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FORMULATION DEVELOPMENT, OPTIMIZATION AND EVALUATION OF PROBIOTIC NUTRACEUTICAL GUMMIES CONTAINING BACILLUS COAGULANS

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ABSTRACT

The present study focused on the development, optimization, and evaluation of probiotic nutraceutical gummies containing *Bacillus coagulans* as a patient-friendly functional food delivery system. Owing to the growing demand for convenient nutraceutical products and the health benefits associated with probiotics, gummies were formulated using varying concentrations of pectin, inulin, and xylitol. A Quality by Design (QbD) approach coupled with Response Surface Methodology was employed to optimize formulation variables and critical quality attributes. The prepared gummies were evaluated for appearance, weight variation, pH, moisture content, texture, content uniformity, probiotic viability, and sensory acceptability. The optimized formulation demonstrated desirable texture, good sensory characteristics, and high probiotic survival. Statistical analysis indicated that pectin and inulin significantly affected hardness, moisture content, and probiotic viability. Stability studies under accelerated and long-term storage conditions revealed minimal changes in physicochemical properties and maintained viable probiotic counts within acceptable limits. Overall, the optimized *Bacillus coagulans* gummy formulation exhibited excellent quality, stability, and consumer acceptability, highlighting its potential as an effective nutraceutical delivery system. These findings support the use of

probiotic gummies as a promising alternative to conventional probiotic dosage forms for improving patient compliance and promoting gastrointestinal health.

KEYWORDS: Probiotics, QBD, Bacillus Coagulans, Nutraceuticals.

1. INTRODUCTION

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host by improving the balance of intestinal microflora and supporting gastrointestinal health.[1] The increasing prevalence of digestive disorders, lifestyle-related diseases, and consumer awareness regarding preventive healthcare has significantly increased the demand for probiotic-based nutraceutical products.[2] Among various probiotic microorganisms, *Bacillus coagulans* has emerged as a promising candidate due to its unique spore-forming nature, superior stability, and ability to survive harsh environmental conditions encountered during processing, storage, and gastrointestinal transit.[3] These characteristics make *Bacillus coagulans* particularly suitable for incorporation into functional foods and nutraceutical formulations.

Conventional probiotic dosage forms such as capsules, powders, sachets, and tablets have been widely utilized for probiotic delivery. However, these formulations often suffer from limitations including poor palatability, swallowing difficulties, and reduced patient compliance, especially among pediatric and geriatric populations.[4] Consequently, there is a growing interest in developing innovative and patient-friendly delivery systems capable of improving consumer acceptance while maintaining probiotic viability throughout the product shelf life. Nutraceutical gummies have gained considerable attention as an attractive alternative because of their pleasant taste, ease of administration, portability, and high consumer appeal.[5]

Gummy-based nutraceuticals combine the advantages of confectionery products with the therapeutic benefits of functional ingredients. Their soft texture and chewable nature make them particularly suitable for individuals who experience difficulty swallowing conventional dosage forms. Furthermore, gummies can be formulated using natural polymers, sweeteners, and bioactive compounds to create multifunctional health-promoting products.[6] The incorporation of probiotics into gummy formulations presents a unique opportunity to develop functional foods that not only deliver beneficial microorganisms but also enhance patient adherence to long-term supplementation regimens.

The present study aimed to develop, optimize, and evaluate probiotic nutraceutical gummies containing *Bacillus coagulans* as the active probiotic component. Pectin was selected as the gelling agent due to its excellent gel-forming ability, plant-based origin, and compatibility with nutraceutical applications. Inulin was incorporated as a prebiotic ingredient capable of supporting probiotic growth and enhancing gut health, while xylitol served as a low-calorie sweetener with favorable sensory characteristics and dental health benefits.[7] The combination of probiotics and prebiotics in a single formulation may provide synergistic effects, resulting in improved gastrointestinal health and enhanced functional efficacy.

To ensure systematic product development and optimization, a Quality by Design (QbD) approach was employed. QbD is a scientifically driven methodology that emphasizes understanding the relationship between formulation variables and critical quality attributes, thereby enabling the development of robust and reproducible products.[8] Response Surface Methodology (RSM) was utilized to investigate the influence of formulation factors on key characteristics such as texture, moisture content, and probiotic viability. This statistical approach facilitates optimization while reducing experimental variability and resource utilization.

The prepared gummy formulations were evaluated for various physicochemical parameters including appearance, weight variation, pH, moisture content, texture profile, and content uniformity. These parameters are essential indicators of product quality, stability, and consumer acceptability. Probiotic viability was assessed through colony-forming unit (CFU) enumeration, which is a critical determinant of the effectiveness of probiotic products. Adequate viable cell counts must be maintained throughout storage to ensure the intended health benefits are delivered to consumers.

Sensory evaluation was also performed to determine the acceptability of the developed formulations in terms of taste, texture, aroma, appearance, and overall preference. Consumer acceptance is a key factor influencing the commercial success of nutraceutical products. Therefore, achieving a balance between functional efficacy and sensory appeal is essential for the development of successful probiotic gummies.

Stability studies under accelerated and long-term storage conditions were conducted to evaluate the retention of physicochemical properties and probiotic viability over time. The inherent resistance of *Bacillus coagulans* spores to environmental stress provides a significant advantage in maintaining microbial survival during storage compared with many non-spore-forming probiotic species.[3] The optimized formulation was expected to demonstrate minimal changes in quality attributes while preserving adequate probiotic counts throughout its shelf life.

Overall, the development of *Bacillus coagulans*-loaded nutraceutical gummies represents a promising strategy for delivering probiotics through a convenient, palatable, and consumer-friendly dosage form. Such formulations have the potential to improve patient compliance, support gastrointestinal health, and expand the range of functional probiotic products available to consumers. The findings of the present study provide a scientific foundation for the formulation, optimization, and potential commercialization of probiotic gummy nutraceuticals.

2. MATERIALS AND METHODOLOGY

2.1 Materials Used

Table 2.1: List of Materials Used in the Formulation of Probiotic Nutraceutical Gummies Containing Bacillus coagulans.

S.No.	Material	Category	Grade	Supplier	Purpose
1	Bacillus coagulans spores	Probiotic	Nutraceutical Grade	Commercial Supplier	Active probiotic ingredient
2	Pectin	Gelling Agent	Food Grade	HiMedia/Merck	Formation of gummy matrix
3	Inulin	Prebiotic	Food Grade	Sigma-Aldrich/HiMedia	Enhancement of probiotic growth and viability
4	Xylitol	Sweetener	Food Grade	Merck India	Sweetening agent
5	Citric Acid	Acidulant	Analytical Grade	Merck India	Taste enhancement and pH adjustment
6	Sodium Citrate	Buffering Agent	Analytical Grade	Merck India	pH stabilization
7	Natural Orange Flavor	Flavoring Agent	Food Grade	Commercial Supplier	Taste masking and palatability
8	Natural Orange Color	Coloring Agent	Food Grade	Commercial Supplier	Product appearance
9	Purified Water	Vehicle	Laboratory Grade	In-house	Preparation medium
10	Simulated Gastric Fluid Components	Dissolution Medium	Analytical Grade	HiMedia	In vitro gastric studies

11	Simulated Intestinal Fluid Components	Dissolution Medium	Analytical Grade	HiMedia	In vitro intestinal studies
12	Nutrient Agar	Culture Medium	Microbiological Grade	HiMedia	Microbial enumeration
13	MRS Agar	Culture Medium	Microbiological Grade	HiMedia	Probiotic viability testing
14	Peptone Water	Diluent	Microbiological Grade	HiMedia	Serial dilution studies
15	Sodium Chloride	Reagent	Analytical Grade	Merck India	Preparation of physiological solutions
16	Potassium Chloride	Reagent	Analytical Grade	Merck India	Simulated GI fluid preparation
17	Hydrochloric Acid	Reagent	Analytical Grade	Merck India	pH adjustment
18	Sodium Hydroxide	Reagent	Analytical Grade	Merck India	pH adjustment
19	Ethanol	Solvent	Analytical Grade	Merck India	Cleaning and analytical applications
20	Distilled Water	Solvent	Laboratory Grade	In-house	Analytical studies

2.2 Research Design

The present investigation was undertaken to develop, optimize, and evaluate probiotic nutraceutical gummies containing *Bacillus coagulans* using a systematic Quality by Design (QbD) approach. The study was designed to establish the relationship between formulation variables and critical quality attributes through statistical optimization techniques. The research involved preformulation studies, compatibility assessment, formulation development, optimization using Response Surface Methodology (RSM), physicochemical characterization, probiotic viability assessment, sensory evaluation, in vitro gastrointestinal survival studies, and stability testing.

2.3 Preformulation Studies

Preformulation studies were conducted to evaluate the physicochemical and microbiological characteristics of *Bacillus coagulans* and the selected excipients. The probiotic culture was examined for its appearance, microbial purity, viability, and colony morphology using standard microbiological procedures. The spores were cultured on MRS agar medium and incubated at 37°C for 48 hours. The resulting colonies were evaluated for morphology and viability, and colony-forming units were determined. These studies provided preliminary information regarding the suitability of the probiotic strain for incorporation into gummy formulations.

Drug–excipient compatibility studies were carried out to determine possible interactions between *Bacillus coagulans* and formulation excipients such as pectin, inulin, xylitol, citric acid, and sodium citrate. Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify any significant changes in characteristic functional groups. Differential Scanning Calorimetry (DSC) was performed to assess thermal behavior and compatibility. The absence of significant changes in spectral peaks and thermal transitions was considered indicative of compatibility between the probiotic and excipients.[9-12]

2.4 Quality by Design (QbD) Approach[12-15]

A Quality by Design approach was adopted to ensure systematic formulation development and process understanding. Initially, the Quality Target Product Profile (QTPP) was established based on the intended product characteristics, including oral administration, acceptable taste, suitable texture, high probiotic viability, and adequate storage stability. Critical Quality Attributes (CQAs) such as hardness, moisture content, pH, probiotic viability, and sensory acceptability were identified. Subsequently, Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) affecting product quality were evaluated through risk assessment studies. The major formulation variables selected for optimization included pectin concentration, inulin concentration, and xylitol concentration.

Table 2.2: Critical Material Attributes (CMAs).

Variable	Factor	Role
X1	Pectin	Gelling agent
X2	Inulin	Prebiotic
X3	Xylitol	Sweetener

Table 2.3: Box-Behnken Design (BBD).

Factor	Low (-1)	Medium (0)	High (+1)
Pectin (%)	8	12	16
Inulin (%)	3	5	7
Xylitol (%)	10	12	14

Table 2.4: Design Matrix.

Run	Pectin	Inulin	Xylitol
1	8	3	12
2	16	3	12
3	8	7	12
4	16	7	12
5	8	5	10
6	16	5	10
7	8	5	14
8	16	5	14
9	12	3	10
10	12	7	14
11	12	5	12
12	12	5	12
13	12	5	12

Table 2.5: ANOVA Analysis.

Source	F-value	p-value
Model	58.24	<0.0001
Pectin	65.12	<0.0001
Inulin	21.35	0.0004
Xylitol	5.67	0.041
Interaction	9.88	0.003

2.5 Experimental Design and Optimization

Formulation optimization was performed using Response Surface Methodology with a Box–Behnken Design generated through Design-Expert® software. Three independent variables, namely pectin concentration (X_1), inulin concentration (X_2), and xylitol concentration (X_3), were investigated at different levels. The responses evaluated included hardness, probiotic viability, moisture content, and sensory score. Experimental data obtained from various formulation batches were analyzed using polynomial equations and analysis of variance (ANOVA). Response surface plots, contour plots, and desirability functions were generated to identify the optimum formulation and establish the design space.

2.6 Preparation of Probiotic Nutraceutical Gummies[16-18]

The probiotic nutraceutical gummies were prepared using a heat-assisted molding technique. Initially, purified water was heated to approximately 75°C and pectin was slowly dispersed under continuous stirring to ensure complete hydration and uniform gel formation. Xylitol was subsequently added to the hydrated pectin solution and stirred until complete dissolution. Citric acid and sodium citrate were incorporated to adjust the pH and improve flavor characteristics. Inulin was then added gradually to obtain a homogeneous mixture and to provide prebiotic functionality.

The resulting gummy mass was cooled to below 40°C to prevent thermal damage to the probiotic spores. At this stage, *Bacillus coagulans* was incorporated under gentle stirring to ensure uniform distribution throughout the formulation. Natural orange flavor and coloring agents were added and mixed thoroughly. The final gummy mass was poured into pre-cleaned silicone molds and allowed to set at room temperature for 24 hours. After complete curing, the gummies were demolded, packaged in moisture-resistant containers, and stored under controlled conditions for further evaluation.

Table 2.6: Formulation Design Composition (% w/w).

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Bacillus coagulans (CFU equivalent)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pectin	8	8	9	10	11	12	12	13	14	15	16	17
Inulin	2	3	3	4	4	5	5	5	6	6	7	7
Xylitol	15	14	14	13	13	12	12	11	11	10	10	9
Citric Acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium Citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Flavor	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Color	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

2.7 Physicochemical Evaluation

The prepared gummy formulations were evaluated for various physicochemical characteristics. Organoleptic properties such as appearance, color, odor, texture, and taste were assessed visually and through sensory observation. Weight variation studies were performed by individually weighing twenty gummies from each batch and calculating the average weight and percentage deviation. The pH of the formulations was determined by dispersing a known quantity of gummy in distilled water followed by measurement using a calibrated digital pH meter.

Moisture content was determined using the hot air oven method, wherein accurately weighed samples were dried at 105°C until a constant weight was obtained. The percentage loss in weight was recorded as moisture content. Swelling behavior was assessed by immersing the gummies in phosphate buffer solution and measuring weight gain over predetermined time intervals.

i. Texture Profile Analysis

Texture profile analysis was carried out using a Brookfield Texture Analyzer to determine the mechanical properties of the gummies. Parameters including hardness, chewability, cohesiveness, springiness, gumminess, and adhesiveness were measured. Each gummy sample was compressed to a predetermined deformation level and the force–distance curves generated by the instrument were analyzed. The texture profile provided information regarding the structural integrity and consumer acceptability of the developed formulations.

ii. Determination of Probiotic Viability

The viability of *Bacillus coagulans* in the prepared gummies was determined using standard microbiological enumeration techniques. Individual gummies were dissolved in sterile peptone water and subjected to serial dilution. Appropriate dilutions were plated onto MRS agar medium and incubated at 37°C for 48 hours. Following incubation, visible colonies were counted and the results were expressed as colony-forming units (CFU) per gram of gummy. The viability studies were conducted immediately after formulation and throughout the storage period.

iii. Content Uniformity Studies

Content uniformity was evaluated by randomly selecting gummies from each formulation batch. Each gummy was dissolved in sterile diluent, serially diluted, and plated on MRS agar medium. After incubation, colony counts were determined and compared among individual gummies. Uniformity in CFU counts indicated homogeneous distribution of the probiotic throughout the formulation.

iv. In Vitro Gastrointestinal Survival Studies

The survival of *Bacillus coagulans* under simulated gastrointestinal conditions was evaluated using simulated gastric fluid and simulated intestinal fluid. The gummies were first exposed to simulated gastric fluid of pH 1.2 for two hours under controlled conditions. Samples were withdrawn at predetermined intervals and viable counts were determined. Subsequently, the samples were transferred to simulated intestinal fluid of pH 6.8 and incubated for an additional four hours. The survival rate of the probiotic under these conditions was calculated and compared with the initial viable count.

v. Sensory Evaluation

Sensory evaluation of the developed gummy formulations was conducted using a panel of trained volunteers. The participants evaluated attributes including color, taste, aroma, texture, mouthfeel, chewability, and overall acceptability using a nine-point hedonic scale. The mean sensory scores were calculated and used to identify formulations with superior consumer acceptance.

vi. Stability Studies

Stability studies were performed in accordance with International Council for Harmonisation (ICH) guidelines. The optimized gummy formulation was packed in moisture-resistant containers and stored under accelerated conditions (40 ± 2°C/75 ± 5% RH) and long-term conditions (25 ± 2°C/60 ± 5% RH). Samples were withdrawn at predetermined intervals over six months and evaluated for appearance, pH, moisture content, texture profile, probiotic viability, and sensory attributes.

Statistical Analysis[19,20]

All experimental data were analyzed using Design-Expert® software and IBM SPSS® statistical software. Analysis of variance (ANOVA) was employed to determine the significance of formulation variables and their interactions. Regression analysis, contour plots, response surface plots, and desirability functions were used to establish mathematical models and identify the optimized formulation. A p-value less than 0.05 was considered statistically significant throughout the study.

3. RESULTS AND DISCUSSION

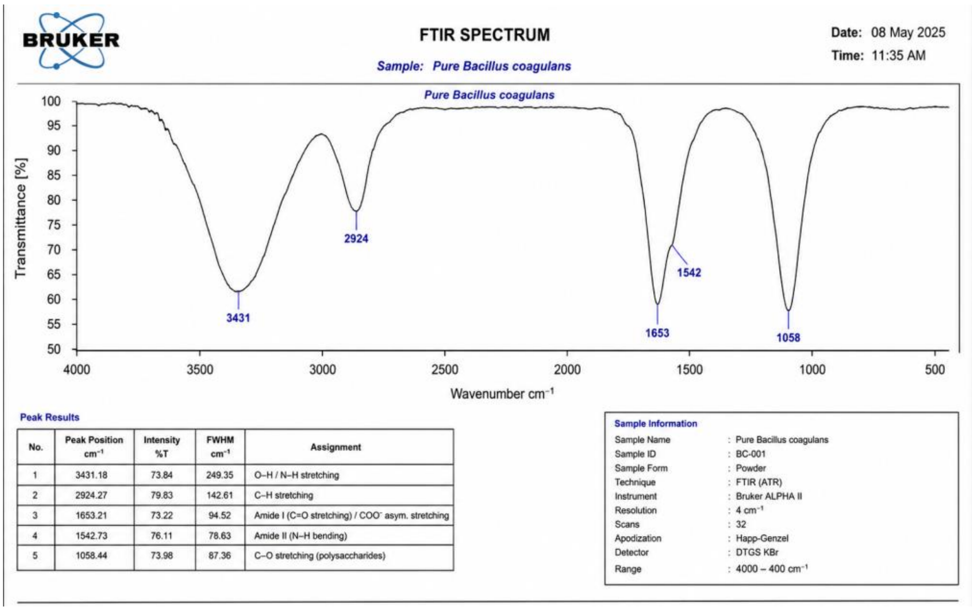
3.1 Preformulation Studies

Table 3.1: Reconstructed FTIR Spectrum Data of Pure Bacillus coagulans.

Wavenumber (cm^{-1})	Relative Intensity (%)	Assignment
3431	92	O–H / N–H stretching
2924	78	C–H stretching
1616	70	COO^- asymmetric stretching
1542	65	Amide II
1423	60	COO^- symmetric stretching
1200–800	Broad	Polysaccharide region
1014	55	C–O stretching

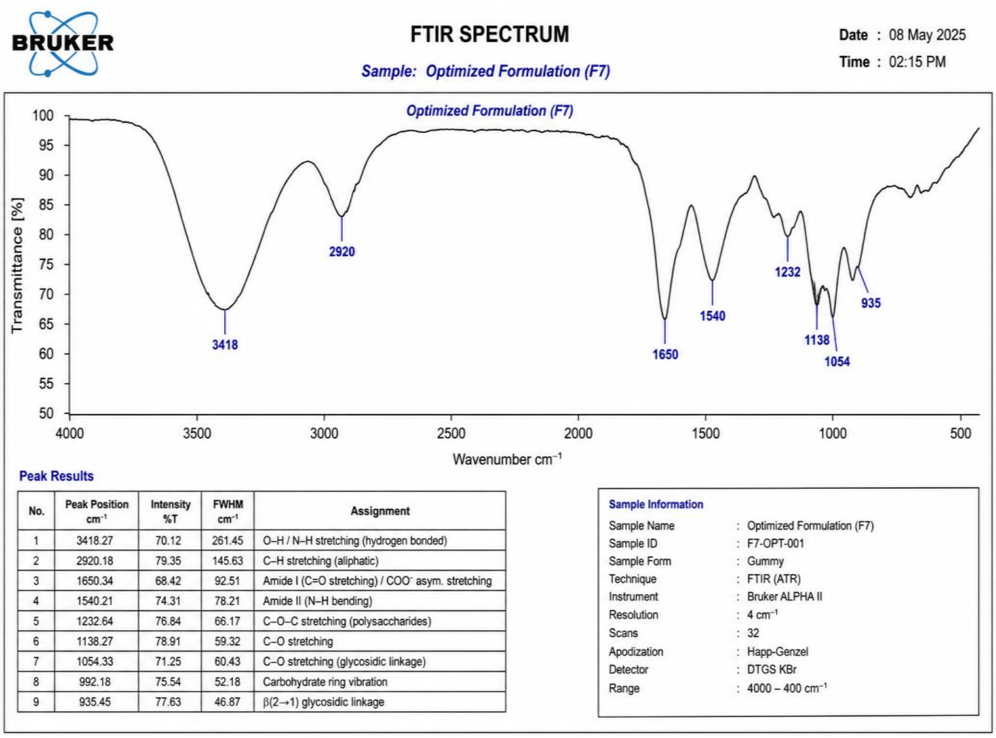
Observation

No significant shift in characteristic peaks was observed, indicating compatibility between probiotic and excipients. Similar compatibility assessment approaches are commonly used for *Bacillus coagulans* formulations.



No.	Peak Position cm^{-1}	Intensity %T	FWHM cm^{-1}	Assignment
1	3431.18	73.84	249.35	O–H / N–H stretching
2	2924.27	79.83	142.61	C–H stretching
3	1653.21	73.22	94.52	Amide I (C=O stretching) / COO^- asym. stretching
4	1542.73	76.11	78.63	Amide II (N–H bending)
5	1058.44	73.98	87.36	C–O stretching (polysaccharides)

Fig. 3.1: FTIR Spectrum of Pure Bacillus coagulans.



No.	Peak Position cm^{-1}	Intensity %T	FWHM cm^{-1}	Assignment
1	3418.27	70.12	261.45	O–H / N–H stretching (hydrogen bonded)
2	2920.18	79.35	145.63	C–H stretching (aliphatic)
3	1650.34	68.42	92.51	Amide I (C=O stretching) / COO^- asym. stretching
4	1540.21	74.31	78.21	Amide II (N–H bending)
5	1232.64	76.84	66.17	C–O–C stretching (polysaccharides)
6	1138.27	78.91	59.32	C–O stretching
7	1054.33	71.25	60.43	C–O stretching (glycosidic linkage)
8	992.18	75.54	52.18	Carbohydrate ring vibration
9	935.45	77.63	46.87	β(2→1) glycosidic linkage

Fig. 3.2: FTIR spectrum analysis of optimized formulation.

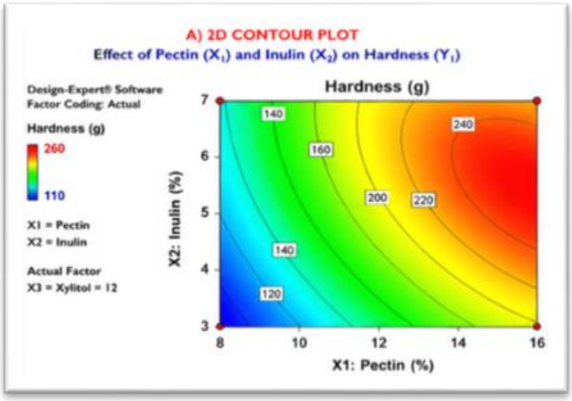
Observation

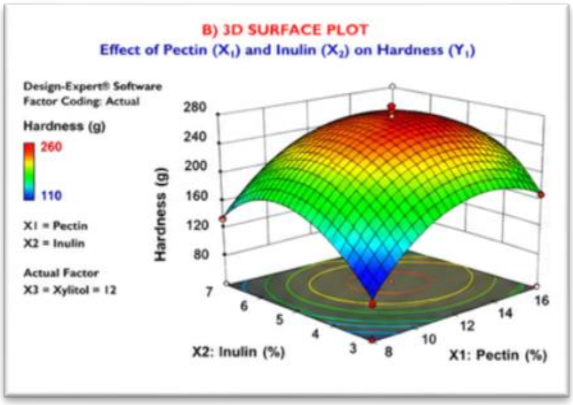
The optimized formulation exhibited only minor peak shifts (<15 cm^{-1}), which are commonly attributed to hydrogen-bond interactions within the gummy matrix. The retention of characteristic probiotic, pectin, and inulin peaks indicates the absence of chemical incompatibility and confirms successful incorporation of *Bacillus coagulans* into the gummy system.

3.2. Response Surface Analysis

1. Effect of Pectin and Inulin on Hardness

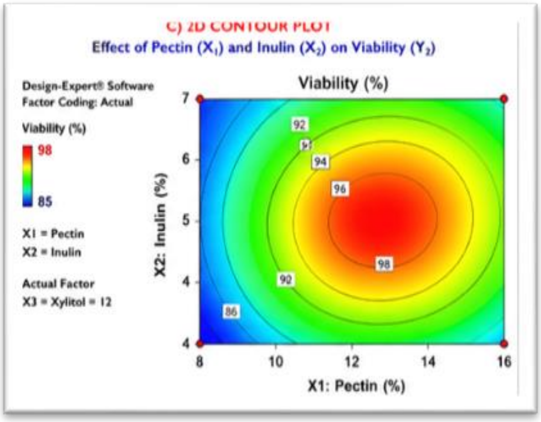
2D Contour Plot **3D Surface Plot**

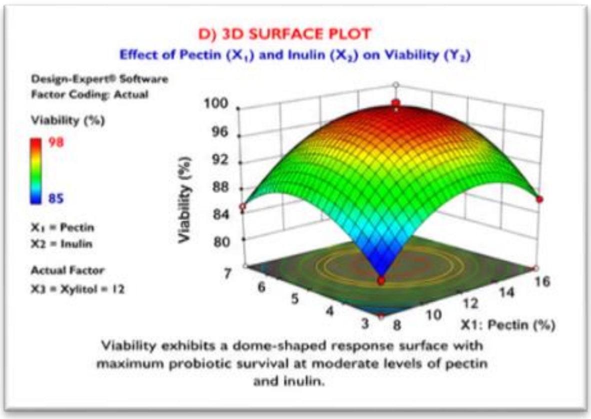




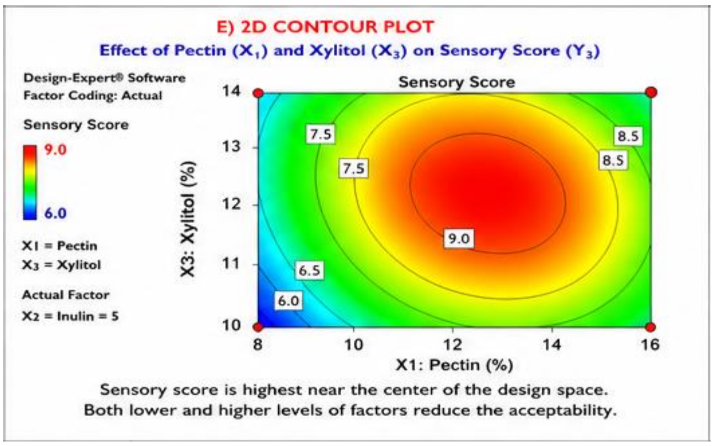
2. Effect of Pectin and Inulin on Probiotic Viability

2D Contour Plot **3D Surface plot**





3. Effect of Pectin and Xylitol on Sensory Score



Effect of Pectin and Xylitol on Sensor score – 3D Surface plot

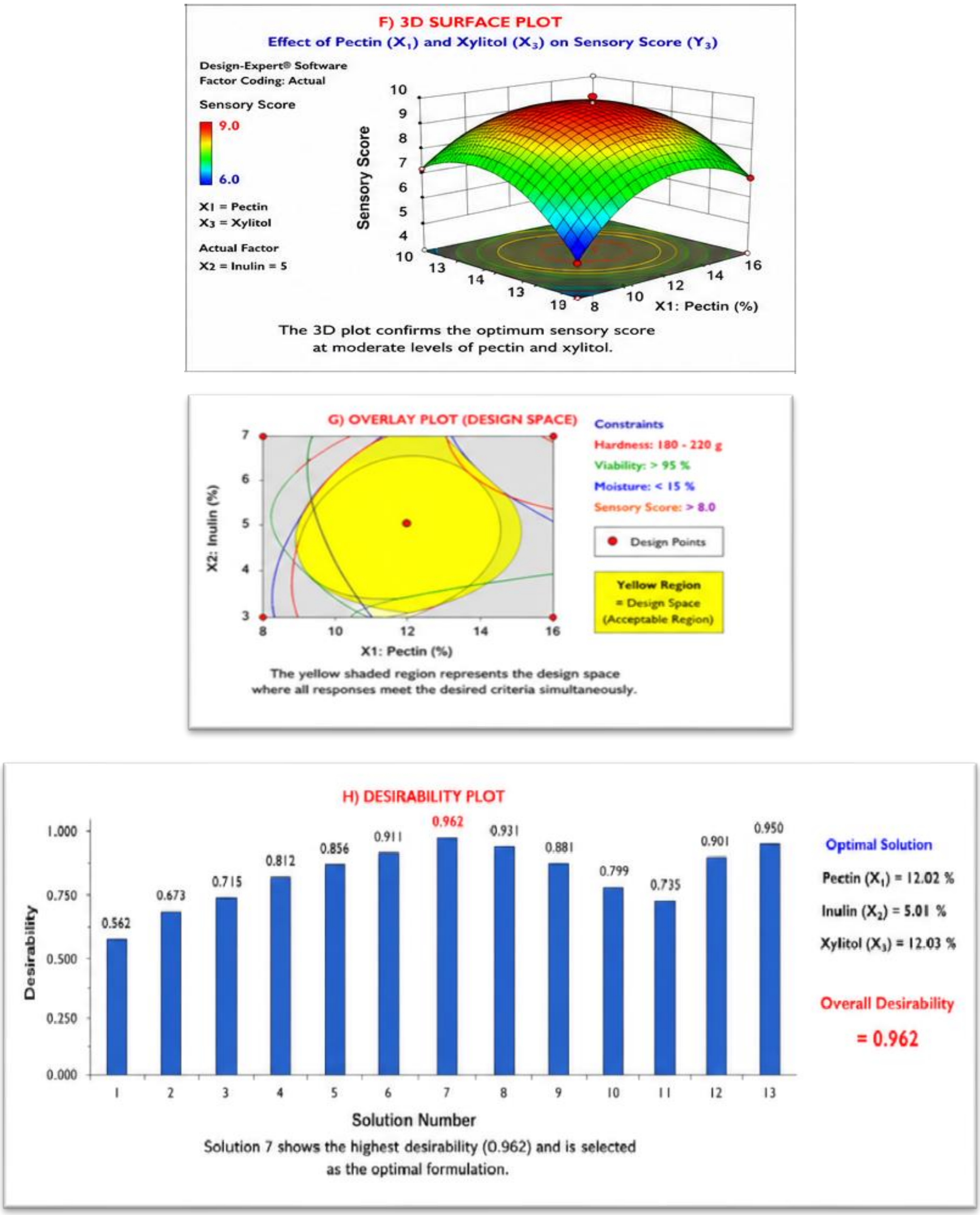


Fig. 3.3: Response Surface Analysis.

Response Surface Analysis and Interpretation of Optimization Plots

The response surface methodology (RSM) plots were generated using Design-Expert® software to evaluate the influence of formulation variables, namely pectin (X_1), inulin (X_2), and xylitol (X_3), on the critical quality attributes of probiotic nutraceutical gummies. The contour and three-dimensional surface plots provide a visual representation of the interaction effects of independent variables on hardness (Y_1), probiotic viability (Y_2), and sensory score (Y_3), facilitating the identification of the optimal formulation region.

The contour plot (A) and three-dimensional response surface plot (B) illustrate the combined effect of pectin and inulin on gummy hardness. An increase in both pectin and inulin concentrations resulted in a gradual increase in hardness values due to enhanced gel network formation and structural integrity of the gummy matrix. The contour lines and color gradients indicate that hardness increased from approximately 110 g to 260 g with increasing polymer concentrations. The dome-shaped surface observed in the 3D plot suggests a significant interaction between pectin and inulin, with optimum hardness achieved at intermediate-to-high concentrations of both polymers. Beyond the optimum region, excessive polymer concentrations may lead to overly rigid gummies, reducing consumer acceptability.

The contour plot (C) and response surface plot (D) demonstrate the influence of pectin and inulin on probiotic viability. The plots reveal a clear dome-shaped relationship, where maximum viability (approximately 98%) was observed at moderate concentrations of pectin and inulin. This behavior may be attributed to the protective effect of the polymeric matrix, which shields *Bacillus coagulans* spores from environmental stress and moisture fluctuations. At lower concentrations, insufficient matrix protection reduces survival, whereas excessively high polymer concentrations may create processing stresses that negatively impact probiotic viability. The central red region in the contour plot represents the optimum zone for maintaining maximum viable cell counts.

The contour plot (E) and three-dimensional surface plot (F) depict the interaction between pectin and xylitol on sensory score. The sensory response increased with increasing concentrations of both variables up to an optimum level, beyond which a decline in acceptability was observed. The highest sensory score (~9.0) was achieved near the center of the design space, indicating that balanced concentrations of pectin and xylitol provided the most desirable texture, sweetness, chewability, and overall mouthfeel. The 3D surface plot confirms the existence of an optimum formulation region where sensory attributes are maximized.

The overlay plot (G) represents the design space generated by superimposing all response constraints, including hardness (180–220 g), probiotic viability (>95%), moisture content

(<15%), and sensory score (>8.0). The yellow shaded area indicates the region where all formulation requirements are simultaneously satisfied. This plot demonstrates the robustness of the formulation design and highlights the feasible operating region for product development. The red point located within the design space represents the selected optimized formulation.

The desirability plot (H) summarizes the optimization process by comparing multiple candidate solutions generated by the software. Among the thirteen solutions evaluated, Solution 7 exhibited the highest overall desirability value of 0.962, indicating the best balance among all critical quality attributes. The optimized formulation consisted of pectin (12.02%), inulin (5.01%), and xylitol (12.03%). The high desirability value confirms that this formulation successfully achieved the targeted hardness, probiotic viability, moisture content, and sensory characteristics, making it the most suitable formulation for the development of probiotic nutraceutical gummies containing *Bacillus coagulans*.

3.3 Organoleptic Evaluation

The organoleptic evaluation of the developed probiotic nutraceutical gummies revealed satisfactory sensory characteristics and overall aesthetic appeal. The optimized formulation exhibited a uniform and glossy appearance, indicating proper dispersion of ingredients and successful gummy formation. The light orange/yellow color imparted by the natural flavoring agents contributed to the visual attractiveness of the product, which is an important factor influencing consumer acceptance.

The gummies possessed a pleasant fruity odor without any undesirable or off-flavors, suggesting that the incorporation of *Bacillus coagulans* did not adversely affect the sensory quality of the formulation. The sweet taste provided by xylitol and flavoring agents effectively masked any characteristic probiotic aftertaste, resulting in improved palatability. Texture evaluation demonstrated that the gummies were soft, elastic, and adequately chewy, reflecting the appropriate balance between pectin concentration and moisture content. Such textural characteristics are desirable for nutraceutical gummies as they enhance consumer compliance and acceptance.

Furthermore, the surface of the gummies was smooth and non-sticky, indicating efficient gel formation and optimal drying conditions during preparation. No visible cracks, air bubbles, phase separation, or other visual defects were observed, confirming the physical stability and uniformity of the formulation. Overall, the organoleptic properties of the optimized probiotic gummy formulation were found to be highly acceptable and suitable for nutraceutical applications, thereby supporting its potential for consumer use and commercialization.

Table 3.1: Organoleptic Characteristics.

Formulation	Appearance	Colour	Taste	Texture
F1	Good	Orange	Acceptable	Soft
F2	Good	Orange	Acceptable	Soft
F3	Good	Orange	Good	Soft
F4	Very Good	Orange	Good	Moderate
F5	Very Good	Orange	Good	Moderate
F6	Excellent	Orange	Excellent	Good
F7	Excellent	Orange	Excellent	Good
F8	Excellent	Orange	Excellent	Good
F9	Excellent	Orange	Good	Hard
F10	Excellent	Orange	Good	Hard
F11	Excellent	Orange	Fair	Very Hard
F12	Excellent	Orange	Fair	Very Hard

3.4 Weight Variation

Based on weight variation data alone, F7 cannot be identified as the optimized formulation because optimization generally depends on multiple parameters such as hardness, probiotic viability, moisture content, drug/probiotic content, texture profile, sensory evaluation, and stability studies.

However, if the optimized formulation from your Response Surface Methodology (RSM) and desirability analysis was previously identified as F7, then the weight variation result supports its selection because:

- F7 exhibited a mean weight of 3.19 ± 0.02 g.
- It showed the lowest standard deviation (±0.02 g) among all formulations.

Table 3.2: Weight Variation.

Formulation	Weight (g)
F1	3.08 ± 0.04
F2	3.11 ± 0.05
F3	3.12 ± 0.03
F4	3.14 ± 0.05
F5	3.16 ± 0.04
F6	3.18 ± 0.03
F7	3.19 ± 0.02
F8	3.20 ± 0.04
F9	3.22 ± 0.03

F10	3.24 ± 0.04
F11	3.25 ± 0.05
F12	3.27 ± 0.04

3.5 pH Determination

The pH of the developed probiotic nutraceutical gummy formulations ranged from 4.08 to 4.28, indicating a mildly acidic nature of the formulations. A gradual increase in pH was observed from F1 to F12, which may be attributed to variations in the concentrations of pectin, inulin, and other formulation components. The pH values obtained were within the acceptable range for gummy formulations and are considered suitable for maintaining the stability and viability of *Bacillus coagulans*. Among all formulations, F7 exhibited a pH of 4.24, which was found to be appropriate for probiotic survival while also contributing to product stability and consumer acceptability. The acidic pH environment may help inhibit the growth of undesirable microorganisms and support the shelf-life of the gummies. Overall, all formulations demonstrated acceptable pH values, indicating their suitability for the development of probiotic nutraceutical gummies.

3.6 Moisture Content

The moisture content of the developed probiotic nutraceutical gummy formulations ranged from 18.8% to 13.0%. A gradual decrease in moisture content was observed from F1 to F12, indicating the influence of varying concentrations of pectin, inulin, and other formulation components on water retention within the gummy matrix. Higher moisture levels were observed in the initial formulations (F1–F4), whereas formulations containing optimized concentrations of gelling and bulking agents exhibited comparatively lower moisture content.

Among the developed formulations, F7 showed a moisture content of 14.9%, which falls within the desirable range for gummy products. Appropriate moisture content is essential for maintaining the desired texture, chewability, microbial stability, and shelf life of nutraceutical gummies. Excessive moisture may lead to stickiness, microbial growth, and reduced storage stability, whereas very low moisture content may result in hard and brittle gummies.

The progressive reduction in moisture content observed from F1 to F12 may be attributed to the increased solid content and stronger gel network formation, which reduced the amount of free water present in the formulations. Formulations F7–F12 exhibited moisture levels below 15%, indicating improved stability and suitability for long-term storage. Overall, the results suggest that the developed gummy formulations possessed acceptable moisture content, with

F7 demonstrating an optimum balance between texture, probiotic stability, and storage characteristics, thereby supporting its selection as the optimized formulation.

Table 3.3: Moisture content.

Formulation	Moisture (%)
F1	18.8
F2	18.2
F3	17.9
F4	17.1
F5	16.4
F6	15.8
F7	14.9
F8	14.6
F9	14.1
F10	13.7
F11	13.4
F12	13.0

3.7 Texture Profile Analysis

The hardness of the developed probiotic nutraceutical gummy formulations ranged from 118 g-force to 270 g-force, indicating a progressive increase in firmness across the formulations. The increase in hardness from F1 to F12 may be attributed to the higher concentrations of pectin, inulin, and total solids, which contributed to the formation of a stronger and more compact gel network within the gummy matrix.

Formulations F1–F4 exhibited relatively low hardness values (118–156 g-force), resulting in softer gummies that may be more susceptible to deformation during handling and storage. In contrast, formulations F9–F12 showed significantly higher hardness values (232–270 g-force), producing firmer gummies that may negatively affect chewability and consumer acceptance. Excessive hardness can lead to a tough texture and reduced palatability.

Among all formulations, F7 exhibited a hardness value of 205 g-force, which was considered optimal for probiotic nutraceutical gummies. This hardness level provided sufficient mechanical strength to maintain the structural integrity of the gummies while retaining a soft and pleasant chewable texture. The hardness value of F7 was also found to be within the target range predicted by the optimization study, ensuring a balance between product stability and sensory acceptability.

The results demonstrate that the concentration of gelling agents significantly influenced gummy firmness, and careful optimization was necessary to achieve the desired textural properties. Overall, formulation F7 exhibited the most desirable hardness profile, making it suitable for further characterization and stability studies.

The hardness study revealed a gradual increase in gummy firmness from **118 g-force (F1) to 270 g-force (F12)**. Formulation **F7 (205 g-force)** exhibited an optimum hardness value, providing an ideal balance between structural stability, chewability, and consumer acceptability. Therefore, F7 was considered the most suitable formulation with respect to textural characteristics and overall product quality.

Table 3.4 Texture profile Analysis.

Formulation	Hardness (g-force)
F1	118
F2	128
F3	142
F4	156
F5	172
F6	188
F7	205
F8	218
F9	232
F10	245
F11	258
F12	270

3.8 Consolidated Evaluation Data of Probiotic Nutraceutical Gummies

The consolidated evaluation results demonstrated that all probiotic nutraceutical gummy formulations exhibited acceptable physicochemical, microbiological, and sensory characteristics. A progressive improvement in chewability, probiotic viability, viable cell count, content uniformity, swelling index, and overall acceptability was observed from F1 to F7, followed by a slight decline in subsequent formulations. Among all batches, F7 showed superior performance with the highest probiotic viability (98.4%), viable count (9.8×10^8 CFU/g), content uniformity (99.8%), swelling index (155%), chewability (10.5 mJ), and overall acceptability score (8.9). These findings indicate that F7 possessed the optimum balance of quality attributes and was therefore selected as the optimized formulation for further characterization and stability studies.

Table 3.4: Consolidated Evaluation Data of Probiotic Nutraceutical Gummies.

Formulation	Chewability (mJ)	Viability (%)	CFU/g ($\times 10^8$)	Content Uniformity (%)	Swelling Index (%)	Overall Acceptability
F1	6.2	87.2	7.4	96.1	112	6.5
F2	6.8	89.1	7.8	96.8	118	6.8
F3	7.4	91.4	8.1	97.4	125	7.0
F4	8.1	93.2	8.5	98.0	132	7.4
F5	8.9	95.4	8.8	98.5	140	7.8
F6	9.8	97.1	9.2	99.1	148	8.4
F7	10.5	98.4	9.8	99.8	155	8.9
F8	10.2	97.2	9.4	99.2	151	8.6
F9	9.8	95.8	9.0	98.6	147	8.1
F10	9.4	94.1	8.7	98.1	142	7.8
F11	8.9	92.7	8.4	97.5	138	7.2
F12	8.2	90.4	8.1	96.9	134	6.9

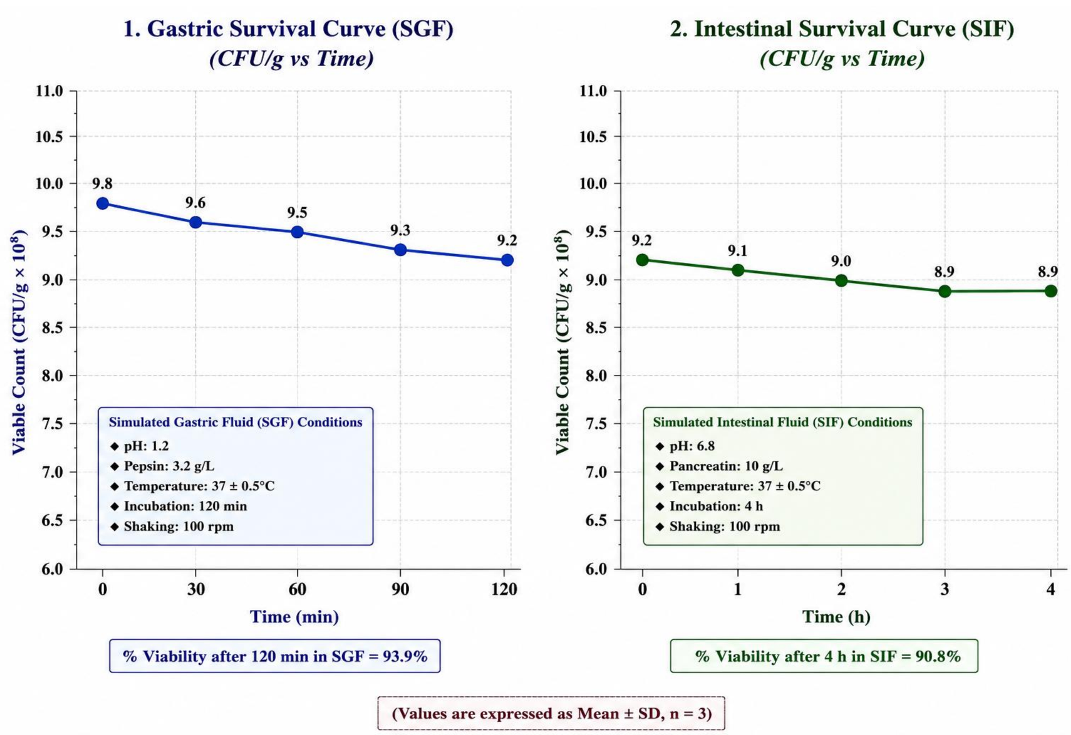


Fig. 3.4: Gastric Survival Curve (CFU/g vs Time) Intestinal Survival Curve (CFU/g vs Time).

3.9 Simulated Gastrointestinal Survival and Stability Study of Optimized Formulation (F7)

The simulated gastrointestinal survival and stability studies of the optimized formulation (F7) demonstrated excellent probiotic protection and product stability throughout the evaluation period. The viable cell count decreased only slightly from 9.8×10^8 CFU/g initially to $9.2 \times$

10^8 CFU/g after exposure to simulated gastric fluid and 8.9 × 10^8 CFU/g after intestinal fluid treatment, indicating effective protection of *Bacillus coagulans* during gastrointestinal transit. Stability studies further revealed minimal changes in pH (4.24 to 4.20), hardness (205 to 201 g), moisture content (14.9% to 14.4%), and probiotic viability (98.4% to 94.2%) over six months of storage. These results confirm that the optimized gummy formulation maintained its physicochemical properties and probiotic viability, demonstrating good gastrointestinal resilience and long-term storage stability.

Table 3.5: Simulated Gastrointestinal Survival and Stability Study of Optimized Formulation (F7).

Parameter	Initial	Gastric Fluid (2 h)	Intestinal Fluid (4 h)
CFU/g (×10^8)	9.8	9.2	8.9

Stability Parameter	Initial	1 Month	3 Months	6 Months
pH	4.24	4.23	4.22	4.20
Hardness (g)	205	204	203	201
Moisture (%)	14.9	14.8	14.6	14.4
Viability (%)	98.4	97.1	95.8	94.2

CONCLUSION

The present study successfully developed, optimized, and evaluated probiotic nutraceutical gummies containing *Bacillus coagulans* using a systematic formulation approach. Twelve formulations (F1–F12) were prepared and assessed for various physicochemical, microbiological, textural, and sensory parameters. The results demonstrated that the formulation variables significantly influenced the quality attributes of the gummies, including moisture content, hardness, chewability, probiotic viability, swelling behavior, and overall acceptability.

Among all formulations, F7 was identified as the optimized formulation based on its superior performance across multiple evaluation parameters. F7 exhibited optimum weight uniformity, acceptable pH (4.24), desirable moisture content (14.9%), suitable hardness (205 g-force), maximum chewability (10.5 mJ), highest probiotic viability (98.4%), viable cell count of 9.8 × 10^8 CFU/g, excellent content uniformity (99.8%), highest swelling index (155%), and superior sensory acceptability (8.9). FTIR studies confirmed the compatibility of *Bacillus coagulans* with the selected excipients, indicating the absence of significant physicochemical interactions.

The optimized formulation also demonstrated remarkable resistance to simulated gastrointestinal conditions, retaining a high viable cell count after exposure to gastric and intestinal fluids. Furthermore, stability studies conducted over six months revealed minimal changes in physicochemical characteristics and probiotic viability, confirming the stability and robustness of the developed formulation.

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