mmol.l^{-1}, p = 0.32). Liver enzyme levels (AST and ALT) were stable through the trials and no serious adverse events were observed.

Table 5. Characteristics and stability of the 100 kg pilot-scale nutritional coffee product (Mean ± SD, n=5).

Parameter	Initial	After 3 months	After 6 months
Moisture Content (%)	4.80 ± 0.20	4.90 ± 0.20	5.00 ± 0.20
Water Activity (a, 25 °C)	0.28 ± 0.01	–	–
Particle Size D50 (µm)	~120.00	–	–
Aerobic Microorganisms (CFU.g^{-1})	<10³	–	–
Heavy Metals (Pb, As, Cd)	Pb<0.10, As<0.05, Cd<0.01 ppm	–	–
Dissolution Time (s)	24.00 ± 2.00	–	–
Polyphenol (mg GAE.g^{-1})	9.10 ± 0.30	8.90 ± 0.30	8.70 ± 0.30
DPPH (%)	77.00 ± 3.00	76.00 ± 3.00	75.00 ± 3.00
Lipase Activity (U.g^{-1})	28.50 ± 1.00	27.70 ± 0.90	27.00 ± 1.01
Sensory Score (/9)	7.60 ± 0.30	7.50 ± 0.30	7.40 ± 0.40

Note: Stability tests indicate minimal reductions (<5%) in polyphenol content, lipase activity, DPPH activity, and sensory scores over 6 months, confirming the stability of the pilot-scale product.

Table 6. Cost comparison between small-scale (1 kg) and pilot-scale (100 kg) batches.

Scale	Time (hours)	Electricity & Water (USD)	Labor (USD)	Total Cost (USD)	Cost per kg (USD/kg)
1 kg	0.50–1.00	5.00–8.00	~5.00	~13.00	~13.00
100 kg	2.00–3.00	~200.00	~100.00	~600.00	~6.00

Note: Pilot-scale production significantly reduced the unit cost, demonstrating economic feasibility at larger production volumes.

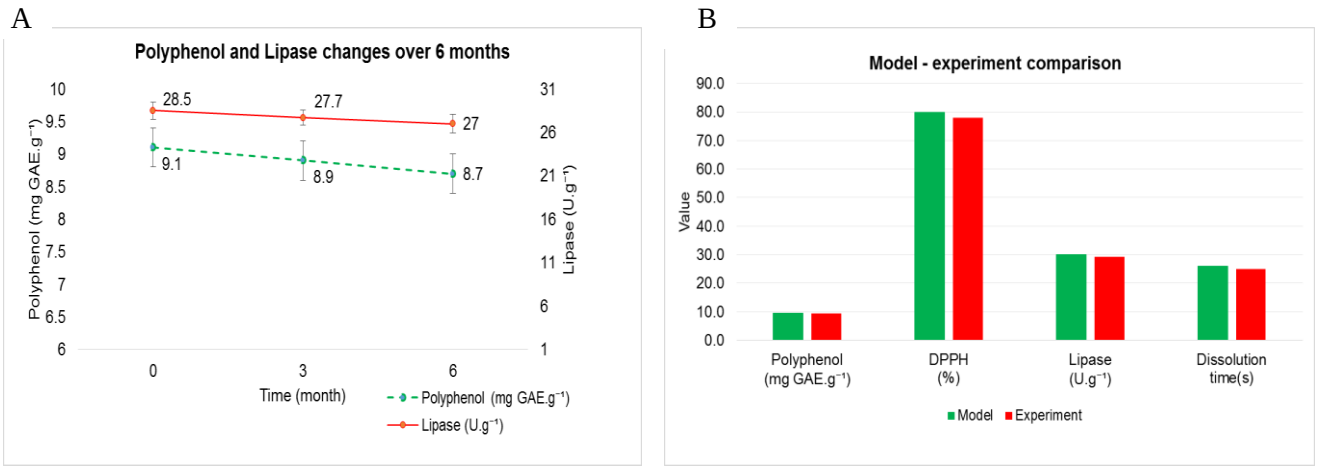


Figure 5. Stability and validation of the pilot-scale nutritional coffee product: (A) Changes in polyphenol content (mg GAE.g⁻¹) and lipase activity (U.g⁻¹) over 6 months storage at 25 ± 2 °C and relative humidity ~60%, demonstrating minimal reduction (<5%) and confirming stability of key bioactive compounds; (B) Comparison between model-predicted and experimentally measured values for polyphenol content (mg GAE.g⁻¹), DPPH (%), lipase activity (U.g⁻¹), and dissolution time (s), showing deviations below 5% ($p > 0.050$), confirming accuracy of the developed Response Surface Methodology (RSM) model.

Table 7. Baseline Characteristics of the Two Groups (n = 127 after 6 Months) (Mean ± SD; $p > 0.05$ indicates no significant differences.)

Parameter	Intervention (n = 66)	Control (n = 61)	p-value
Age (years)	40.90 ± 10.80	41.30 ± 10.20	0.772
Female (%)	78.80	80.30	0.864
BMI (kg.m^{-2})	26.70 ± 3.80	26.90 ± 3.90	0.741
Hypertension (%)	20.00	19.70	0.963
Baseline Glucose (mmol.L^{-1})	5.60 ± 1.50	5.50 ± 1.60	0.801
Baseline LDL-C (mg.dL^{-1})	138.20 ± 34.10	137.30 ± 33.50	0.912

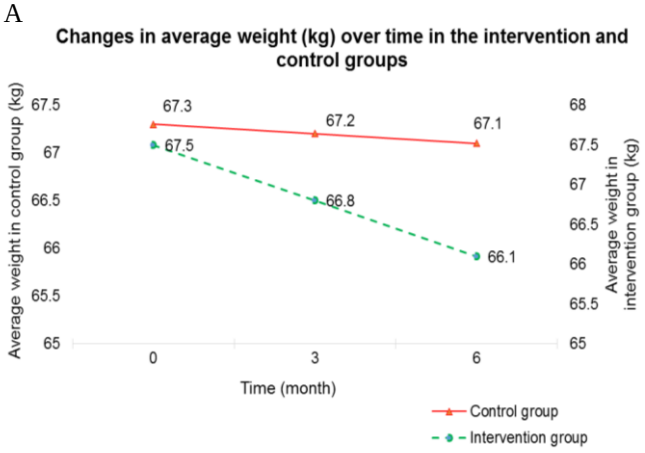
Table 8. Changes in weight, BMI, waist circumference, body fat, lipid profiles, and glucose after 6 months of intervention (Mean ± SD, n=127).

Parameter	Group	Baseline (M0)	6 Months (M6)	Change (Δ)	p-value
Weight (kg)	Intervention	67.50 ± 12.90	66.10 ± 12.70	–1.40 ± 2.10	<0.05
	Control	67.30 ± 13.10	67.10 ± 13.00	–0.20 ± 2.00	0.76
BMI (kg.m^{-2})	Intervention	26.70 ± 3.80	26.20 ± 3.70	–0.50 ± 0.90	<0.05
	Control	26.90 ± 3.90	26.80 ± 3.80	–0.10 ± 0.80	0.63
Waist Circumference (cm)	Intervention	86.10 ± 9.80	85.10 ± 9.60	–1.00 ± 3.50	<0.05
	Control	85.90 ± 10.00	86.20 ± 10.20	+0.30 ± 3.20	0.58
Body Fat (%)	Intervention	35.20 ± 6.70	33.80 ± 6.90	–1.40 ± 2.60	<0.05
	Control	35.00 ± 6.60	34.90 ± 6.60	–0.10 ± 2.50	0.82
Visceral Fat (cm²)	Intervention	118.30 ± 42.10	110.00 ± 40.30	–8.30 ± 12.50	<0.05
	Control	117.40 ± 41.90	115.30 ± 41.80	–2.10 ± 10.70	0.66
LDL-C (mg.dL^{-1})	Intervention	138.00 ± 34.10	126.00 ± 30.00	–12.20 ± 15.80	<0.01
	Control	137.30 ± 33.50	135.90 ± 33.20	–1.40 ± 13.50	–
Total Cholesterol (mg.dL^{-1})	Intervention	235.50 ± 58.60	218.20 ± 50.10	–17.30 ± 24.20	<0.01
	Control	236.00 ± 59.10	233.40 ± 56.80	–2.60 ± 20.30	–
HDL-C (mg.dL^{-1})	Intervention	63.00 ± 14.20	60.90 ± 13.50	–2.10 ± 5.50	0.14
	Control	62.70 ± 14.00	59.20 ± 13.60	–3.50 ± 5.80	–
Triglycerides (mg.dL^{-1})	Intervention	181.50 ± 188.00	170.30 ± 164.70	–11.20 ± 38.00	0.08
	Control	179.00 ± 185.50	178.60 ± 179.20	–0.40 ± 35.50	–
Glucose (mmol.L^{-1})	Intervention	5.60 ± 1.50	5.40 ± 1.60	–0.20 ± 0.60	<0.05
	Control	5.50 ± 1.60	5.60 ± 1.70	+0.10 ± 0.50	–

Note: Significant improvements were observed in weight, BMI, waist circumference, body fat, visceral fat, LDL-C, total cholesterol, and glucose in the intervention group compared to the control group.

Figure 6A illustrates the changes in average body weight at baseline (M0), 3 m (M3) and 6 m (M6) for the two groups. The intervention group weight decreased from approximately 67.50 kg at the baseline to 66.80 kg at M3 and 66.10 kg at M6, while the control group weight was essentially unchanged (67.30 kg at M0, 67.20 kg at M3 and 67.10 kg at M6). Error bars represent SD.

A



B

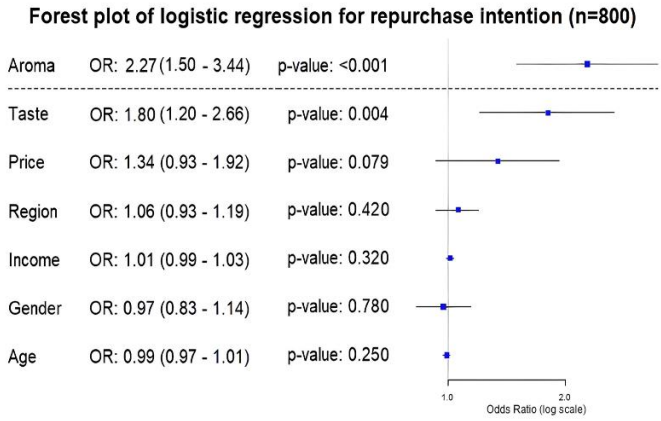


Figure 6. Clinical outcomes and consumer survey results: (A) Changes in average body weight (kg) of intervention and control groups over the 6-month study period, measured at baseline (M0), 3 months (M3), and 6 months (M6), illustrating modest but consistent weight reduction in the intervention group compared to the stable control group; (B) Forest plot illustrating logistic regression results for repurchase intention (n=800), with odds ratios (OR) and 95% confidence intervals (CI) for factors influencing consumer decisions. Significant positive influences are observed for aroma (OR = 2.27 [1.50–3.44], $p < 0.001$) and taste (OR = 1.80 [1.20–2.66], $p = 0.004$), while price (OR = 1.34 [0.93–1.92], $p = 0.079$) and demographic factors (age, gender, income, location) show no significant impact.

In summary, the "Nutrition Coffee Love World" intervention led to a modest but statistically significant increase in body weight (-1.40 kg) and waist circumference (-1.00 cm) in the intervention group, compared to the control group. Additionally, improvements in lipid profiles (particularly increases in LDL-C and total cholesterol) were observed. Although the weight loss was modest, these findings suggested that nutritional coffee supplementation might support weight management and lipid profile improvement. These clinical benefits could be attributed directly to the bioactive compounds in the nutritional coffee formulation. Specifically, chlorogenic acids from coffee enhance lipid and glucose metabolisms; thus, supporting increases in body weight and improvements in lipid profiles. Polyphenols and flavonoids derived from fermented lotus leaves and breadfruit leaves included potent antioxidative and anti-inflammatory effects, which contributed to decreased oxidative stress and inflammation associated with obesity and dyslipidemia. Additionally, saponins from *P. notoginseng* enhanced lipid metabolism through activation of lipase enzyme activity, increasing triglyceride hydrolysis and fat oxidation. Furthermore, cordycepin from *C. militaris* modulated lipid biosynthesis pathways, collectively supporting the significant metabolic improvements in this trial. Future studies should include longer-term trials (≥ 12 m), larger sample sizes, stricter monitoring of dietary and exercise adherence and further precise body composition measurements (e.g., DEXA or MRI) to better assess long-term efficacy. Additionally, future studies should incurporate intention-to-treat analysis approaches to provide unbiased estimates of intervention effectiveness and enhance generalizability. Systematic use of imputation methods for handling missing data is recommended to minimize bias from participant dropouts. Additionally, further studies should include detailed analyses of loss characteristics to better understand reasons for attrition, identify loss predictors and improve retention strategies; thus, ensuring further robust and reliable outcomes.

3.7. Consumer Survey Results

A market survey was carried out for 1,000 consumers aged 18–60 y, predominantly from urban areas (70%), with an average monthly income of 10–15 million Vietnamese Dong (approximately 400–600 USD). Participants were instructed to use "Nutrition Coffee Love World" for 3 m by mixing 15 g of powder with 120–150 ml of hot water per serving. From the participants, 20.00% (200/1000) did not complete the survey, primarily due to relocation or scheduling conflicts. Demographic characteristics, including age, gender, income and coffee consumption frequency, were analyzed and no significant differences were detected between losses and 800 respondents, who completed the survey ($p > 0.05$). Table 9 details the demographic profile of 800 respondents, who had an average age of 34.60 y ±8.20, with 52.00% of them were female, 70.00% residing in urban areas, an average monthly income of 12.70 ±4.00 million Vietnamese Dong and 85.00% held at least a technical or college-level education. The loss rate was consistent with similar market surveys [26].

Participants assessed the product using five-point Likert scale, with average ratings of 4.20 ±0.60 for aroma, 4.00 ±0.70 for taste and 3.70 ±0.80 for price. Approximately 65.00% (520/800) of respondents indicated a willingness to repurchase the product (coded as 1 = yes and 0 = no). A logistic regression analysis was carried out with repurchase intention as the dependent variable, controlling for age, gender, income and location. The model goodness-of-fit was verified using Hosmer–Lemeshow test (p = 0.21) and it demonstrated moderate explanatory power with a Nagelkerke R^2 of approximately 0.35 and an overall correct classification rate of 72.00%. Table 10 indicates that aroma (OR = 2.27, $p < 0.001$) was the strongest predictor of repurchase intention, followed by taste (OR = 1.80, p = 0.004). Price did not include statistical significance (OR = 1.34, p = 0.079) and demographic factors included no significant effects ($p > 0.05$). This suggested that consumers prioritized sensory attributes over pricing when deciding to repurchase.

Table 9. Demographic Profile of the Survey Respondents (n = 800). (Mean ± SD or Percentage, n = 800).

Parameter	Value
Age (years)	34.60 ± 8.20
Female (%)	52.00
Urban Area (%)	70.00
Monthly Income (million VND)	12.70 ± 4.00
Education ≥ Technical/College (%)	85.00
Dropout Rate (%)	20.00

Table 10. Logistic Regression Results for Repurchase Intention (n = 800) (OR: Odds Ratio; CI: 95% Confidence Interval).

Variable	Coefficient β ± SE	OR (95% CI)	p-value
Aroma	0.82 ± 0.22	2.27 (1.50–3.44)	<0.001
Taste	0.59 ± 0.21	1.80 (1.20–2.66)	0.004
Price	0.29 ± 0.18	1.34 (0.93–1.92)	0.079
Age	–0.01 ± 0.01	0.99 (0.97–1.01)	0.250
Gender	–0.03 ± 0.09	0.97 (0.83–1.14)	0.780
Income	0.01 ± 0.01	1.01 (0.99–1.03)	0.320
Location	0.06 ± 0.07	1.06 (0.93–1.19)	0.420

Figure 6B presents a forest plot summarizing the odds ratios for all variables. The confidence intervals for aroma and taste were entirely greater than 1, whereas those for price and demographic variables were 1. Additionally, while the analysis was based on 800 completers, the 20.00% loss rate suggested that future studies should consider using intention-to-treat analyses or imputation methods to address missing data. Understanding whether losses differed in their initial product perceptions or consumption habits could help refine future market segmentation. As the survey sample predominantly represents urban consumers, generalizability of the findings to rural populations might be limited. Future studies should aim to include a further geographically and socioeconomically diverse sample. Specifically, extending consumer acceptance surveys to rural areas significantly enhanced the robustness, generalizability and applicability of market insights derived from the present study. Carrying out comparative analyses between urban and rural consumer responses could provide valuable information for targeted marketing strategies and broader commercial viability

Overall, the logistic regression model incorporating age, gender, income and location yielded a Nagelkerke R^2 of 0.35, indicating moderate explanatory power. The findings clearly demonstrated that sensory attributes, particularly aroma and taste were significant determinants of repurchase intention. With 65% of respondents expressing willingness to repurchase "Nutrition Coffee Love World," the product showed significant commercial potentials. Further studies should expand the geographical scope, extend the use time and incorporate a further diverse consumer base to provide a further comprehensive market assessment. This study addressed the increasing public health issue of overweight and obesity in Vietnam by developing a novel multiple-component nutritional coffee formulation. The present formulation integrated fermented *N. nucifera* (lotus leaves), *A. altilis* (breadfruit leaves), lotus seeds, *P. notoginseng* (notoginseng flowers), *C. militaris* (caterpillar fungi), collagen and coffee. The study combined rigorous technical assessments—including *B. subtilis* fermentation and formulation optimization via a second-order mixture design—with practical uses such as a 6-m clinical trial and a comprehensive market survey.

The fermentation conditions optimized through BBD—35 °C, 65.00–70.00% RH and 72 h—not only improved key bioactive metrics such as polyphenol content and lipase activity but also demonstrated robustness within multiple runs, reinforcing reliability of the process for further scale-up. While these improvements are promising, it is important to acknowledge potential confounding factors such as batch-to-batch variability and fluctuations in environmental conditions during fermentation. Further studies should address these uncertainties by incorporating tighter process controls and additional replicates to further decrease variability. The mixture design model, with a robust fit (R^2 > 0.95, error < 5.00%), yielded an optimized formulation that achieved a sensory score of approximately 7.50/9. Despite the model high predictive accuracy, the relatively small number of replicates (n = 2–3 per formulation) might increase the risk of overfitting, particularly when additional bioactive components are considered. Furthermore, the sensory assessment was based on a panel of only 8–10 participants, which might limit the generalizability of consumer preferences in a product where taste is highly subjective. In the clinical trial (n = 127), the intervention group experienced a modest but statistically significant

weight increase of −1.40 kg, with improvements in LDL-C and total cholesterol.

However, this weight loss, representing nearly 2.00% of body weight, were less than the 5.00% threshold recommended by the American Diabetes Association (ADA) for clinically significant benefits [27]. This finding suggested that while the formulation showed potentials, its clinical effects might be limited under the current intervention time and monitoring conditions. Further trials should address longer times or further stringent controls of dietary intake and physical activity to potentially achieve greater weight loss. From a product stability and industrial perspective, the increase in production cost observed when scaling up from 1 to 100 kg suggested strong economic feasibility. Nonetheless, it must be assessed if the process parameters optimized at the pilot scale are held when production is further scaled (e.g. to 500 kg or further). Detailed analyses of enzyme stability during brewing and long-term shelf-life assessments are necessary to ensure that product quality is preserved at larger scales.

The consumer survey results, indicating a 65.00% repurchase intention, provided encouraging evidence of commercial potential. However, the survey sample predominantly represented urban consumers through convenience sampling and a loss rate of 20.00% was recorded. These factors limited the generalizability of the findings and suggested that further market assessments should include further geographically and socioeconomically diverse samples. Furthermore, while the present study focused on quantifying polyphenol, lipase and collagen levels, other important bioactive compounds such as saponins and chlorogenic acid were not assessed. Their omission might limit understanding of the full metabolic benefits of the formulation. Further studies should use advanced analytical methods such as LC-MS or HPLC to quantify these compounds and investigate potential synergistic interactions. In summary, the present interdisciplinary approach-which integrates microbiology, biochemistry, food technology, nutrition, clinical research and market analysis-provides a solid foundation for the development of a functional nutritional coffee products. Despite limitations linked to sample size, intervention time and the scope of bioactive analysis and improvements in polyphenol content, enzyme activity and lipid profile as well as the positive consumer response underscore the potential of this formulation. Further studies should focus on larger and longer-term clinical trials, enhanced process optimization and further comprehensive bioactive profiling to fully realize the product benefits and ensure its scalability in industrial production.

4. Conclusion

This study effectively created a multiple-nutrient coffee product that combined fermented lotus leaves, breadfruit leaves, lotus seeds, notoginseng flowers, *C. militaris*, hydrolyzed collagen and coffee. Stringent optimization procedures such as *B. subtilis* fermentation, mixture design, 6-m human trial and consumer questionnaire were verified with beneficial effects in weight loss, better lipid profiles and high consumer preferences. With weaknesses in sample size and trial time, its commercial viability is strongly suggested by its good metabolic effects, scalability and economic benefits. Large-scale validation and further fine-tuning are needed to investigate its potential as an extended metabolic health improvement solution. This study results are useful for scientists, providing valuable practical directives for food industries worldwide to design novel functional drinks for better metabolic health and weight control.

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6. Conflict of Interest

The authors report no conflict of interest.

7. Authors' Contributions

Conceptualization, HDT and HCN; methodology, HCN and HSD; software, BTN; validation, HCN, PPTH and HP; formal analysis, HCN and QDQ; investigation, HSD, PPTH and QTL; resources, HDT; data curation, HCN and TLL; writing—original draft preparation, HCN, PPTH and HSD; writing—review and editing, HDT and HP; visualization, QTL; supervision, HDT; project administration, HDT; funding acquisition, HCN.

8. Using Artificial Intelligent Chatbots

No AI chatbot has been used in this study.

9. Ethical Consideration

The authors declare no conflict of interest. Ethical approval no. 16/HDDDNCYSH dated June 20, 2024 was issued by the Biomedical Research Ethics Council of Military Hospital 7A.

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بهینه‌سازی و ارزیابی بالینی قهوه تغذیه‌ای حاوی برگ‌های تخمیر شده نیلوفر آبی و ترکیبات زیست فعال گیاهی منتخب

فان-فونگ-ترانگ هوینه[1]، هو-کونگ نگویین[2]، هونگ-سونگ دینه[3]، توآن-لوک لی[4]، کوئک-دانگ کوآن [5]، کوآنگ-تری لی [6]، با-توی نگویین[2]، هونگ فا[7]، تانه-کونگ نگویین [4]، مای-نگوک بویی[8]، هوانگ-دانگ ترن[1]

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چکیده

سابقه و هدف: چاقی مشکل بهداشت عمومی و رو به شیوع است لذا به مداخلات تغذیه‌ای عملی و علمی نیاز دارد. هدف این مطالعه فرموله کردن قهوه‌های فراسودمند[1] غنی شده با برگ‌های تخمیر شده نیلوفر آبی[2] با *باسیلوس سوبتیلیس* و به منظور افزایش غلظت پلی‌فنول و فعالیت آنزیم لیپاز است. اجزای اضافی شامل برگ‌های میوه نان[3]، دانه‌های نیلوفر آبی، نوعی گل[4] قارچ‌های کاترپیلار[5] و کلاژن بودند که به‌دلیل اثر بر عملکردهای متابولیک، حمایت‌های ایمنی، ویژگی‌های حسی و ملاحظات بازار انتخاب شدند.

مواد و روش ها: فرموله کردن چند جزء با استفاده از طراحی ترکیبی[6] ادغام شده با روش سطح پاسخ بهینه شد. اثربخشی از طریق یک کارآزمایی تصادفی کنترل شده m- ۶ شامل ۱۲۷ بزرگسال دارای اضافه وزن ارزیابی شد. در این کارآزمایی، برای اطمینان از قابلیت اطمینان و به حداقل رساندن سوگیری، از یک طرح کنترل شده با دارونما دوسوکور[7] استفاده شد. علاوه بر این، از ۸۰۰ شرکت کننده، یک نظرسنجی پذیرش مصرف‌کننده برای ارزیابی قصد خرید مجدد و درک محصول انجام شد.

یافته‌ها و نتیجه‌گیری: تخمیر (10^{7} CFU.g^{-1} ، ۳۵°C، رطوبت نسبی ٪ ۷۰/۰۰-۶۵/۰۰، ۷۲ ساعت) منجر به افزایش ۲/۵ برابری محتوای پلی‌فنول و دو برابر شدن فعالیت آنزیم لیپاز شد. در کارآزمایی بالینی، شرکت‌کنندگانی که قهوه تغذیه‌ای مصرف می‌کردند، میانگین افزایش وزنی ۱/۴۰کیلوگرم، کاهش کلسترول لیپوپروتئین با چگالی کم تقریباً ۱۰/۰۰ mg.dl^{-1} و افزایش کلسترول لیپوپروتئین با چگالی بالا را نزدیک به ۳/۰۰ mg.dl^{-1} داشتند. این بهبودها از نظر آماری معنی‌دار بود ($p<0/05$) و با عوارض جانبی جدی همراه نبود. نظرسنجی مصرف‌کننده نشان داد که ۶۵ درصد قصد خرید مجدد دارند که نشان‌دهنده پتانسیل امیدوارکننده بازار است. اگرچه این مطالعه شامل محدودیت‌هایی مانند حجم نمونه، خروج نمونه از جامعه ارزیابی طرح و زمان مداخله بود، یافته‌ها حاکی از مزایای متابولیکی و امکان‌سنجی صنعتی است. این مطالعه یک پایه محکم برای توسعه و تجاری سازی قهوه کاربردی با هدف مدیریت وزن و حمایت قلبی عروقی فراهم می کند. این یافته‌ها بینش‌های با ارزشی را برای محققان و صنایع در سراسر جهان که علاقه‌مند به توسعه نوشیدنی‌های کاربردی نوآورانه با هدف مدیریت چاقی و بهبود سلامت قلب و عروق هستند، ارائه می‌کند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] Functional
[2] *Nelumbo nucifera*
[3] *Artocarpus altilis*
[4] notoginseng (*Panax notoginseng*)،
[5] *Cordyceps militaris*
[6] Mixture design
[7] Double-blind placebo-controlled design