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Design and Optimization of a Plant-Based Synbiotic Beverage from Sprouted Buckwheat: A Multi-Response Approach for Enhancing Functional Properties

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Abstract

Fermented plant-based beverages represent promising functional foods due to their content of bioactive compounds (polyphenols, prebiotics) and viable probiotic microorganisms. Sprouted buckwheat is a rich source of bioactives and nutrients, which makes it a promising ingredient for the development of synbiotic formulations. This study aimed to optimize the fermentation process of a plant-based beverage composed of germinated buckwheat, honey, inulin, and *Lactiplantibacillus plantarum* (*Lpb. plantarum*), using Box–Behnken experimental design (BBD) and Response Surface Methodology (RSM) tools. The influence of three independent variables (inulin, honey, and inoculum concentration) was evaluated on five key response variables: total polyphenol content, flavonoid content, antioxidant activity (RSA%), pH, and starter culture viability. The optimal formulation—comprising 3% inulin, 10% honey, and 6.97 mg/100 mL inoculum—demonstrated functional stability over 21 days of refrigerated storage (4 °C), maintaining high levels of antioxidants and probiotic viability in the fermented beverage. Kinetic analysis of the fermentation process confirmed the intense metabolic activity of *Lpb. plantarum*, as evidenced by a decrease in pH, active consumption of reducing sugars, and organic acids accumulation.

Keywords: sprouted buckwheat; synbiotic beverage; fermentation; *Lactiplantibacillus plantarum* (*Lpb. plantarum*); Box–Behnken design; Response Surface Methodology (RSM)

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1. Introduction

The increasing awareness of the population regarding health has led to a significant rise in the demand for functional foods rich in natural antioxidants, which play an essential role in supporting well-being and nutritional quality [1]. Functional foods contain constituents with specific physiological actions, and the prevention of diseases associated with modern diets is closely linked to the growing consumption of such products. In

this context, functional beverages represent a rapidly expanding market segment, increasingly focused on products containing probiotic cultures and/or prebiotic ingredients with antioxidant properties.

Sprouted buckwheat (*Fagopyrum esculentum* Moench) stands out as a gluten-free pseudocereal with a valuable phytochemical profile, particularly rich in polyphenols and flavonoids such as rutin, orientin, quercetin, vitexin, and isoorientin [2], which are known not only for their antioxidant properties but also for their ability to inhibit lipid peroxidation and protect cellular membranes from oxidative damage [3]. Additionally, it provides dietary fiber, resistant starch, B-complex vitamins, vitamin A, and essential minerals, including zinc, copper, iron, and sodium [4,5]. Moreover, buckwheat is also cultivated as a melliferous plant, and the honey derived from it is recognized for its high content of bioactive compounds with therapeutic value [6]. Both buckwheat and sprouted buckwheat intake have been associated with significant reductions in total cholesterol, LDL cholesterol, and triglycerides in both human and animal studies, primarily due to their high content of soluble fiber, resistant starch, and bioactive compounds such as rutin and quercetin. Moreover, its low glycemic index and high satiety-inducing properties suggest a potential role in appetite regulation and metabolic health improvement [7].

Sprouting is a traditional, simple, and effective method that improves the nutritional value of seeds by reducing antinutritional compounds and activating the synthesis of secondary metabolites with potential health benefits [8]. Buckwheat, especially in its sprouted form, represents an underutilized source of bioactive compounds with antioxidant and prebiotic potential. However, its application in fermented functional beverages remains limited. The multiple biochemical transformations of the substrate during germination and fermentation are driven by the activity of exogenous enzymes and the metabolic properties of the starter culture, leading to the formation of bioactive metabolites (such as organic acids, phenolic compounds, antimicrobials, etc.). These compounds play an essential role in enhancing the stability, safety, and bioavailability of the resulting synbiotic fermented product [9]. Among lactic acid bacteria, strains from the *Lactiplantibacillus* genus (especially *Lpb. plantarum*) are notable for their ecological versatility and metabolic adaptability to pH and temperature variations [10]. *Lpb. plantarum* is frequently isolated from fermented foods and the human gastrointestinal tract, possessing a relatively large genome that allows adaptation to diverse environments [11]. This species holds QPS (EFSA) and GRAS (FDA) status, being considered safe for food and supplement use [12]. *Lpb. plantarum* strains contribute to enhancing the nutritional and sensory value of products, exhibit antimicrobial and antioxidant activity, and may extend their shelf life [13]. Their ability to survive under harsh gastrointestinal conditions (acidic pH, bile salts) makes them valuable probiotic candidates. Oral administration of *Lpb. plantarum* has led to significant reductions in body weight, cholesterol, and triglycerides, suggesting favorable metabolic effects supported by its proven ability to lower serum lipids and enhance satiety through interactions with the gut microbiota and host metabolism [14,15].

The antioxidant activity of *Lpb. plantarum* strains is remarkable due to their ability to produce enzymes and metabolites that neutralize free radicals and reduce oxidative stress [16]. Recent studies have demonstrated that fermentation with lactic acid bacteria may significantly modify the content of the polyphenols and flavonoids, thereby enhancing the bioactive characteristics (antioxidant and antimicrobial activities) of the final fermented product [17,18]. For a food product to be considered probiotic, it must contain at least 6 log CFU/mL of viable bacteria throughout its shelf life, ensuring a daily therapeutic dose of 8–9 log CFU/g or mL for probiotics to confer health benefits [19,20]. However, simultaneously maintaining the bioactivity of antioxidant compounds and microbial viability during storage remains a major technological challenge.

Inulin is a natural prebiotic from the fructooligosaccharide class, found in fruits and vegetables, which serves as a substrate for microbial fermentation. It promotes the production of short-chain fatty acids, such as butyrate, which is the primary energy source for colonocytes, covering 60–70% of the colon's energy requirements [21]. Dietary supplementation with inulin has been associated with stimulation of beneficial bacteria, detoxification of carcinogenic compounds, and reinforcement of the intestinal barrier, offering significant health benefits for the colon and intestinal microbiome balance [22]. Additionally, inulin supplementation significantly reduces triglycerides and contributes to appetite regulation by modulating the gut microbiota and increasing the production of short-chain fatty acids (SCFAs), which promote satiety [23,24]. Despite these well-documented benefits, current market-available beverages rarely combine these three components in a synergistic and stable formulation. Moreover, limited efforts have been made to optimize their combined fermentation parameters to preserve bioactivity and ensure functional stability. These gaps, coupled with the growing burden of metabolic disorders, prompted the design of this study.

The aim of this study was to optimize the fermentation conditions for developing a functional fermented beverage based on germinated buckwheat, with enhanced synbiotic and bioactive properties, ensuring nutritional and functional stability throughout its shelf life. A Box–Behnken experimental design (BBD) combined with Response Surface Methodology tools was used to evaluate the influence of three independent variables—concentrations of inulin, honey, and inoculum—on five responses: pH, total polyphenol content, flavonoid content, antioxidant activity, and lactic acid bacteria viability.

Within this framework, the synergistic interaction between germinated buckwheat, inulin, honey, as fermentation substrates, and the biochemical properties of *Lpb. plantarum* inoculum was optimized to maximize the product's functional potential and preserve its bioactivity over 21 days of refrigerated storage. The study was, therefore, designed to formulate a synbiotic beverage with the biotic potential in modulating gut health, supporting immune function, and potentially contributing to appetite regulation and lipid metabolism—key targets in preventive nutrition and food biotechnology.

2. Materials and Methods

2.1. Ingredients

The starter culture *Lpb. plantarum*, Vege Start 60 (lyophilized), provided by Christian Hansen Company, Hørsholm, Denmark, had a concentration of 4.8×10^{10} CFU/g.

The lyophilized inulin was provided by SC Enzymes and Derivates, Bistrita, Romania.

Buckwheat honey was purchased from beekeepers in Bălți, Republic of Moldova.

The biological material analyzed consisted of achenes of the "Gigant" cultivar of Sakhalin buckwheat (*Fallopia sachalinensis Fr. Schmidt*), as listed in the National Catalogue of Plant Varieties.

Buckwheat seeds were subjected to a 7-day germination process using the Easy-Green automatic sprouting system (Seed & Grain Tech Inc., Zhengzhou, China), which maintained constant humidity through periodic water spraying. After germination, the seeds were dried at temperature of 40 °C for cca. 18 h until reaching a moisture content below 10%, ensuring microbial stability and preventing spoilage, contamination, and undesirable fermentations. The dried sprouts were then ground to a fine powder using a ball mill. The resulting powder was sieved through a fine mesh to ensure uniform particle size, then mixed with sterile water to obtain a suspension at a final concentration of 6 g/100 mL.

The buckwheat suspension was heated at 45 °C for 30 min in a water bath, followed by a heat treatment at 80 °C for 10 min to inactivate spoilage microorganisms prior to controlled fermentation. Honey and inulin were first dissolved in water at the concentrations

indicated in Table 1 and the resulting solutions were subsequently mixed with the sprouted buckwheat suspension, respecting the concentrations for all ingredients according to the fermentation medium formula resulting from the designed experiments.

Table 1. Independent variables and levels of variation using the BBD design of the experiments.

Independent Variables	Code Symbol	Levels		
		−1	0	+1
Inulin concentration (g/100 mL)	A	1	2	3
Honey concentration (g/100 mL)	B	1	5.5	10
Inoculum concentration (mg/100 mL)	C	0.2	10.1	20

2.2. Fermentation Process

The CFU values (10^5, 5×10^6, and 10^7 CFU/mL) refer to the viable cell concentration per mL of the fermented beverage. The corresponding values of 0.2, 10.1, and 20 mg/100 mL represent the amounts of lyophilized starter culture powder used as inoculum. The CFU values (10^5, 5×10^6, and 10^7 CFU/mL) refer to the viable cell concentration per mL of the fermented beverage.

The prepared samples were maintained under static conditions at 30 °C until a pH of 4.6 was reached. The fermented samples were then stored at 4 °C. At 7, 14, and 21 days of storage, five functional parameters were analyzed and compared to the freshly fermented product.

2.3. Experimental Design

To optimize the composition of the fermented functional beverage, the BBD was applied. This multifactorial statistical model is based on quadratic regression and allows for an efficient evaluation of the influence and interactions of independent variables while reducing the number of experiments required. The resulting data were analyzed using RSM, a statistical technique that models and interprets relationships between experimental factors and measured responses, aiming to identify the optimal processing conditions.

In this study, three independent variables were investigated: inulin concentration (A, g/100 mL), honey concentration (B, g/100 mL), and inoculum concentration (C, mg/100 mL).

2.4. Analytical Determinations

After fermentation, the samples were centrifuged at 5000 rpm for 10 min to separate the solid residue from the liquid phase. The resulting supernatant was carefully collected and used for all subsequent chemical analyses.

2.4.1. Determination of Total Polyphenol Content

The total polyphenol content (TPC) was determined by spectrophotometric analysis using a T80 UV/VIS spectrophotometer (PG Instruments Ltd., Leicestershire, UK), with gallic acid as the standard, following the method described by the International Organization for Standardization (ISO 14502-1) [25]. Briefly, 1.0 mL of the centrifuged beverage supernatant was transferred, in duplicate, into separate test tubes containing 5.0 mL of a 1:10 dilution of Folin–Ciocalteu reagent (Sigma-Aldrich, Darmstadt, Germany) in water. Subsequently, 4.0 mL of sodium carbonate ((Sigma-Aldrich, Darmstadt, Germany) solution (7.5% w/v) were added. The mixture was incubated at room temperature for 60 min, after which the absorbance was measured at 765 nm using 1 cm path length quartz cuvettes.

Results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 mL of beverage [26].

2.4.2. Determination of Total Flavonoid Content

The total flavonoid content (TFC) in the buckwheat suspensions was determined by a spectrophotometric method based on the formation of flavonoid–aluminum complexes, which exhibit maximum absorbance between 420 and 430 nm, as described by Bozin et al. [27]. Briefly, 1 mL of the centrifuged beverage supernatant was mixed with 1 mL of 2.5% $AlCl_3$ (Sigma-Aldrich, Darmstadt, Germany) solution, 2 mL of 10% sodium acetate (Sigma-Aldrich, Darmstadt, Germany) solution, and 70% ethanol (Chimopar, București, Romania) to reach a final volume of 10 mL. The reaction mixtures were prepared in duplicate and incubated at room temperature for 30 min. Absorbance was measured at 420 nm against a control sample. The flavonoid content was calculated using a calibration curve generated with quercetin and rutin standards (10–50 µg/mL) and expressed as mg quercetin or rutin equivalents per 100 mL of beverage.

2.4.3. Determination of Antioxidant Activity by DPPH Method

The free radical scavenging activity (RSA) was assessed using the method described by Brand-Williams et al. [28], based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. A volume of 3.975 mL of DPPH (Sigma-Aldrich, Darmstadt, Germany) working solution (6×10^{-5} M) was transferred into a 1 cm quartz cuvette, and the initial absorbance (A_0) was recorded at 515 nm using a T80+, UV/VIS spectrophotometer. Subsequently, 25 µL of the centrifuged beverage supernatant were added to the cuvette, and the absorbance (A) was measured after 10 min of incubation at room temperature. The antioxidants present in the sample reduced the DPPH radical, leading to a decrease in absorbance proportional to the antioxidant concentration. The RSA was calculated using the following equation:

$$\text{RSA, \%} = [(\text{Acontrol} - \text{Asample})/\text{Acontrol}] \times 100$$

where: Acontrol is the absorbance of the control sample (methanol with DPPH), Asample is the absorbance of the analyzed buckwheat suspension.

2.4.4. Determination of Total Lactic Acid Bacteria Count

The total number of lactic acid bacteria was determined by cultivation on MRS agar using a double-layer method in sterile Petri dishes. Incubation was performed at 30 °C for 48 h. Viability was established by colony counting and expressed as log CFU/mL of fermented product [29].

2.4.5. Determination of Titratable Acidity (TTA)

Titratable acidity was determined according to the AOAC method, with minor modifications [30]. A known volume of the fermented sample (10 mL) was titrated with 0.1 N NaOH (Merck, Darmstadt, Germany) using phenolphthalein as an indicator until a persistent pink endpoint was reached. The results were expressed as grams of lactic acid per 100 mL of sample.

2.4.6. Determination of Reducing Sugars

The content of reducing sugars was determined using the 3,5-dinitrosalicylic acid (DNS) method, as described by AOAC (1995) [31]. Briefly, the reaction mixture was heated to allow color development, and the absorbance was measured spectrophotometrically at 540 nm. Glucose was used as a standard, and the concentration of reducing sugars in the

samples were calculated from a calibration curve constructed using known concentrations of glucose.

2.4.7. Determination of Starch Content

Starch content was determined according to the AOAC (1995) method [32], which involves acid hydrolysis of starch under heat to convert it into glucose. The resulting glucose was quantified using the 3,5-dinitrosalicylic acid (DNS) method, and absorbance was measured spectrophotometrically at 540 nm. Starch concentration was calculated based on a glucose standard curve.

To ensure accuracy, the reducing sugar content of a non-hydrolyzed (blank) sample was also measured, and this value was subtracted from the hydrolyzed sample to account only for starch-derived glucose.

2.4.8. Determination of pH

The pH was determined according to the AOAC (1984) AOAC Official Method 981.12 [33] method using a Mettler Toledo SevenEasy S20K pH meter equipped with a standard glass combination electrode, suitable for general applications (Switzerland). Prior to measurement, the instrument was calibrated with standard buffer solutions (pH 4.0 and 7.0) (Sigma-Aldrich, Darmstadt, Germany). Measurements were conducted at room temperature.

2.5. Mathematical Modeling and Statistical Analysis

The results are expressed as mean values of samples analyzed in triplicate. A factorial analysis of variance (ANOVA) was performed using Design-Expert® software version 8.0.7.1 (Stat-Ease. Inc. Design-Expert® Software, Version 8.0.7.1; Stat-Ease. Inc.: Minneapolis, MN, USA, 2010) to evaluate the main effects and interactions of the independent variables within the BBD. Statistical significance was established at $p < 0.05$. The desirability function approach was employed for the simultaneous optimization of all response variables.

For each response variable, a second-order polynomial regression model was fitted according to the following equation:

$$Y = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$$

where A, B, and C are the independent variables, b_0–b_9 are the regression coefficients, and Y is the predicted response.

3. Results and Discussion

Based on the BBD experimental, 15 distinct experimental combinations were generated, each representing a unique combination of the three factors at coded levels (−1, 0, +1), as shown in Table 2. This experimental setup enabled the evaluation of the linear, quadratic, and interaction effects of the variables on the analyzed responses: total polyphenol content (mg gallic acid/100 mL), total flavonoid content (mg rutin/100 mL), antioxidant activity (RSA%), and viability of lactic acid bacteria (log CFU/mL). All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

In the experiments, the 6% buckwheat suspension was mixed with honey and inulin solutions, as well as the *Lpb. plantarum* inoculum, and then subjected to fermentation at 30 °C until a pH of 4.6 was reached. This pH value is considered a microbiological safety threshold in the food industry and is associated with enhanced functional health benefits of the final product for consumers [34,35].

Table 2. The designed matrix variation of independent variable and the experimental values of obtained responses according to the Box–Behnken technique.

Experiment No.	Independent Variables, Coding Level			Independent Variables, Real Values			Experimental Values Answers			
	A	B	C	Inulin Concentration g/100 mL	Honey Concentration g/100 mL	Inoculum Concentration mg/100 mL	Polyphenols mg/100 mL	Flavonoids mg/100 mL	Antioxidant Capacity %	Viability of Culture, log CFU/mL
1	−1	−1	0	1	1	10.1	250.18 ± 15.34	68.54 ± 5.98	79.03 ± 5.00	8.16 ± 0.09
2	+1	−1	0	3	1	10.1	256.91 ± 13.55	70 ± 8.88	79.4 ± 4.70	8.37 ± 0.10
3	−1	+1	0	1	10	10.1	317.41 ± 19.80	78.4 ± 6.60	75.25 ± 3.20	8.25 ± 0.07
4	+1	+1	0	3	10	10.1	340.18 ± 15.50	78.52 ± 5.56	79.4 ± 2.90	8.6 ± 0.11
5	−1	0	−1	1	5.5	2	259.82 ± 10.32	75.24 ± 3.35	69.84 ± 4.98	7.34 ± 0.09
6	+1	0	−1	3	5.5	2	272.38 ± 11.41	77.21 ± 6.70	81.25 ± 6.87	7.61 ± 0.06
7	−1	0	+1	1	5.5	20	270.54 ± 13.14	67.28 ± 4.45	70.87 ± 6.72	8.88 ± 0.08
8	+1	0	+1	3	5.5	20	288.8 ± 14.14	66.98 ± 5.55	63.84 ± 4.80	9.2 ± 0.09
9	0	−1	−1	2	1	2	252.77 ± 10.90	70.1 ± 8.70	74.97 ± 7.32	6.53 ± 0.06
10	0	+1	−1	2	10	2	319.98 ± 20.20	83.11 ± 2.56	78.84 ± 4.50	7.06 ± 0.08
11	0	−1	+1	2	1	20	259.66 ± 19.40	65.41 ± 4.45	73.65 ± 6.00	8.42 ± 0.08
12	0	+1	+1	2	10	20	342.35 ± 18.00	69.3 ± 5.00	64.2 ± 3.76	8.21 ± 0.08
13	−1	0	0	1	5.5	10.1	3174.76 ± 10.60	73.52 ± 4.20	71.58 ± 5.65	8.07 ± 0.06
14	+111	0	−1	3	1	2	276.55 ± 14.98	74.51 ± 3.90	68.98 ± 5.90	8.09 ± 0.11
15	0	0	+1	2	5.5	20	325.66 ± 20.21	78.21 ± 4.00	75.65 ± 4.00	8.11 ± 0.21

3.1. Adequacy of Mathematical Models and Statistical Validation

All five studied responses—polyphenol content, flavonoid content, antioxidant capacity, log CFU/mL, and pH—were modeled using a quadratic regression model. The models were validated through ANOVA analysis, in which:

- p values < 0.05 confirmed the statistical significance of the models;
- Coefficients of determination (R^2) ranged from 0.9955 to 0.9997, and adjusted R^2 values from 0.9873 to 0.9994, indicating excellent model fit;
- Lack-of-fit p values were >0.05, confirming that the models did not significantly deviate from the experimental data;

A summary of the determination coefficients and the significant factors identified for each response is presented in Table 3, highlighting both the positive and negative influences of each variable, as well as notable interaction effects observed within the quadratic regression models.

Table 3. Values of the determination coefficient (R^2) adjusted coefficient (R^2 adj) and significant regression coefficients ($p < 0.05$) for each analyzed response (polyphenols, flavonoids, RSA%, log CFU/mL, pH).

No.	Response	R^2	Adjusted R	Significant Coefficients
1	Polyphenols, mg/100 mL	0.995	0.9876	A (+), B (++), C (+). AB, AC, BC, A^2 (−), B^2 (+++)
2	Flavonoids, mg/100 mL	0.9961	0.9905	B (++), C (−−). AC, BC, C^2 (−−)
3	Antioxidant capacity, RSA%	0.9982	0.9947	B^2 (+++), C (−−), AB, AC (−−), BC, A^2 (++), B^2 (++), C^2 (−−)
4	Log UFC/mL	0.9997	0.9994	C (+++), A (+), B (+), A^2 (++), B^2 (−), C^2 (−)
5	pH (day 7)	0.9941	0.9855	A (−), C (−−), B^2 (+)
6	pH (day14)	0.9958	0.9898	C (−−), AC (−−), B^2 (+)
7	pH (day 21)	0.9972	0.9927	A^2 (++), C^2 (−−), AB

Table 3 summarizes the performance of the quadratic regression models for each response. It presents the R^2 and adjusted R^2 values, as well as the statistically significant regression coefficients, which reflect individual effects (A—inulin, B—honey, C—inoculum),

interaction effects (AB, AC, BC), and quadratic effects (A^2, B^2, C^2). The symbols "(+)" and "(–)" indicate the direction of the effect. The table clearly highlights the key determinants for each functional variable within the fermentation process.

3.2. Individual Results for Each Response

3.2.1. Total Polyphenol Content (mg GAE/100 mL)

The quadratic regression model generated for polyphenol content was statistically significant with a coefficient of determination R^2 = 0.9955, indicating an excellent fit between the experimental and predicted data. The most substantial effect on polyphenol accumulation was exerted by honey (B), followed by inulin (A), while the inoculum (C) initially had a positive effect that became negative in the later stages of fermentation. The response surface plots shown in Figure 1 illustrate the combined influence of the variables on polyphenol concentration. Maximum values were observed in the presence of high honey concentrations and moderate inulin levels. Additionally, the interactions between variables (AB. AC. BC) significantly contributed to the response variation.

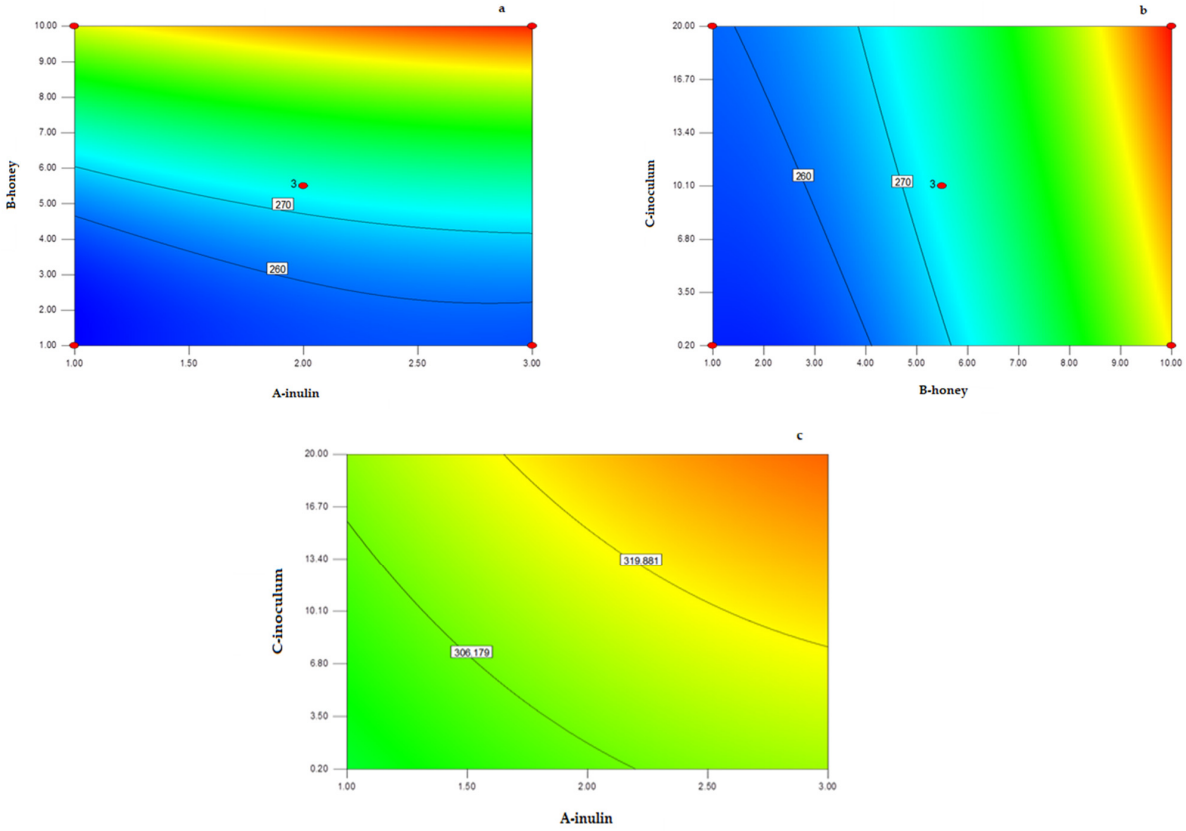

Figure 1. Response surface plots showing the combined effects of: a-inulin (A) and honey (B), b-honey (B) and inoculum concentration (C), c-inulin (A) and inoculum concentration (C), on polyphenol concentration in the fermented buckwheat beverage The color gradients in each plot represent the predicted polyphenol concentration, with cooler colors (blue) indicating lower concentrations, and warmer colors (green to yellow to red) indicating progressively higher concentrations. (**a**) illustrates the interaction between inulin and honey, highlighting that maximum polyphenol levels are achieved at moderate-to-high inulin concentrations combined with elevated honey levels. This outcome suggests a synergistic effect between these two components in enhancing polyphenol accumulation. (**b**) shows the combined influence of honey and inoculum, where a significant increase in polyphenol content is associated with high honey concentrations, regardless of the inoculum level. (**c**) completes the analysis, demonstrating that the positive effect of inulin on polyphenol content is enhanced at low to moderate inoculum levels, supporting the observation of its limiting effect during the advanced stages of fermentation.

The regression equation for total polyphenol content (mg GAE/100 mL), based on the actual values of the factors, is:

$$Y = 240.68173 + 14.4351A - 4.08886B + 6.7493 \times 10^{-3}C + 0.89111AB + 0.14394AC + 0.086869BC - 2.8125A^2 + 0.88852B^2$$

3.2.2. Total Flavonoid Content (mg Rutin/100 mL)

The quadratic regression model used to estimate the total flavonoid content was statistically validated, with a coefficient of determination (R^2) of 0.9961 and an adjusted R^2 of 0.9905. These values indicate an excellent correlation between the experimental data and the predicted values, confirming the accuracy and robustness of the model.

The regression equation, expressed in terms of the actual values of the independent variables, is as follows:

$$Y = 64.54 + 2.43A + 1.62B - 0.37C - 0.057xAxC - 0.051BC - 0.022C^2$$

where Y represents the predicted flavonoid content, and A, B, and C are the actual values of inulin, honey, and inoculum concentration, respectively.

Among the studied factors, honey (B) had the most pronounced positive effect on flavonoid accumulation, which may be attributed to its inherent phenolic compound content and its potential interactions with the plant matrix, enhancing flavonoid retention or stability. Inulin (A) exerted a moderate positive influence. In contrast, the bacterial inoculum (C) negatively affected flavonoid concentration (coefficient = −0.37), likely due to the enzymatic degradation of flavonoids during fermentation, possibly mediated by β-glucosidases that hydrolyze flavonoid glycosides.

The combined effects of the variables are illustrated in the response surface plots: Figure 2a (A × B) shows a slight increase in flavonoid content with increasing honey concentration. Figure 2b,c (A × C) and (B × C) highlight the inhibitory effect of higher inoculum concentrations on flavonoid content, with the effect being more pronounced when combined with elevated honey levels.

Overall, the proposed model is statistically significant ($p < 0.05$), suitable for describing and optimizing flavonoid accumulation in the fermented beverage, and reveals that careful dosing of honey and limiting the inoculum level may favor the preservation of a high flavonoid content in the final product.

3.2.3. Antioxidant Capacity

The quadratic regression model obtained for antioxidant capacity showed remarkable accuracy, with a coefficient of determination $R^2 = 0.9982$ and an adjusted $R^2 = 0.9947$. These values confirm an almost perfect correlation between the experimental and predicted data, indicating the robustness and reliability of the model.

The regression equation expressed in terms of the actual values of the independent variables is:

$$Y = 78.50 - 6.81A - 2.28B + 1.45C + 0.21AB - 0.47AC - 0.075BC + 2.87A^2 + 0.21B^2 - 0.025C^2$$

Among all the regression terms, the most influential was the quadratic component of honey (B^2), which exhibited a significant positive effect. This finding suggests that moderate to high honey levels optimize antioxidant capacity, likely due to honey's natural content of phenolic compounds and its potential involvement in enzymatic reactions that facilitate the release of antioxidant-active metabolites during fermentation.

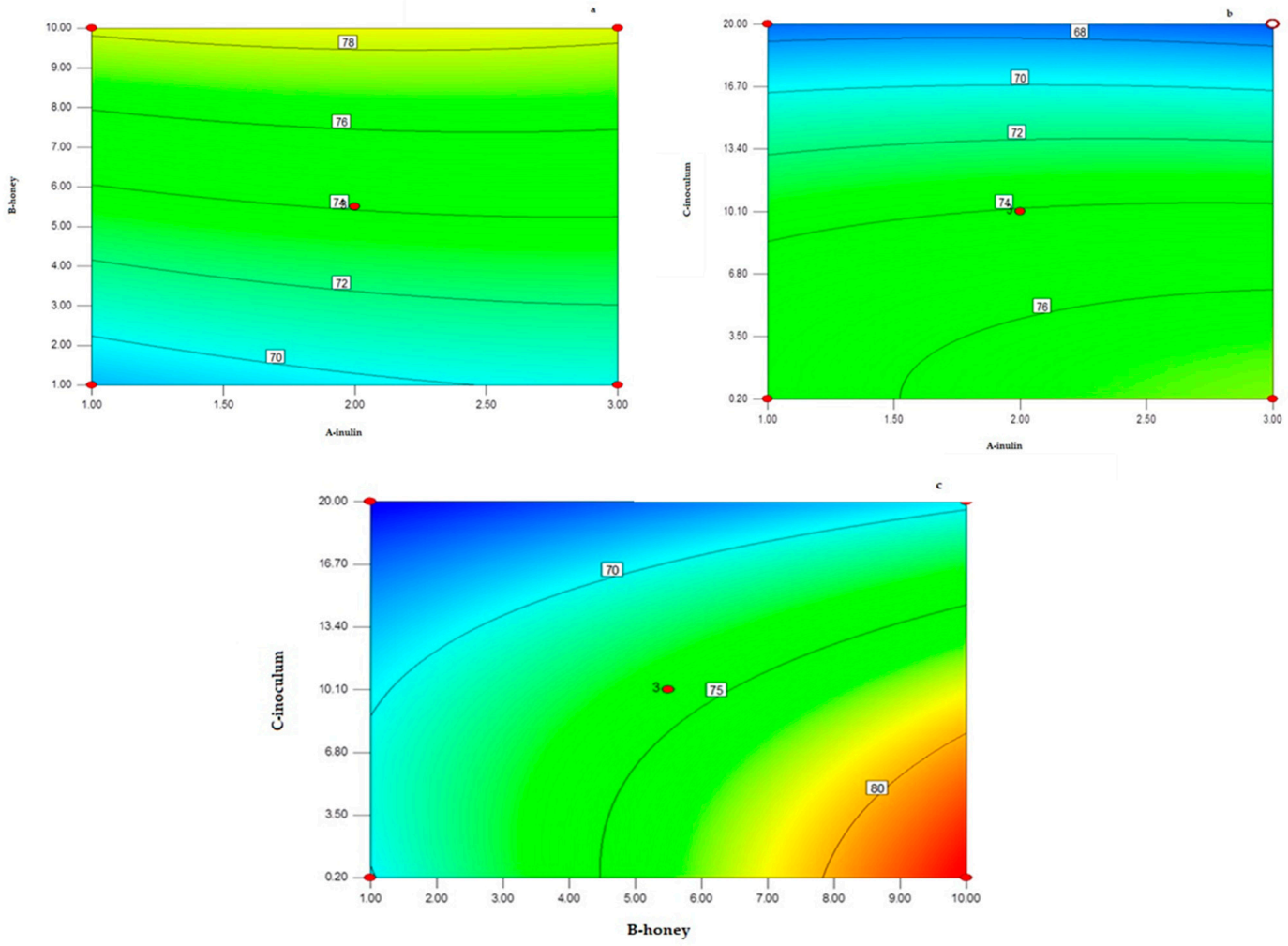

Figure 2. Response surface plots showing the combined effects of: a-inulin (A) and honey (B), b-inulin (A) and inoculum concentration (C), c-honey (B) and inoculum concentration (C), on flavonoid content in the fermented buckwheat-based beverage. The color gradients in the plots represent the predicted flavonoid concentrations: cooler colors (blue) indicate lower values, while warmer colors (green to yellow to red) indicate higher flavonoid content. (**a**) (A × B) indicates a positive trend in flavonoid content with increasing honey concentration in the presence of inulin. (**b**) (A × C) and (**c**) (B × C) clearly highlight the inhibitory effect of increased inoculum doses, especially when combined with high levels of honey, suggesting a possible synergistic degradation of phenolic compounds.

Inulin (A) also contributed significantly through its quadratic term (A^2), indicating a beneficial stabilizing role. Although *Lpb. plantarum* is known to partially metabolize inulin during fermentation, as supported by previous studies indicating its ability to use inulin as a carbon source, the relatively short duration of the fermentation process, and the structural complexity of inulin, suggesting that a substantial fraction likely remained unfermented. This unconsumed portion may have contributed to the overall prebiotic content of the beverage and exerted a stabilizing effect by forming a protective matrix within the food system. Such a network could reduce oxidative degradation of antioxidant compounds, thereby enhancing their stability and retention throughout the fermentation process.

In contrast, the inoculum (C) exerted an overall negative influence on antioxidant capacity—both through its linear term and via interactions with inulin (AC) and honey (BC). However, the quadratic term (C^2) was not statistically significant, suggesting a primarily linear effect. Although *Lpb. plantarum* is known to synthesize phenolic derivatives via phenyl acid decarboxylase activity, it is assumed that, under the current experimental

conditions, the production of postbiotic metabolites with low or unstable antioxidant potential may have contributed to the observed decrease in antioxidant activity.

Graphical Analysis of the Response Surfaces

Figure 3a (A × B) reveals a distinct region of elevated antioxidant capacity associated with moderate inulin concentrations combined with high honey levels. This suggests a synergistic interaction that enhances antioxidant activity, likely due to optimal phenolic release and stabilization within the matrix.

Figure 3b (A × C) illustrates a marked decrease in antioxidant capacity at higher inoculum concentrations, particularly when combined with increased inulin levels. This pattern may indicate a disruption of the antioxidant matrix, potentially caused by microbial enzymatic activity that degrades or transforms bioactive compounds.

Figure 3c (B × C) confirms a negative interaction between high concentrations of honey and inoculum, reflecting an unfavorable synergy that reduces antioxidant potential. This may be attributed to excessive microbial metabolism of honey-derived phenolics or a shift in fermentation dynamics toward the formation of less active metabolites.

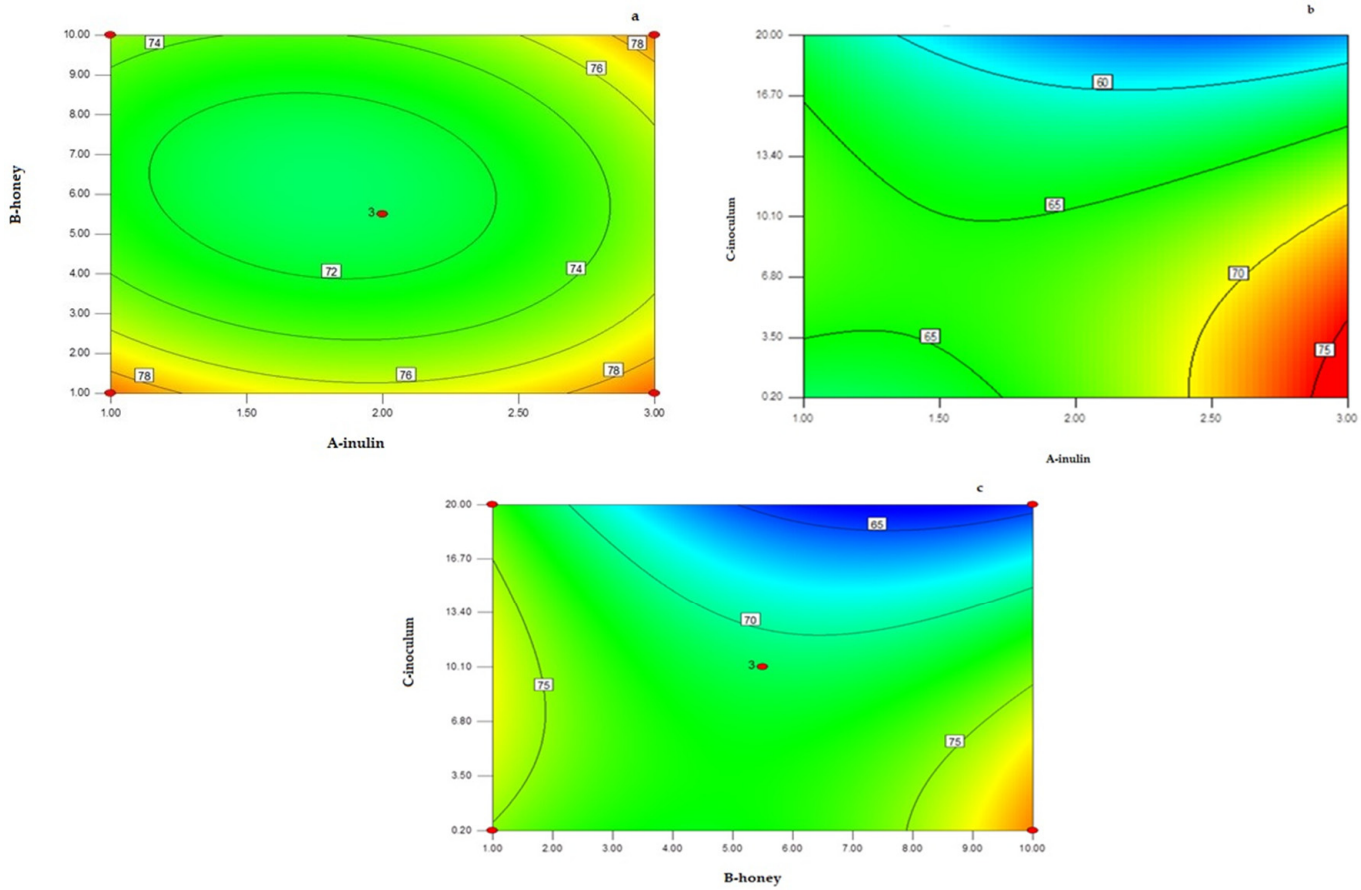

Figure 3. Response surface plots illustrating the interactive effects of: a-inulin (A) and honey (B), b-inulin (A) and inoculum concentration (C), c-honey (B) and inoculum concentration (C), on the antioxidant capacity of the fermented buckwheat-based beverage. Color gradients indicate predicted antioxidant capacity: cooler colors (blue to green) represent lower antioxidant levels, warmer colors (yellow to red) indicate higher antioxidant activity. These visual gradients allow for a clear interpretation of how the interactions between variables affect antioxidant performance during fermentation.

3.2.4. Viability of Lactic Acid Bacteria in the Fermented Product

The quadratic regression model developed for estimating bacterial viability (expressed as log CFU/mL) demonstrated exceptional precision, with a coefficient of determination (R^2) of 0.9997. This value confirms an almost perfect fit between the experimental and predicted data, indicating the robustness and reliability of the model in describing probiotic cell development within the fermented product.

The regression equation, expressed in terms of the actual values of the variables, is:

$$Y = 8.06 - 1.83A + 0.17B + 0.16C + 0.0078AB + 0.0013AC - 0.0042BC + 0.48A^2 - 0.011B^2 + 0.00032C^2$$

The high goodness-of-fit values indicate the model's strong predictive capacity for microbial growth dynamics during fermentation. Statistically significant coefficients ($p < 0.05$) highlight the dominant role of the inoculum (C), as well as a synergistic effect between inulin and the microbial culture in enhancing probiotic cell proliferation within the plant-based substrate.

Analysis of the coefficients reveals that the primary influential factor on bacterial viability was the inoculum (C), with a significant direct positive effect (coefficient +0.16), which promoted the growth of viable cells, particularly in the early stages of fermentation. Additionally, inulin contributed favorably to maintaining viability through its quadratic effect (A^2 = +0.48), suggesting its prebiotic role in creating a suitable environment for lactic acid bacteria development.

An analysis of the regression coefficients identifies the inoculum (C) as the primary driver of bacterial viability, exerting a direct positive linear effect (+0.16). This supports the growth of viable cells, particularly during the early stages of fermentation, when microbial adaptation and proliferation are most active.

In addition, inulin (A) exhibited a significant quadratic effect (A^2 = +0.48), underscoring its prebiotic role in sustaining bacterial viability.

This result suggests that, at optimal concentrations, inulin contributes to the formation of a protective or nutritive environment, facilitating the continued development of lactic acid bacteria throughout the fermentation process.

In contrast, the negative quadratic effect of honey ($B^2 = -0.011$) and the negligible effect of the inoculum's quadratic term ($C^2 \approx 0$) suggest that excessive levels of these components may become unfavorable in the later stages of fermentation, potentially due to the accumulation of organic acids or secondary metabolites that inhibit cell growth. Moreover, the negative interaction between honey and inoculum (B × C) may indicate a suboptimal balance of fermentable sugars, which could transiently affect microbial activity or metabolic efficiency under specific fermentation conditions. Such conditions could suppress cell proliferation or trigger a premature transition into the stationary phase.

The response surfaces illustrated in:

Figure 4a (A × B) shows a maximum viability zone at moderate inulin levels and slightly increased honey concentrations.

Figure 4b (A × C) confirms the beneficial effect of high inoculum concentrations in combination with inulin, supporting the synergistic interaction between the prebiotic substrate and the bacterial culture.

Figure 4c (B × C) highlights an inhibitory effect at high combinations of honey and inoculum, suggesting the occurrence of a metabolic imbalance in the medium.

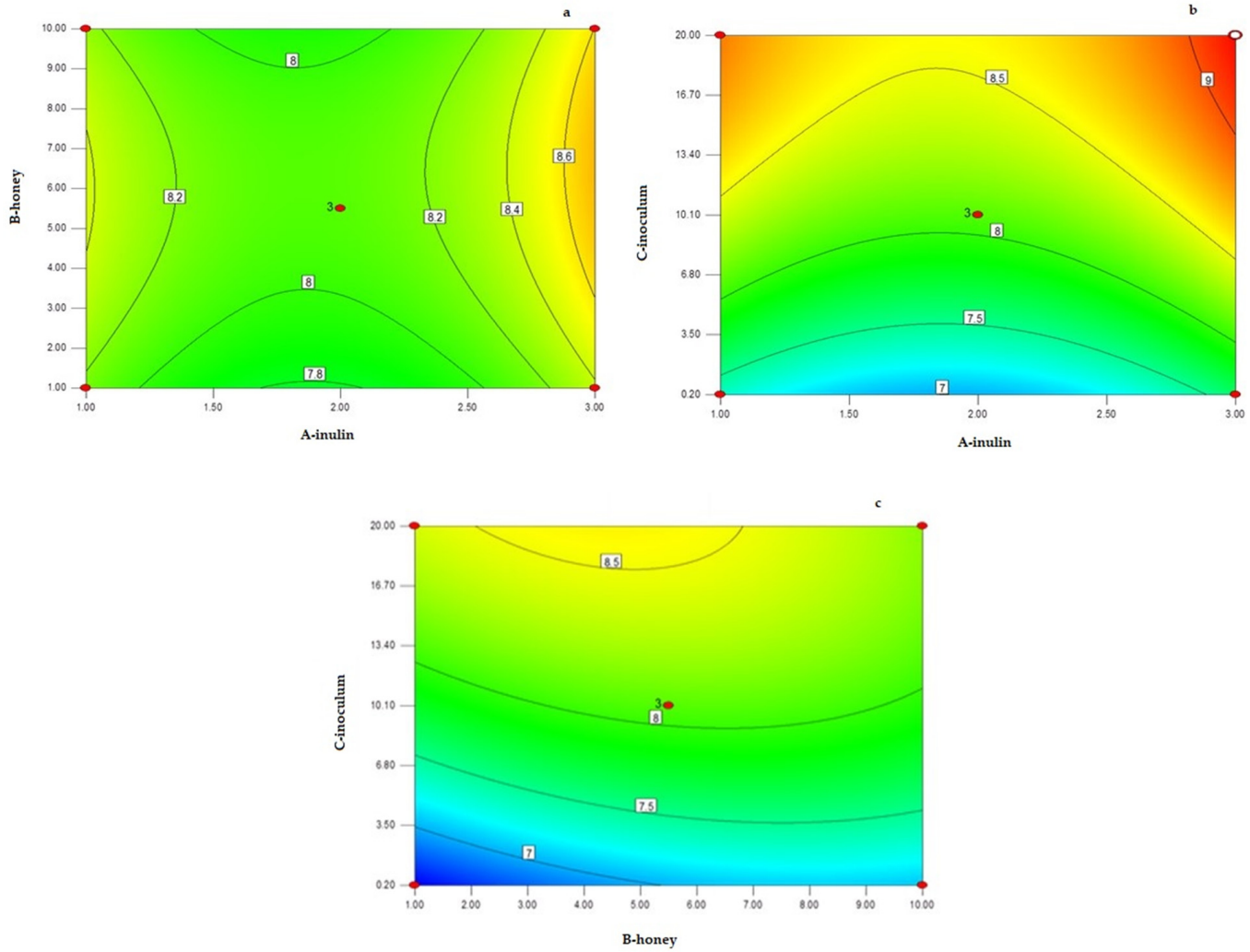

Figure 4. Response surface plots illustrating the interactive effects of: a-inulin (A) and honey (B), b-inulin (A) and inoculum concentration (C), c-honey (B) and inoculum concentration (C), on the number of viable cells in the fermented buckwheat-based beverage. Color gradients represent the predicted number of viable cells: cooler colors (blue to green) indicate lower microbial counts, warmer colors (yellow to red) correspond to higher viable cell concentrations. Subfigure labels (**a**–**c**) are indicated for clarity.

3.2.5. Evolution of pH in the Fermented Product During Storage

The decrease in pH during fermentation serves as an indirect indicator of lactic acid bacterial activity, being closely associated with the production of organic acids and the reduction of the buffering capacity of the matrix. Regression models for pH on days 7, 14, and 21 yielded R^2 values ranging from 0.9941 to 0.9972, confirming the high predictive accuracy of the models.

On day 7, the inoculum (C) exerted a significant negative effect on pH, reflecting intense fermentative activity. Inulin (A) initially exhibited a mild acidifying effect. However, its quadratic term (A^2) became positive by day 21, suggesting a potential buffering role or a gradual substrate release over time. The quadratic term for honey (B^2) was also positive, though biologically insignificant. A statistically significant interaction between inulin and the inoculum (AC) was observed on day 14, indicating a combined influence on acid stabilization (Table 4).

Table 4. Summary of regression model performance and dominant factors influencing the response at different fermentation times.

No.	Day	R^2	Adjusted R^2	Significant Coefficients ($p < 0.05$)	Dominant Influence
1	7	0.9941	0.9855	A (−), C (−−), B^2 (+)	C și B^2
2	14	0.9958	0.9893	C (−−), AC (−−), B^2 (+)	C
3	21	0.9972	0.9927	A^2 (++), C^2 (−−), AB	A^2

The symbols "(+)" and "(–)" indicate the direction of the effect.

3.3. Optimization of the Fermentation Process

The simultaneous optimization of the five analyzed responses was performed using the desirability function approach, a robust method for addressing multiple objectives in experimental design. Each response variable was assigned a partial desirability function, and the combination of these functions resulted in the determination of a global desirability function (D), calculated as a weighted geometric mean.

The optimization criteria focused on maximizing polyphenol and flavonoid content, enhancing antioxidant capacity, increasing viable cell counts (log CFU/mL), and maintaining pH stability throughout storage.

The optimal formulation was obtained with 3 g/100 mL inulin, 10 g/100 mL honey, and 6.97 mg/100 mL inoculum. Under these conditions, the desirability function value reached 0.883 at 24 h and slightly increased to 0.891 on day 21.

Response modeling was conducted using statistically significant quadratic regression equations for all variables ($p < 0.05$; $F > 1$), with non-significant lack-of-fit values ($p > 0.05$) and coefficients of determination (R^2) ranging from 0.9955 to 0.9997. The high adjusted R^2 values (>0.9873) and low coefficients of variation (<1.16%) confirm the robustness, precision, and predictive accuracy of the models.

3.4. Global Optimization and the Importance of the Desirability Function Method

The desirability function approach enabled the simultaneous integration of multiple responses, yielding a global desirability (D) value greater than 0.88—an indicator of a near-optimal combination of variables.

This method offers clear advantages over single-factor optimization, as it facilitates a rational compromise between competing responses. Moreover, it accounts for both interactive and quadratic effects of the variables, making it a powerful tool for comprehensive modeling. Its applicability for long-term prediction was demonstrated in this study, with consistent optimization outcomes observed over a 21-day storage period.

For evaluation of the starter culture functionality, a correlation between cell counts and carbohydrate metabolism was evaluated (Figure 5) during the fermentation process and, also, storage of the fermented product.

3.5. Evolution of Biochemical and Microbiological Parameters in the Optimized Variant During Fermentation and Throughout 21 Days of Storage at 4 °C

The evolution of biochemical and microbiological parameters in the optimized variant during fermentation and throughout 21 days of storage at 4 °C is illustrated in Figure 5.

Figure 5 shows the real-time evolution of key biochemical and microbiological parameters during fermentation and cold storage of the germinated buckwheat-based beverage. The reducing sugars decreased progressively from approximately 8.4 g/100 mL at the start of fermentation to 7.8 g/100 mL at the end of fermentation and further to 6.5 g/100 mL after 21 days of storage, highlighting their active utilization by *Lpb. plantarum*. In contrast, starch was metabolized more slowly, declining from 2.0 g/100 mL to 1.8 g/100 mL during

fermentation and reaching 1.0 g/100 mL by the end of storage. This pattern suggests a metabolic preference for easily fermentable carbohydrates in the early stages, followed by progressive enzymatic starch hydrolysis.

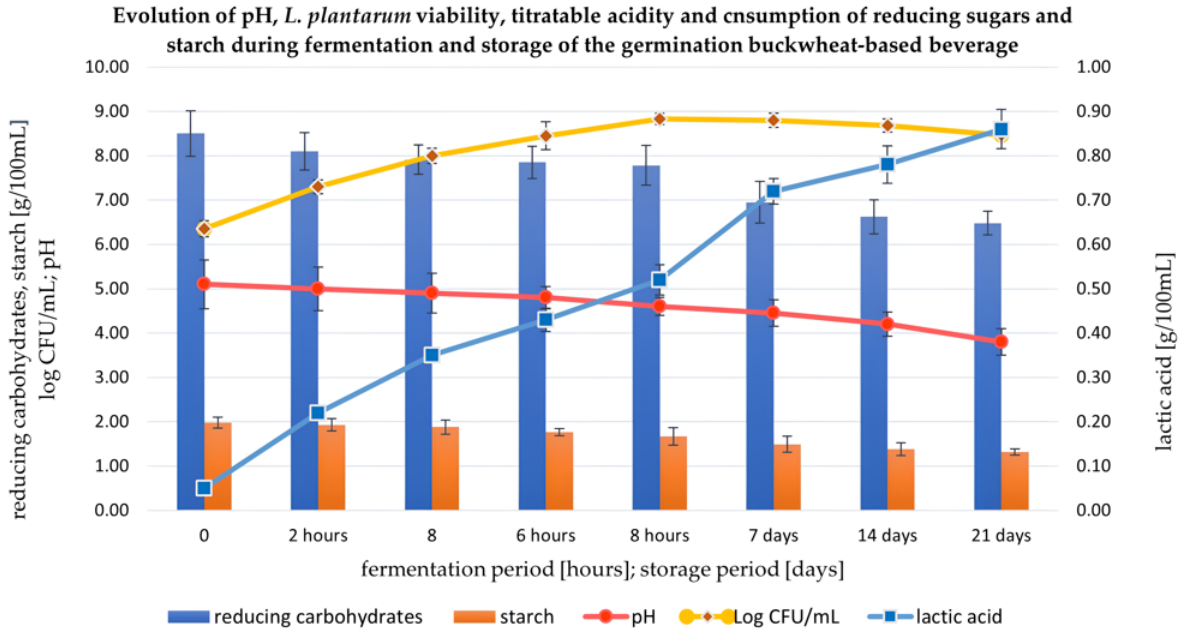

Figure 5. Evolution of pH, *Lpb. plantarum* viability, titratable acidity, and consumption of reducing sugars and starch during fermentation and storage of the germinated buckwheat-based beverage. Initial conditions for the optimized formulation were: 3 g/100 mL inulin, 10 g/100 mL honey, and 6.97 mg/100 mL lyophilized *Lpb. plantarum* inoculum.

The pH decreased from 5.1 to 4.6 during fermentation, then remained relatively stable. This acidification was accompanied by a consistent rise in lactic acid levels—from 0.05 g/100 mL initially, to 0.52 g/100 mL at the end of fermentation, and further to 0.88 g/100 mL after 21 days—indicating ongoing metabolic activity during storage.

Microbial viability, expressed as log CFU/mL, increased rapidly during the first 8 h, from 6.35 to 8.83, with no lag phase observed—likely due to the high availability of fermentable sugars. The peak value (8.80 log CFU/mL) was recorded after 7 days of storage and remained above 8.47 log CFU/mL thereafter, confirming the probiotic character of the beverage.

These findings are in agreement with Kun et al. [36], who noted that the absence of a lag phase is characteristic of carbon-rich matrices. In this study, the synbiotic composition—based on germinated buckwheat and honey (7.576 g/100 mL of reducing sugars)—facilitated rapid fermentation onset. This is further supported by Shamala et al. [37], who reported the stimulatory effect of honey on *Lpb. plantarum* and *L. acidophilus*, in contrast to sucrose, which did not yield the same effect.

Although high cell viability was maintained throughout storage, a slight decrease was noted after 14 days, potentially due to nutrient exhaustion, metabolite accumulation (e.g., lactic acid, phenolic acids), and pH values approaching the tolerance threshold of the bacteria. Such behavior is typical in fermented food systems and should be considered when establishing product shelf life [38].

3.6. Correlative Effects on the Independent Variables

3.6.1. Correlation Between Starch Consumption and Bacterial Growth

Figure 6 illustrates an inverse relationship between starch content (g/100 mL) and the cell density of *Lpb. plantarum* (log CFU/mL) during the fermentation of the synbiotic bever-

age. The extremely high correlation coefficient (R = 0.9998) and coefficient of determination (R^2 = 0.9996) confirm the excellent fit of the regression model, indicating that 99.96% of the variation in bacterial growth can be explained by starch consumption.

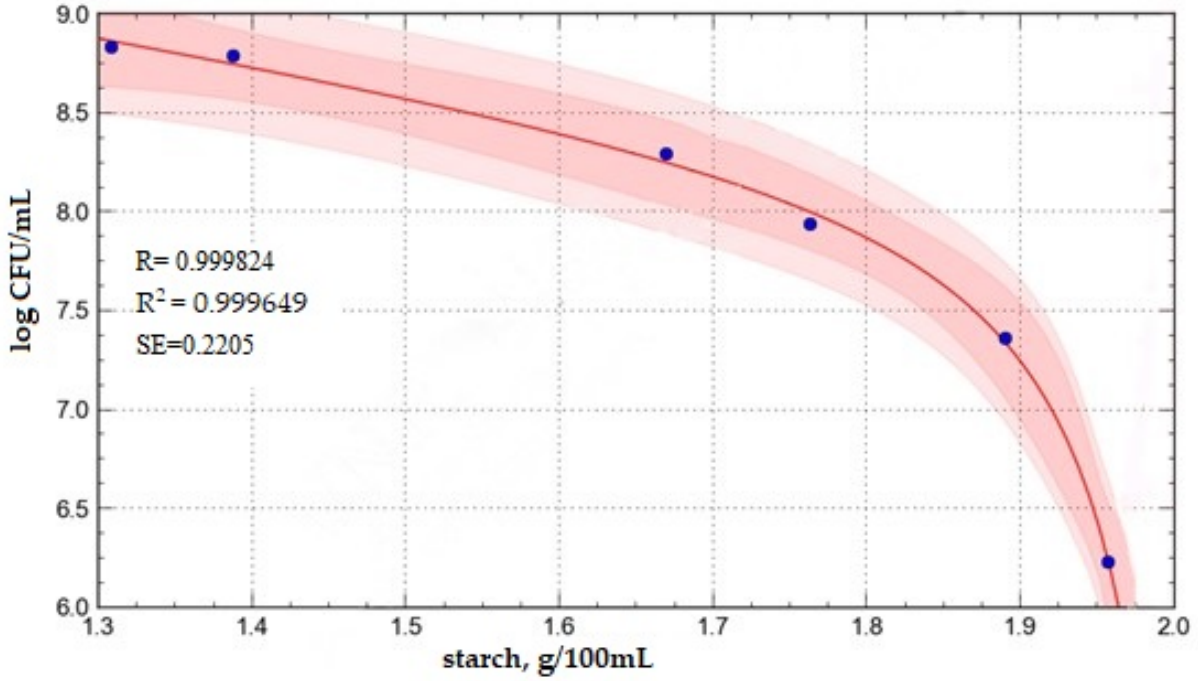

Figure 6. Correlation between starch content and bacterial growth (log CFU/mL) in the fermented buckwheat-based beverage. Blue dots represent experimental data points; the red line indicates the fitted regression curve; the shaded red area shows the 95% confidence interval of the model. R—correlation coefficient; R^2—coefficient of determination; SE—standard error of the regression.

This strong association suggests that the starch in the plant matrix is efficiently hydrolyzed by *Lpb. plantarum* into monoglucides, which are then utilized as a carbon source to support bacterial proliferation.

Thus, the decrease in starch concentration is directly correlated with the increase in bacterial density, confirming the amylolytic activity of the strain. These results are supported by the study conducted by Kusnadi et al. [39], which demonstrated that 87.6% of starch reduction during the fermentation of skim milk with taro flour in their optimized beverage was due to the metabolic activity of *Lpb. plantarum*. In comparison, our data indicate an even higher efficiency of starch utilization in the experimental system containing germinated buckwheat, honey, and inulin, highlighting the strong potential of this matrix to support intense probiotic fermentation.

3.6.2. Correlation Between pH Decrease and Starter Culture Growth

Figure 7 illustrates the relationship between the pH of the medium and the cell density of *Lpb. plantarum* (log CFU/mL) during the fermentation of the synbiotic beverage based on sprouted buckwheat, honey, and inulin. *Lpb. plantarum* maintains optimal viability (≥8.5 log CFU/mL) within a relatively stable pH range of 4.6 to 3.8. However, a further drop in pH below 3.8 is associated with a marked decline in cell density, highlighting the strain's sensitivity to excessive acidification of the medium.

During fermentation (8 h), lactic acid accumulation was modest, reaching only 0.470 g/100 mL, which is a result comparable to that reported by Iancu et al. [40] and Mousavi [41]. This low lactic acid production may be attributed to inulin metabolism,

which, in the presence of *Lpb. plantarum*, can lead to the formation of butyric acid (butyrate), according to hypotheses proposed by Nazzaro, Kaplan, and Saulnier [42–44].

The presence of butyrate in the fermented product is particularly beneficial from a functional perspective, as it is a primary energy source for colonocytes, with positive effects on colonic mucosa regeneration and potential in preventing malignant transformations by stimulating the expression of the glutathione-S-transferase enzyme [45,46].

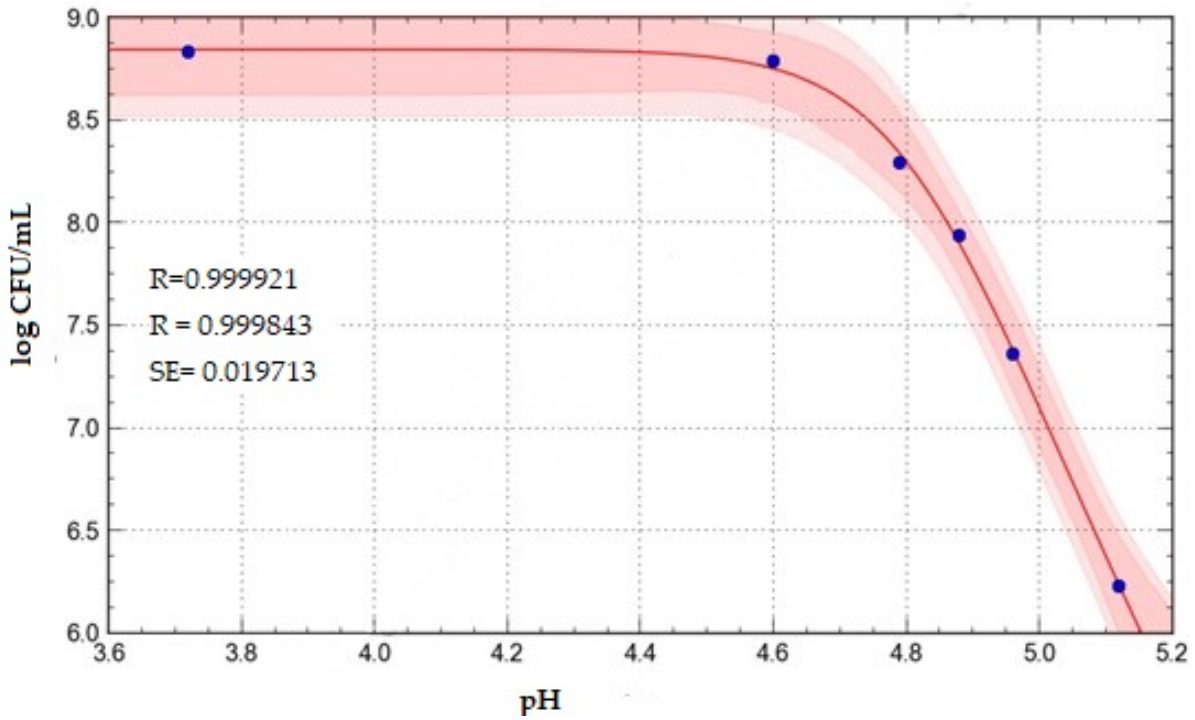

Figure 7. Correlation between pH decrease and starter culture growth (log CFU/mL). Blue dots represent experimental data; the red curve corresponds to the fitted regression model; the shaded red area indicates the 95% confidence interval for the prediction. R—correlation coefficient; R^2—coefficient of determination; SE—standard error.

Thus, the limited decrease in pH, accompanied by the sustained high cell density, confirms the adaptability of *Lpb. plantarum* to a plant-based synbiotic environment, as well as the potential of this beverage to deliver postbiotic metabolites with protective effects at the intestinal level. Thus, the limited pH decrease accompanied by the maintenance of high cell density confirms the adaptability of *Lpb. plantarum* to a plant-based synbiotic environment, as well as the potential of this beverage to provide postbiotic metabolites with protective effects at the intestinal level.

3.6.3. Correlation Between Bacterial Growth and Lactic Acid Production

Figure 8 illustrates the direct proportional relationship between cell density (expressed as log CFU/mL) and lactic acid concentration (g/100 mL) in the synbiotic beverage fermented with *L plantarum*. The high correlation coefficient (R = 0.9705) and coefficient of determination (R^2 = 0.9418) confirm a significant association between bacterial proliferation and lactic acid accumulation, reflecting the efficiency of lactic fermentation metabolism in this functional plant-based matrix. The low standard error (SE = 0.5191) further supports the validity of the applied regression model.

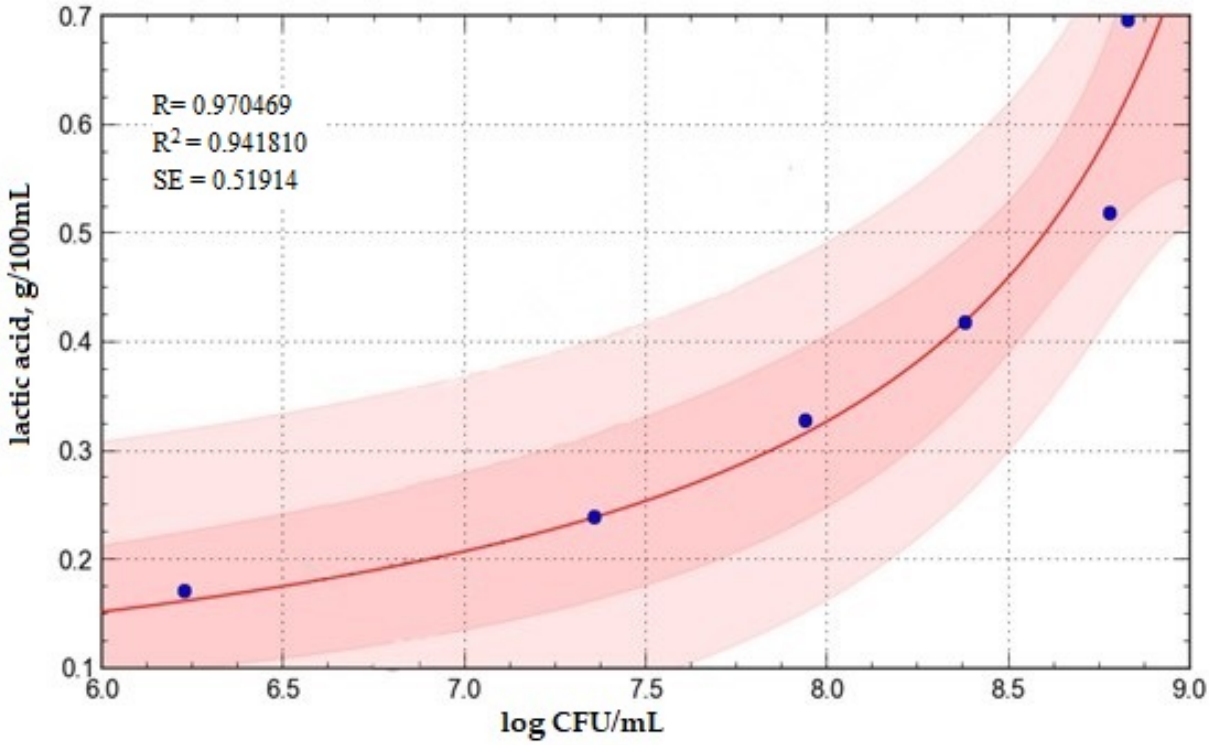

Figure 8. Correlation between starter culture growth (log CFU/mL) and lactic acid production (g/100 mL). Blue dots represent experimental data points; the red curve corresponds to the fitted regression model; the shaded red area indicates the 95% confidence interval for the predicted values. R—correlation coefficient; R^2—coefficient of determination; SE—standard error.

Maximum cell growth was recorded at the end of fermentation, when the bacterial density reached 8.83 log CFU/mL, exceeding the minimum threshold recommended by FAO/WHO for probiotic foods (≥6 log CFU/mL at the time of consumption). By the end of the storage period. a moderate decrease of 0.36 log CFU/mL was observed, resulting in a final value of 8.47 log CFU/mL, which confirms the product's microbial stability and probiotic viability.

These results are consistent with findings reported by Boeni and Pourahmad [47] and demonstrate superior performance compared to the study by Jaiswal et al. [48], in which a probiotic product based on fermented cabbage with *Lpb. plantarum* exhibited a 2.14 log CFU/mL reduction during storage. This contrast underscores the capacity of the plant-based substrate composed of sprouted buckwheat, honey, and inulin to maintain cell viability and metabolic activity within optimal parameters, supporting the development of high-quality probiotic products.

3.6.4. The Correlation Between Reducing Sugar Consumption and Lactic Acid Production During the Fermentation and Storage of the Buckwheat-Based Beverage

Figure 9 illustrates the inverse proportional relationship between the content of reducing sugars and the concentration of lactic acid (g/100 mL) observed during the fermentation and storage of the synbiotic beverage obtained from germinated buckwheat. The very high correlation coefficient (R = 0.9941) and coefficient of determination (R^2 = 0.9882) indicate an extremely strong association between the reduction of sugars and the accumulation of lactic acid, reflecting the intensity of *L. plantarum*'s metabolic activity within this prebiotic, plant-based matrix.

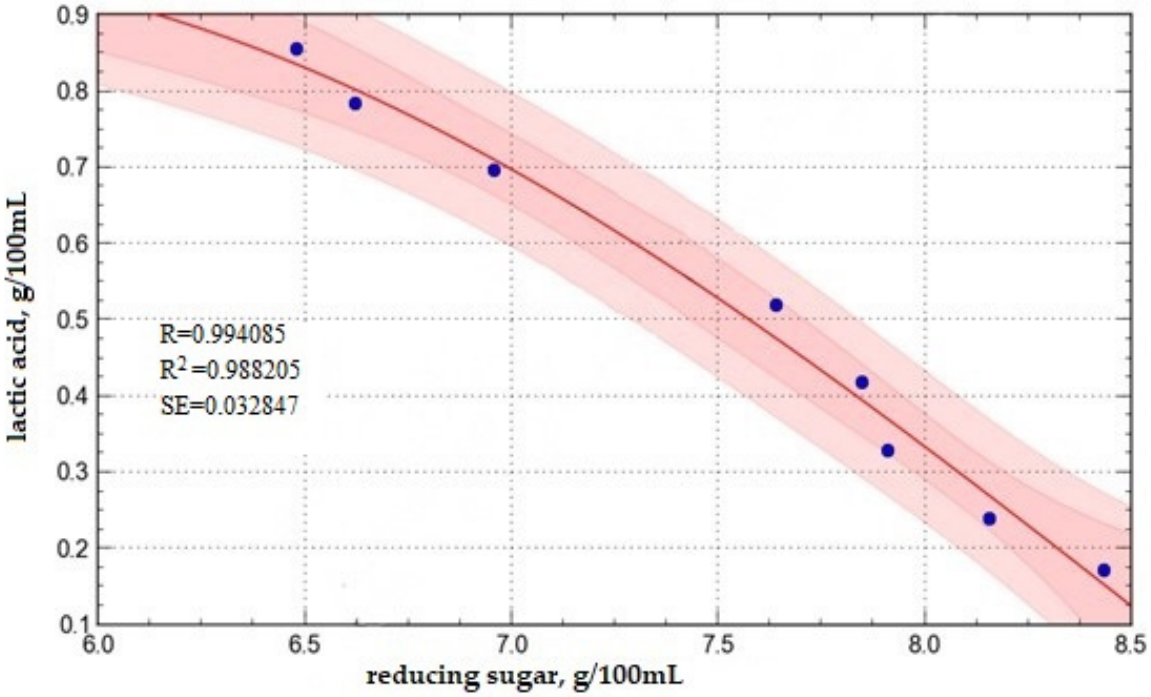

Figure 9. Correlation between reducing sugar consumption (g/100 mL) and lactic acid production (g/100 mL) during fermentation. Blue dots represent experimental values;the red line shows the fitted regression curve;the shaded red area indicates the 95% confidence interval of the prediction model. R—correlation coefficient; R^2—coefficient of determination; SE—standard error. This model confirms that as the bacteria utilize the available sugars as an energy source, they are efficiently converted into lactic acid, with a minimal standard error (SE = 0.0328), indicating a robust prediction of the fermentation dynamics. By comparison, Buruleanu et al. [49], using the Luedeking–Piret model, reported R^2 values of 0.6687 for carrot juice fermentation and 0.9236 for cabbage juice, highlighting the superior predictive accuracy and metabolic efficiency achieved in the experimental system with sprouted buckwheat.

This relationship further supports that the buckwheat-based beverage, enriched with honey and inulin, serves as an optimal fermentation matrix, promoting an efficient and stable lactic fermentation process with high functional potential.

3.6.5. The Correlation Between the Consumption of Reducing Sugars and the Production of Lactic Acid During the Fermentation and Storage of the Buckwheat-Based Beverage

Figure 10 illustrates the inverse proportional relationship between lactic acid concentration (g/100 mL) and the pH of the synbiotic beverage formulated with sprouted buckwheat, honey, inulin, and *Lpb. plantarum*. The experimental data indicate a very strong correlation between lactic acid production and acidification of the medium, as confirmed by a correlation coefficient (R) of 0.9998 and a determination coefficient (R^2) of 0.9996, showing that 99.96% of the variation in pH values can be explained by the accumulation of lactic acid.

As fermentation progresses, lactic acid production by *Lpb. plantarum* leads to a steady decrease in pH, with a notably steeper decline occurring after the lactic acid concentration exceeds 0.05 g/100 mL. This behavior indicates that the plant-based matrix, composed of sprouted buckwheat and prebiotic additions such as honey and inulin, actively supports the homolactic metabolism of *Lpb. plantarum*, favoring the conversion of fermentable sugars into lactic acid. Furthermore, *Lpb. plantarum* has demonstrated the ability to metabolize prebiotic compounds like inulin, with significantly upregulated gene expression in its presence, pointing to a complex regulatory network involving sugar transport, hydrolysis,

and metabolic pathways [50]. Similar results have been observed in the fermentation of jujube–wolfberry composite juice [51] and in synbiotic systems containing sprouted buckwheat, which displayed comparable lactic acid bacteria metabolism dynamics.

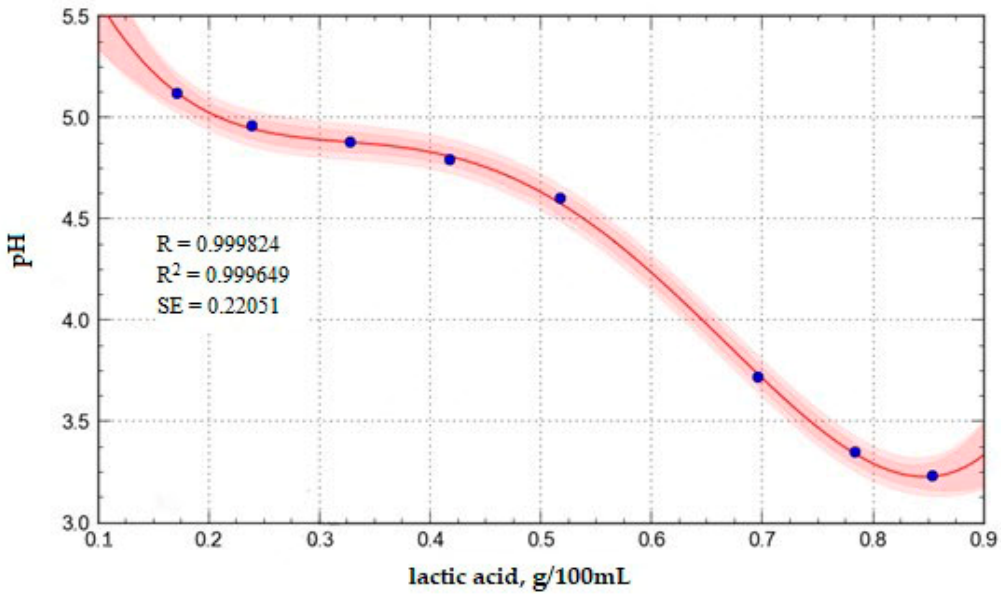

Figure 10. Correlation between lactic acid production (g/100 mL) and pH decrease in the fermented buckwheat-based beverage. Blue dots represent experimental measurements; the red line shows the fitted nonlinear regression model; the shaded red area indicates the 95% confidence interval for the prediction. R—correlation coefficient; R^2—coefficient of determination; SE—standard error.

These findings emphasize the consistent inverse relationship between lactic acid production and pH reduction, supported by high cell viability values. The results further confirm the adaptability of *Lpb. plantarum* to complex, plant-derived fermentation substrates.

The standard error (SE = 0.2205) associated with the model confirms the consistency and reproducibility of the experimental data. Acidification plays a crucial role not only in microbiological stabilization of the beverage but also in sustaining probiotic viability throughout storage. Additionally, the decreased pH contributes to the sensory profile by developing the mildly sour taste characteristic of fermented products. This strong correlation underscores lactic acid as a key marker for monitoring fermentation dynamics and optimizing technological parameters in the development of functional synbiotic beverages.

The low standard error (SE = 0.2205) associated with the model confirms the consistency and reproducibility of the experimental data. Acidification plays a critical role not only in the microbiological stabilization of the beverage but also in sustaining probiotic viability throughout storage. Additionally, the decrease in pH contributes to the sensory profile by imparting the mildly sour taste typical of fermented products.

This strong correlation highlights lactic acid as a key biochemical marker for monitoring fermentation dynamics and optimizing technological parameters in the development of functional synbiotic beverages.

3.7. Influence of Composition on Polyphenol. Flavonoid Content and Antioxidant Capacity

The data obtained highlight the significant contribution of honey to the increase in polyphenol content, especially during the early stages of fermentation—an effect attributed to its natural content of phenolic compounds. These findings are supported by the scientific literature, which indicates considerable variation in the phenolic content of honey depending on its floral origin, with values ranging between 50 and 500 mg GAE/100 g [52,53]. Similar results were reported by Asif Anwar [54] in probiotic milk-based beverages en-

riched with honey, by Dos Santos [55] in a probiotic drink obtained from yerba maté infusion and honey, and by Leite and colleagues [56], who observed significant increases in polyphenol content and antioxidant capacity in mixed beverages based on apple and passion fruit.

The effect of the *Lpb. plantarum* inoculum is dual: in the initial phase, it activates phenolic compounds through enzymatic actions (e.g., tannases. PAD) [35,57–59], but over time, microbial enzymes can also lead to catabolism, which explains the decrease in polyphenol and flavonoid content by day 21. Similarly, the hydrolysis of flavonoids by microbial β-glucosidases leads either to the release of aglycones or to their degradation, justifying the negative effect of the inoculum on this response. These observations are consistent with studies reporting the degradation of rutin and an increase in quercetin in fermented matrices [60].

The antioxidant capacity of the beverage increased significantly after fermentation, reaching values above 82%, compared to the initial buckwheat suspension value (~58%). This effect can be explained by the conversion of phenolic precursors into forms with increased antioxidant activity [32,58], the presence of honey (with its own RSA% of ~18%), and the possible formation of new bioactive compounds during fermentation (e.g., decarboxylated phenolic acids) [54–56].

In this study, a functional beverage with synbiotic properties was developed by combining prebiotic ingredients with probiotic cultures. By using valuable raw materials rich in fermentable fibers and bioactive compounds, and through the optimization of fermentation parameters, a final product with an enhanced bioactive profile was obtained, due to the accumulation of beneficial metabolites generated during the fermentation process.

The study was conducted under controlled laboratory fermentation conditions, and the stability, safety, and functional performance were demonstrated. Some limitations could be evidenced by the increase in the level of production due to the provenance and quality of the raw materials and, also, during the buckwheat germination process, the control of mould growth, which can affect the safety of the fermentation substrate by mycotoxin biosynthesis. In addition, factors such as storage temperature, packaging, and technological stress may affect the viability of probiotic strains and the integrity of bioactive compounds.

To strengthen these findings, further research should include clinical trials to validate the functional health claims, particularly in populations at risk of dyslipidemia, metabolic syndrome, or appetite regulation disorders. Dose–response studies are also needed to determine the optimal intake levels of the beverage. Moreover, omics-based analyses (e.g., metabolomics, microbiome profiling) may provide valuable insights into host–microbiota interactions and help clarify the mechanisms through which the tribiotic effects are exerted.

4. Conclusions

This study is a preliminary one that targets the identification and optimisation of the biotechnological conditions for obtaining a functional fermented beverage based on sprouted buckwheat extract, enriched with inulin and honey, and fermented with a *Lpb. plantarum* commercial strain. Using the experimental design and mathematical modeling, the optimal formulation was identified as containing 6 g/100 mL germinated buckwheat, 3 g/100 mL inulin, 10 g/100 mL honey, and 6.97 mg/100 mL inoculum. Based on model predictions, the final product may contain up to 330 mg/100 mL polyphenols, 80 mg/100 mL flavonoids, and exhibit antioxidant activity exceeding 82%. Probiotic viability, confirmed experimentally, reached 8.5 log CFU/mL and was maintained throughout 21 days of refrigerated storage (4 °C). The optimized fermentation process improved the phenolic profile and ensured both biochemical and microbiological stability of the buckwheat-based beverage, supporting its tribiotic functionality—defined by the synergistic presence of prebiotic,

probiotic, and postbiotic elements. The formulation maintained high probiotic viability and antioxidant potential during 21 days of cold storage, highlighting its functional integrity over time.

The beverage's composition and shelf-life stability make it a promising candidate for further development as a plant-based functional product. This research is relevant to the field of food biotechnology, as it addresses the increasing demand for plant-based functional foods with proven health benefits, in the modern context of the biotication of food by controlled fermentation. The idea of combining sprouted buckwheat, inulin, and honey as fermentation substrates, along with prebiotics and fermentation using a probiotic culture, led to the development of a final product with enhanced bioactive properties. This combination shows strong potential for the formulation of synbiotic beverages aimed at supporting metabolic balance, gut health, and immune function.

Author Contributions: Conceptualization, C.V. and C.N.D.; methodology, C.V.; software, A.O.D.; validation, G.E.B., C.V. and C.N.D.; formal analysis, T.V.G.; investigation, C.N.D.; resources, M.L.; data curation, M.L.; writing—original draft preparation, C.N.D.; writing—review and editing, A.O.D.; visualization, T.V.G.; supervision, G.E.B.; project administration, R.M.D.; funding acquisition, R.M.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Lactoplantibacillus plantarum	*Lpb. plantarum*
Box–Behnken design	BBD
Colony Forming Units per milliliter	CFU/mL
Response Surface Methodology	RSM
The total polyphenol content	TPC
The total flavonoid content	TFC
The free radical scavenging activity	RSA
2,2-diphenyl-1-picrylhydrazyl	DPPH

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