

Optimization of Sensory Responses and Nutritional Evaluation of Carbonated Soykunun-Zaki Beverage

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ABSTRACT

Background: Soykunun-zaki is an improved form of the traditional Kunun-Zaki beverage developed to improve the nutritional and sensory quality. Carbonation was added to add a modern touch and attract consumer interest.

Objective: This study aimed to optimize the formulation of carbonated Soykunun-zaki using response surface methodology for its sensory evaluation and to evaluate its nutritional qualities.

Methodology: Response Surface Methodology was used to optimize the formulation variables (Sample volume, stabilizer (xanthan gum) concentration, preservative (sodium benzoate) concentration, and CO₂ volume) of Soykunun-Zaki. Central Composite Design (CCD) was used to analyze the effects on sensory attributes and nutritional composition. Carbonation was achieved by injecting food-grade carbon dioxide into the beverage under controlled conditions before sensory and nutritional evaluations were conducted. The carbonation was achieved at a pressure–time combination of 50 psi at 30 s for a sample of 250 mL at 10 °C. Proximate analysis was carried out on selected samples: three (3) RSM-optimized samples, the software-predicted formulation, laboratory control, and a commercial beverage.

Results: The RSM model presented variations in the sensory responses, as indicated by the significant model terms and non-significant lack-of-fit. ($p > 0.05$) confirming good model fitness. The predicted sample with 1.5 % stabilizer, 0.15 % preservative, and 2 g CO₂ L⁻¹ produced the highest sensory scores (6.65–7.30). Proximate analysis of RSM-selected samples revealed enhanced protein (2.43–2.73 %) and ash content (1.84–2.10 %) compared with the control and commercial beverages. Anti-oxidant evaluation showed concentration-dependent radical-scavenging and ferric-reducing activities. At 5% concentration, DPPH radical-scavenging activity ranged from 45.64–47.52%, while FRAP values ranged from 35.14–58.88, with Sample S2 exhibiting the highest activity in both assays.

Conclusion: The study proved the efficiency of RSM in optimizing the formulation of traditional beverages and highlights the potential of carbonated Soykunun-zaki as a nutritionally enhanced and consumer-acceptable product.

Keywords: carbonated beverage; soykunun-zaki; response surface methodology; proximate composition; sensory acceptability

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INTRODUCTION

Traditionally, non-alcoholic cereal-based beverages make up an essential part of the food system. In many developing countries, their affordability,

cultural significance, and contribution to daily energy intake have become important. Kunun-zaki is commonly consumed in Nigeria. It is a millet-based beverage that has become a staple in many

homes and localities [1]. However, despite its popularity, traditional *kunun-zaki* production remains largely artisanal. This has resulted in poor quality, shelf instability, and limited nutritional content, overpowered by carbohydrates and relatively low protein content [2, 3]. Protein-energy malnutrition and micronutrient deficiencies remain important public health concerns in many developing countries, particularly among children and low-income populations whose diets rely heavily on cereal-based foods. Traditional *kunun-zaki* is widely consumed because of its affordability and refreshing qualities; however, it is relatively low in protein and some essential nutrients. Soybean fortification provides an effective strategy for improving the nutritional quality of the beverage by increasing its protein content and enhancing its amino acid profile. Consequently, the development of an optimized carbonated *Soykunun-zaki* may contribute to the availability of affordable, nutrient-dense indigenous beverages capable of supporting improved dietary quality and food security.

In addressing low protein content of plant-based foods, soybean fortification has been widely accepted owing to its high protein quality and functional properties [4]. It has also been used as an effective way for improving the protein content of cereal foods and beverages [5, 6]. In beverage production, soy proteins are also employed to enhance mouthfeel and creaminess, thereby influencing sensory perception [7]. Several studies have achieved this technique successfully and hence, from this approach the beverage known as *Soykunun-zaki* originated. *Soykunun-zaki* is a modification of the original *Kunun-zaki*, which evolved by addition of soymilk to fermented millet [8-10].

Furthermore, traditional beverages need a type of processing intervention that is frequently utilized in commercial beverages like soft drinks to improve freshness, texture, consumer appeal and to solve the problem of shelf instability. This application will bridge the gap between local food products and the modern alternatives. The common technique used in soft drink production is Carbonation [11]. It is an established procedure that supports preservation of the sensory properties of beverages and inhibits microbial growth [12, 13]. The main ingredient carbon dioxide (CO₂) used for carbonation is considered as GRAS (Generally Recognized As Safe) [14]. Therefore, it can be used as an easy and affordable method of enhancing shelf life of beverages. However, there is lack of progress and modernization in traditional beverages such as *kunun-zaki*. This has caused setbacks in

improvement, consumer acceptability, commercialization, and large-scale production of such products.

Although previous studies have investigated soybean fortification as a means of improving the nutritional quality of traditional cereal-based beverages, limited attention has been given to the combined application of soybean fortification, controlled carbonation, and statistical optimization for product development. Most existing studies have focused primarily on nutritional enhancement or sensory evaluation without simultaneously optimizing formulation variables and carbonation conditions. Consequently, information on the interactive effects of stabilizer concentration, preservative concentration, and carbon dioxide volume on the nutritional and sensory quality of carbonated *Soykunun-zaki* remains limited. The present study therefore bridges this knowledge gap by applying RSM to optimize the formulation of carbonated *Soykunun-zaki*, with the aim of producing a nutritionally improved beverage with enhanced consumer acceptability and commercial potential.

Response surface methodology (RSM) is a modeling tool used to provide wide data for optimizing multiple formulation and processing variables simultaneously [15]. The procedure has been used to determine interactions between parameters and has also been used to reduce the number of experimental trials, development time and overall cost in product development [16-18]. So many research works have been conducted on the optimization of beverage formulation. A constrained mixture design and multiple responses was used to study the formulation of a functional beverage. This helped in assessing the effects of varying ratios and proportions of beverage ingredients such as fruit juice, yogurt, and functional ingredients such as plant protein, fiber, or other functional compounds [19]. In another study, research work was performed to achieve a pre-determined parameter by choosing formula design based optimized criteria. A sensory test was used to determine the flavor concentration which would be added to the beverage powder and the best formula was chosen according to the highest desirability value [20]. These studies have shown the performance of optimization of the mentioned aspects of beverage formulations. However, none of them have provided information based on the optimization of beverage formulation by considering their ingredient characteristics. Therefore, RSM was employed in this study to optimize the formulation of carbonated *Soykunun-zaki* by evaluating the interactive effects of sample volume, xanthan gum concentration, sodium

benzoate concentration, and carbon dioxide volume on the nutritional quality and sensory characteristics of the beverage. The study aimed to develop an optimized formulation that combines improved nutritional value with enhanced consumer acceptability, thereby providing a scientific basis for the development of a value-added indigenous non-alcoholic beverage with commercial potential.

MATERIALS AND METHODS

Materials

Pearl millet, soybean, ginger, cloves, sugar was purchased from Kure Ultra-Modern Market, Minna, Niger State, Nigeria. Xanthan gum and Sodium benzoate was purchased at PANLAC Nigerian Enterprise both in Minna, Niger State for the preparation of *Soykunun-zaki*. Food-grade carbon dioxide (CO₂) was used for carbonation. Reagents used for proximate and antioxidant analyses were of analytical grade.

Production of Soy Milk

Soybeans were sorted to remove extraneous materials and damaged grains and then subsequently washed thoroughly. The cleaned soybeans were soaked in water, after which the soaked beans were dehulled and washed. The beans were then milled with water to obtain a slurry. The slurry was filtered to separate the milk from the insoluble residue. The resulting soy milk was collected and used in the preparation of *Soykunun-zaki*.

Production of Kunun-zaki

Pearl millet grains were sorted and cleaned to remove stones, dirt, and other foreign materials. The grains were processed with spices and water according to the established preparation procedure. The resulting slurry was filtered to obtain the *kunun-zaki* base, which was subsequently used for preparation of the *Soykunun-zaki* beverage.

Production/Formulation of Soykunun-zaki

As depicted in Figure 1, the prepared soy milk was combined with the *kunun-zaki* to produce the *Soykunun-zaki* beverage. Xanthan gum was incorporated as the stabilizer and sodium benzoate as the preservative. The beverage was thoroughly mixed using a blender to ensure uniform distribution of the ingredients.

Carbonation Levels

The carbonation level was designed based on the RSM matrix, while the infusion of CO₂ into the beverages was achieved by using a modified CO₂

cylinder coupled with a tube, regulators and pressure gauges. Carbonation levels were varied according to the RSM matrix to evaluate their effects on sensory attributes and their interaction with formulation variables. Carbonated samples were equilibrated prior to analysis to ensure uniform carbon dioxide distribution [12].

Carbonation Gas Pressure and Duration Selection

This was carried out based on trials which were conducted on the *soykunun-zaki* samples where injection pressure was applied at increment levels of 10psi for 10 seconds. The pressure application of CO₂ continued until a threshold limit was achieved by a visibly hard bottle of the sample and presence of bubbles. This was achieved at a pressure–time combination of 50 psi at 30 s for a sample of 250 ml at 10 °C [12].

Determination of Carbon Dioxide Volume in Carbonated Soykunun-Zaki Beverage

To confirm the volume of dissolved CO₂ in the *soykunun-zaki* beverage samples, the mass–volume relationships based on the density of carbon dioxide gas under standard condition was used. For this study, 2g per litre was considered which is the carbonation level suitable for non-alcoholic cereal-based beverages. To calculate the density (equation 1) of CO₂ at standard temperature and pressure (STP) is 1.977 g/L, which is equivalent 0.001977 g mL⁻¹ (equation 2). The corresponding result shows that at 250ml of *soykunun-zaki* sample, there is approximately 0.50g of CO₂ dissolved in it (equation 3) [11].

$$\text{Density} = \frac{\text{mass}}{\text{volume}} \quad (1)$$

Where, Volume of CO₂ = 2g / 0.001977gmL⁻¹. This gives a CO₂ volume of approximately 1011 mL, indicating that 2 g of CO₂ occupies about 1.01 L under standard conditions. Therefore, given a 250ml of carbonated *soykunun-zaki* sample, the mass of CO₂ present was proportionally estimated as:

$$CO_2 \text{ mass} = \left(\frac{2g}{1000ml} \right) \times 250ml = 0.50g \quad (2)$$

The corresponding gas volume was calculated as:

$$\text{Volume} = \frac{0.50 \text{ g}}{0.001977 \text{ g mL}^{-1}} \approx 253 \text{ mL} \quad (3)$$

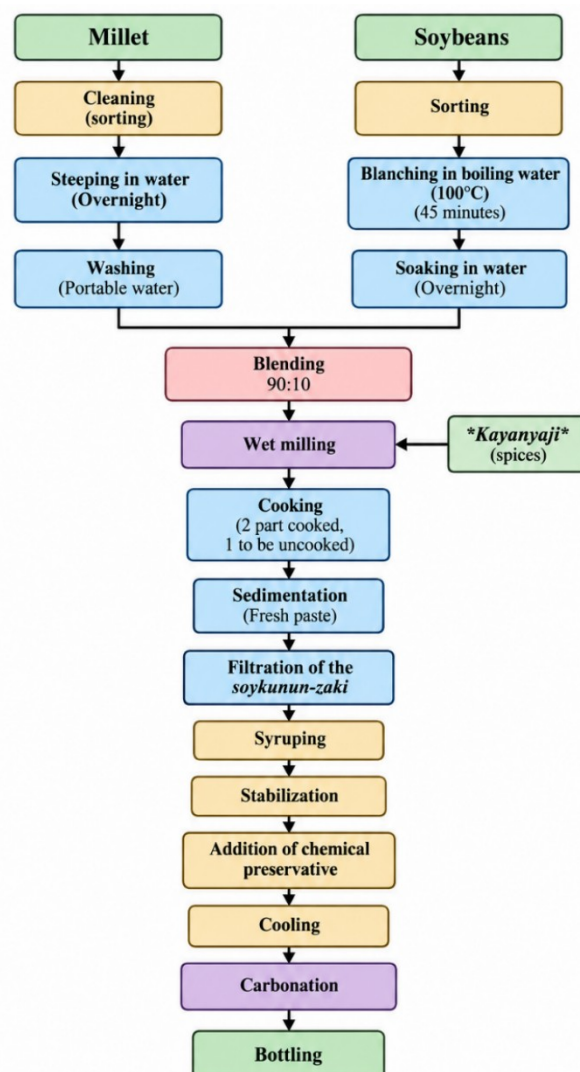


Figure 1. Flow chart for production of stabilized and carbonized Soykunun-zaki

Experimental Design/Response Surface Methodology Design

RSM was used to optimize formulation and processing variables affecting the quality of carbonized soykunun-zaki. The experimental design, factor levels (linear, 2 Factor Interactions (2FI), and Quadratic coefficients), and optimization criteria

were generated using Design-Expert software version 13. Central composite design (CCD) was employed to assess the effects of the volume of sample, stabilizer (xanthan gum), preservative (sodium benzoate), and volume of carbon dioxide. These factors varied at five levels each, presenting a total of 36 experimental runs (Table 1).

Table 1. Experimental Ranges for the Production of Soykunun-zaki.

Factors	Actual Coded Levels				
Volume of water (ml)	150L (-2)	200L (-1)	250L (0)	300L (1)	350L (2)
Stabilizer (Xanthan gum) (g)	0.5% (-2)	1.0% (-1)	1.5% (0)	2.0% (1)	2.5% (2)
Preservative (sodium benzoate) (g)	0.05% (-2)	0.1% (-1)	0.15% (0)	0.2% (1)	0.25% (2)
Volume of CO ₂ (g)	1.0g/L (-2)	1.5g/L (-1)	2.0g/L (0)	2.5g/L (1)	3.0g/L (2)

Sensory Evaluation

Sensory evaluation was carried out using consumer panels. They were subjected to the samples to assess attributes including taste, mouthfeel, aroma, color, and overall acceptability. Sensory evaluation of the carbonated Soykunun-zaki samples was

conducted using 50 panelists comprising staff and students at Federal University of Technology, Minna, Nigeria. The panelists were selected based on their willingness to participate and familiarity with ingredients used in the study. Before participation, the objectives of the study were explained to

all participants, and informed consent was obtained and treated confidentially. To minimize bias during evaluation, the beverage samples were presented in identical transparent cups and identified using randomly generated anonymous codes. The order of sample presentation was randomized for each evaluation session to reduce order and carry-over effects. Drinking water was provided to the panelists between sample tastings to cleanse the palate and minimize flavour carry-over. The panelists evaluated the samples for taste, colour, aroma, mouthfeel, consistency, and overall acceptability using a 9-point hedonic scale, where 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, and 1 = Dislike extremely. The sensory scores obtained were subjected to analysis of variance (ANOVA), and significant differences among the sample means were separated using Duncan's Multiple Range Test at $p < 0.05$ [21].

Proximate Composition Analysis

The Proximate composition was determined using standard Association of Official Analytical Chemists (AOAC) Following sensory evaluation of the 36 experimental formulations, six samples were selected for further analyses based on their overall acceptability scores. Samples S1, S2, and S3 represented the three highest-rated formulations obtained from the RSM experimental runs of the overall acceptability scores, while Sample S4 was the optimum formulation predicted by the Design-Expert software. A laboratory-prepared control (Sample S5) and a commercially available beverage (Sample S6) were included to provide baseline and market comparisons, respectively. These selected samples were subsequently subjected to proximate composition and other quality evaluations.

Moisture content was determined using the oven-drying method by drying the samples at 105 °C to constant weight. Ash content was determined by incinerating the samples in a muffle furnace at 550 °C until a light grey ash was obtained. Crude protein content was determined using the Kjeldahl method, and the nitrogen content obtained was multiplied by a conversion factor of 6.25 to estimate crude protein. Crude fat was determined using the Soxhlet extraction method with petroleum ether as the extraction solvent, while crude fibre was determined by sequential acid and alkali digestion followed by incineration of the residue. Carbohydrate content was calculated by difference using the equation:

$$\begin{aligned} \text{Carbohydrate (\%)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} \\ + \text{Crude Protein (\%)} + \text{Crude Fat (\%)} \\ + \text{Crude Fibre (\%)}] \end{aligned}$$

All analyses were carried out in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Determination of Antioxidant Capacity 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH assay was used to determine the free radical scavenging activity of the beverage samples [22]. Aliquots of diluted samples (0.63–5%, w/v) were mixed with freshly prepared DPPH methanolic solution (0.1 mM). It was incubated in the dark at 37 °C for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Radical scavenging activity was represented as percentage inhibition of DPPH relative to the control according to the equation:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{A_0 - A_s}{A_0} \right) \times 100 \quad (1)$$

where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP was determined using the method as described by Benzie and Strain [23]. The reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM) in a 10:1:1 ratio. Sample aliquots were mixed with the reagent and incubated at room temperature for 30 min. Absorbance was measured at 593 nm, and FRAP values were expressed as ferric reducing power relative to the reagent blank.

Statistical Analysis

Sensory evaluation and proximate data were subjected to one-way analysis of variance (ANOVA), and mean differences were separated using Duncan's Multiple Range Test at a 95% confidence level ($p < 0.05$). Response Surface Methodology (RSM) analysis was performed using Design-Expert® software (Version 13) to evaluate the effects of the independent variables on the sensory responses. Statistical analyses were performed at a 95% confidence level ($\alpha = 0.05$). The adequacy of the fitted quadratic models was evaluated using the coefficient of variation (CV), adequate precision, and lack-of-fit tests generated by Design-Expert® software (Version 13).

RESULTS

Model Fitting and Adequacy of the Response Surface Model

A total of 36 experimental runs (as shown in Table 2) were presented by the Design-Expert software. A Second-order polynomial regression model was used for the RSM of the sensory parameters. This was expressed as:

$$Y = \beta_0 + \beta_{AA}A + \beta_{BB}B + \beta_{CC}C + \beta_{DD}D + \beta_{AB}AB + \beta_{AC}AC + \beta_{AD}AD + \beta_{BC}BC + \beta_{BD}BD + \beta_{CD}CD + \beta_{AA}A^2 + \beta_{BB}B^2 + \beta_{CC}C^2 + \beta_{DD}D^2 + \varepsilon$$

Where:

Y represents the predicted response,

A = volume of sample,

B = stabilizer concentration,

C = preservative concentration,

D = volume of carbon dioxide.

β_0 is the intercept; β_A , β_B , β_C , β_D are linear coefficients; β_{AA} , β_{BB} , β_{CC} , β_{DD} are quadratic coefficients; β_{AB} , β_{AC} , β_{AD} , β_{BC} , β_{BD} , β_{CD} are interaction coefficients; and ε represents experimental error.

Table 2. Coded Experimental Runs and Sensory Scores of Carbonated Soykunun-Zaki Samples

Runs	A	B	C	D	Taste	Colour	Mouth feel	Aroma	Overall Acceptance
1	-1	+1	+1	+1	6.67	5.94	6.40	5.89	6.25
2	0	0	0	0	6.34	6.14	7.00	6.14	6.43
3	+2	0	0	0	6.87	6.57	6.40	7.30	6.81
4	+1	0	0	0	6.57	5.83	7.50	7.00	6.62
5	-1	-1	-1	+1	6.54	6.40	6.30	7.54	6.65
6	-1	-1	+1	+1	6.38	6.21	7.00	7.20	6.74
7	0	+2	0	0	6.40	6.71	6.40	6.66	6.56
8	+1	+2	0	0	6.00	6.57	7.00	5.89	6.47
9	0	0	0	0	6.51	6.60	6.86	6.57	6.70
10	+1	+1	-1	+1	6.46	6.14	6.70	6.71	6.55
11	0	0	0	0	6.57	6.40	6.57	6.43	6.46
12	0	0	0	0	6.60	6.70	6.49	6.86	6.69
13	0	0	0	0	6.00	6.30	6.86	7.66	6.58
14	+1	+1	+1	+1	6.29	5.77	6.30	5.91	6.02
15	0	0	+2	0	6.40	6.38	6.80	6.00	6.22
16	0	0	0	-2	6.00	6.00	6.57	6.30	6.38
17	0	-2	0	0	6.57	6.14	6.90	6.89	6.48
18	0	0	0	0	6.10	6.57	6.23	6.51	6.32
19	+1	-1	-1	+1	6.86	6.57	6.72	7.00	6.77
20	0	0	0	0	6.30	6.60	6.40	7.30	6.56
21	+1	+1	-1	-1	6.00	6.40	6.20	6.30	6.37
22	-1	+1	-1	+1	7.00	6.86	6.23	6.86	6.63
23	-1	-1	+1	-1	6.00	6.40	6.71	6.86	6.60
24	-1	-1	-1	+1	6.71	6.86	6.50	6.57	6.76
25	0	0	0	0	6.86	6.34	6.00	6.43	6.39
26	+1	-1	-1	-1	6.57	6.80	6.57	6.70	6.63
27	0	0	0	0	6.68	6.71	6.00	6.71	6.62
28	-2	0	0	0	6.09	6.60	6.50	6.09	6.41
29	0	0	0	2	6.70	6.71	6.90	6.60	6.77
30	0	0	-2	0	6.83	6.71	7.00	6.57	6.74
31	-1	+1	-1	-1	6.64	6.60	6.70	6.71	6.61
32	0	0	0	0	6.61	6.74	6.57	6.86	6.73
33	0	0	0	0	6.23	6.86	6.57	7.40	6.59
34	-1	+1	+1	-1	6.86	6.50	6.40	7.00	6.48
35	+1	-1	+1	-1	6.40	6.90	6.90	7.14	6.97
36	0	0	0	0	6.00	6.29	6.86	6.90	6.50

A = Volume of Sample; B = Stabilizer; C = Preservative; D = Volume of CO₂

Sensory evaluation of samples

The results of the model validation are presented in Table 3. The validation errors for all responses were low, indicating well-fitting models. Furthermore, 95% confidence intervals were also observed, confirming the adequacy and reliability of the optimization model for acquiring the desired

quality attributes of carbonated Soykunun-zaki. The optimization approach resulted in improvements in several sensory attributes, particularly taste, colour, and overall acceptability, while the performance of the mouthfeel and aroma models was comparatively weaker. These findings suggest that although the developed models adequately described some

responses, additional variables not investigated in this study may also influence mouthfeel and aroma.

Taste

An interactive model was selected for the RSM for taste. Table 2 shows that the Model has an F-value of 2.66 and a P-value of 0.050, which means the model is found to be significant. Other terms such as C, D, and AB were also found to be significant in the model. The Lack of Fit F-value also shows there is an 89.11% chance the model can be used to navigate the design space.

Color

For color, an interactive model was chosen, and the narration in terms of actual factors is shown in Table 3. The Model F-value of 2.25, P-values less than 0.050, and C, AD, CD imply the model is significant. Just like the taste parameter, the Lack of Fit F-value (1.59) was not significant.

Mouthfeel

A linear model was selected for the mouthfeel sensory parameter (Table 2). However, the model was not statistically significant ($p = 0.1126$), indicating that the production variables investigated did not

exert a significant linear effect on mouthfeel within the experimental range. Nevertheless, the model was retained for descriptive purposes because it adequately represented the observed trend and exhibited acceptable model diagnostic statistics, including an adequate precision of 5.80 and a non-significant lack-of-fit ($p = 0.7436$).

Aroma

The result for Aroma, as presented in Table 3, shows a linear model. The Model shows an insignificant F-value (1.86), a significant P-value ($p < 0.05$), and an insignificant Lack of Fit F-value (0.94). The stabilizer concentration (B) was also found to be significant. This could be attributed to the confirmed fact that stabilizers help retain sensory properties.

Overall Acceptance

The overall acceptance of the carbonated *Soykunun-zaki* beverage presented a linear model (Table 3). The significant model indicates that changes in variables collectively influenced the sensory attributes of carbonated *Soykunun-zaki*. The lack of fit was found to be insignificant, which is good for the model.

Table 3: Regression Coefficients of RSM Model for Sensory Attributes of Carbonated Soykunun-Zaki Beverage

Factors	Taste	Color	Mouth feel	Aroma	Overall Acceptance
Intercept					
β_0	+4.45347	+1.5603	+6.2784	+7.6605	+7.02843
Linear					
A (β_A)	+0.0081*	+0.0125*	+0.0012*	+0.0003*	-0.000096*
B (β_B)	+1.6051	+0.9099	-0.272*	-0.4333*	-0.184439*
C (β_C)	-5.1291*	+6.1996	+1.5803	-2.2133*	-1.56082*
D (β_D)	+0.2441	+2.5616	+0.1017	-0.0333*	+0.027602
Interaction					
AB (β_{AB})	-0.0079*	-0.0031*	--	--	--
AC (β_{AC})	+0.008*	+0.020*	--	--	--
AD (β_{AD})	+0.0011*	-0.0058*	--	--	--
BC (β_{BC})	+2.6227	+0.1890	--	--	--
BD (β_{BD})	-0.0217*	-0.0810*	--	--	--
CD (β_{CD})	-1.5577*	-7.1176*	--	--	--
Model					
p-value	0.0230	0.0485	0.1126	0.1419	0.0232
f-value	2.66	2.25	2.04	1.86	3.29
Lack of fit					
p-value	0.8911	0.2233	0.7436	0.5673	0.1339
f-value	0.4965	1.59	0.7254	0.9387	1.92
Mean	6.45	6.47	6.61	6.71	6.55
C.V.%	3.69	3.96	4.57	6.54	2.51
Std. Dev.	0.2384	0.2559	0.3021	0.4389	0.1643
Model Validation					
Predicted	6.46	6.47	6.61	6.70	6.56
Experimental (sample D)	6.48	6.50	6.68	6.77	6.61
Validation Error (%)	0.31	0.46	1.05	1.04	0.76
95% Confidence Interval	6.37–6.55	6.38–6.56	6.51–6.86	6.56–6.86	6.50–6.61

* $p < 0.05$. A = Volume of Sample; B = Stabilizer; C = preservative; D = Volume of CO₂; -- = interaction term not included in the selected model

Effect of the Production Process on the Response Surface Plots

The response surface plots illustrating the effects of the production variables on the sensory attributes of carbonated *Soykunun-zaki* are presented in Figures 1–5. The interaction between sample volume and xanthan gum concentration significantly influenced taste (Figure 1a), with taste scores improving at moderate stabilizer concentrations, indicating that appropriate stabilization enhanced the sensory quality of the beverage through improved viscosity and mouthfeel. Similarly, the interaction between sodium benzoate concentration and carbon dioxide volume (Figure 1b) showed that the highest taste scores were obtained at approximately 0.15% sodium benzoate and 2 g/L carbon dioxide, suggesting that moderate preservative levels combined with controlled carbonation produced the most desirable flavor profile. The response surface plots for color (Figure 2) further demonstrated that color acceptability increased with increasing sample volume and carbon dioxide concentration within the experimental range, while moderate xanthan gum concentrations produced the highest color scores. However, excessive stabilizer levels

resulted in a slight decline in color acceptability, possibly due to increased viscosity affecting the visual appearance of the beverage. The aroma response surface (Figure 4) revealed that increasing xanthan gum concentration beyond the optimum level reduced aroma scores, suggesting that excessive viscosity may have limited the release of volatile aroma compounds responsible for flavor perception. Nevertheless, all formulations maintained acceptable aroma scores, indicating that the processing conditions preserved the characteristic aroma of *Soykunun-zaki*. Similarly, the response surface plot for overall acceptability (Figure 5) showed that formulations containing relatively high sample volumes and lower xanthan gum concentrations received the highest consumer preference. Overall acceptability scores ranged from 6.02 to 6.97 on the nine-point hedonic scale, indicating that all formulations were generally liked by the panelists. These findings demonstrate that optimization of the formulation variables successfully produced carbonated *Soykunun-zaki* with desirable sensory characteristics and high consumer acceptability.

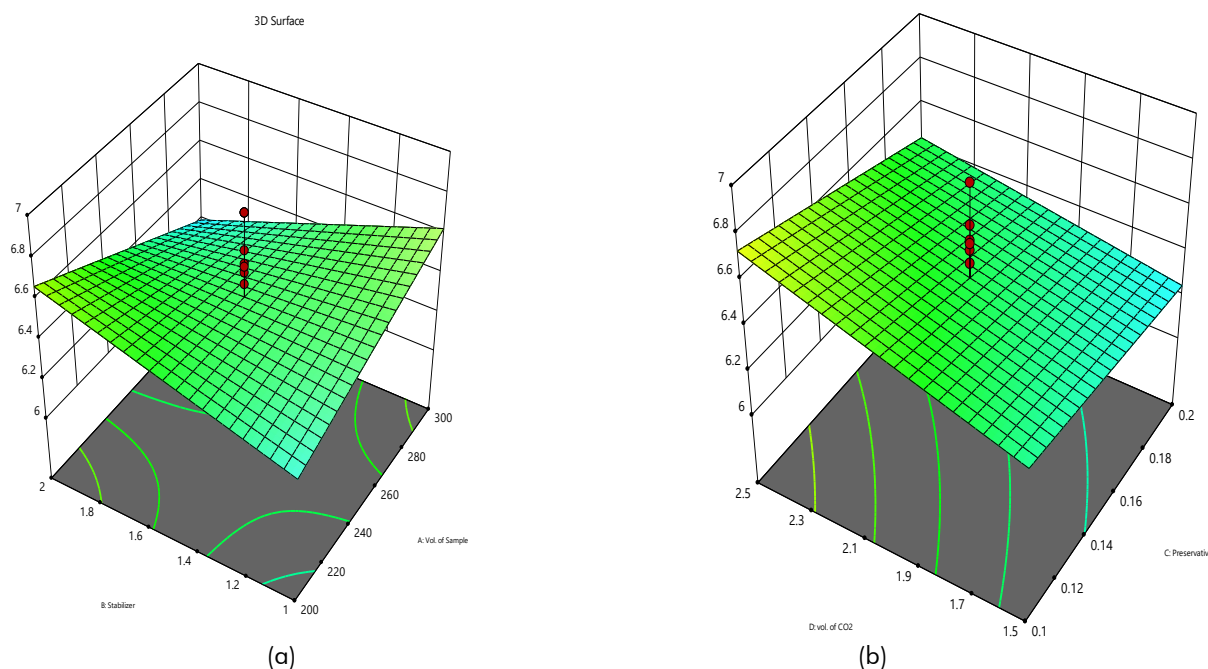
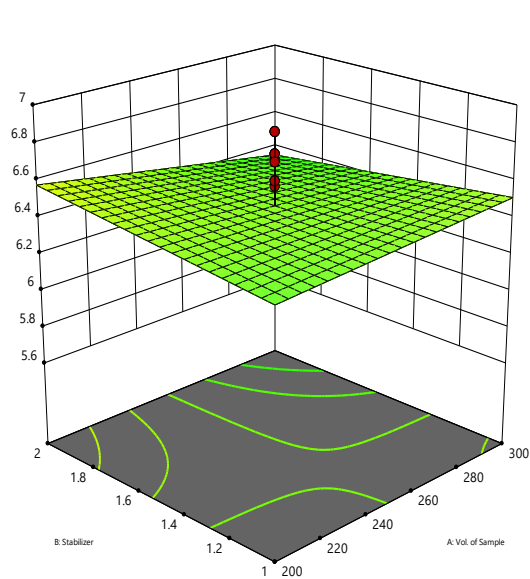
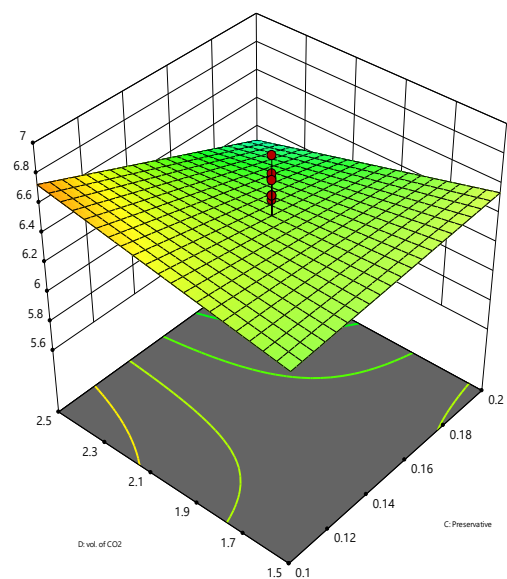


Figure 2. RSM of Taste as affected by A: Volume of sample and B: Stabilizer



(a)



(b)

Figure 3. RSM of color as affected by A: Volume of sample and B: Stabilizer

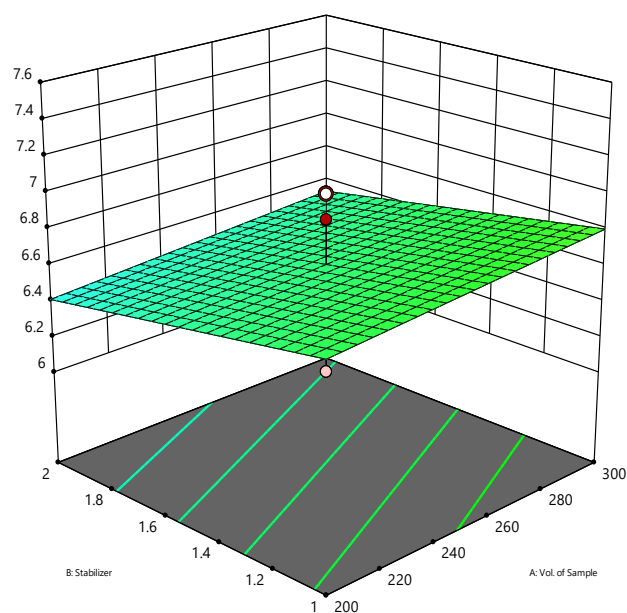


Figure 4. RSM of Mouthfeel as affected by A: Volume of sample and B: Stabilizer

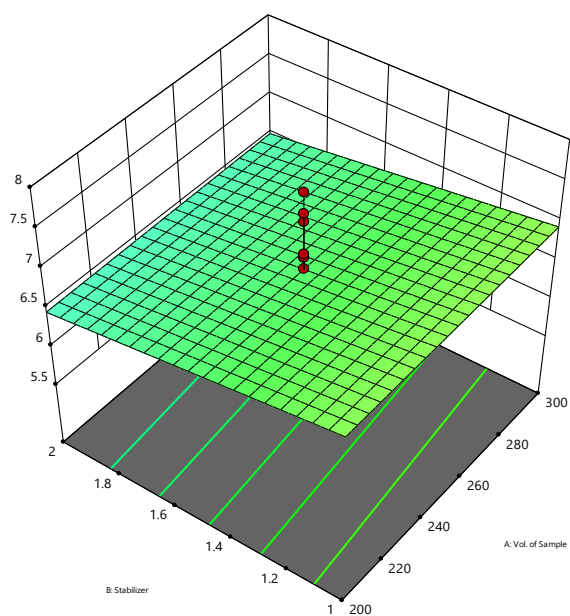


Figure 5. RSM of Aroma as affected by A: Volume of sample and B: Stabilizer

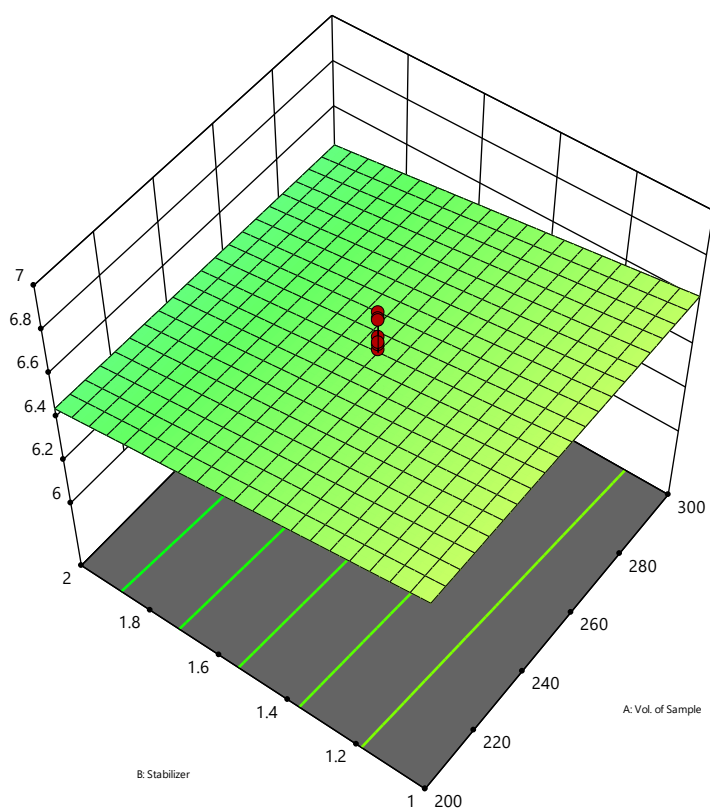


Figure 6. RSM of Overall Acceptance as affected by A: Volume of sample and B: Stabilizer

Proximate Composition Validation on the selected *Soykunun-zaki* samples

To further validate the optimization of *Soykunun-zaki* as predicted by the RSM using Design Expert, proximate analysis was conducted on selected samples. Samples were randomly selected based on the highest sensory scores in overall acceptance (samples 3, 19, 30 in Table 2). A commercial sample, a control sample, and the predicted formulation by the software were also included for the analysis (Table 4). The proximate composition results, including moisture content (MC), protein (CP), ash, fat, and carbohydrates (CHO), are presented in Table 5. The results obtained show how the optimized production process affected the nutritional profile of the beverage. The moisture content of the sample ranges from 86.05% to 91.93%. Sample S2 (91.93%) had the highest moisture content, while Sample S3 had the lowest (86.05%). Notably, the protein content of the sample increased due to the addition of soy milk in samples S1 – S5. It has been established previously that soybean enrichment to *kunun-zaki* elevates its protein content [9]. The predicted sample S4 had the highest protein content as expected, followed by other

samples. Sample S6 had the lowest protein content at 2.13%, which may suggest the intent of a commercial producer prioritizing other factors and reducing costs rather than focusing on the nutrient's composition. The ash content ranged from 1.02% to 2.10%. Samples S1, S2, and S3 have similar and higher ash content (around 2.10%), indicating a higher mineral content, while Sample S6 has the lowest ash content (1.02%) [24]. With the addition of soymilk in the other samples (S1 – S5), there is an expected boost in the ash content. The Fat content was highest in Sample S1 ($2.41 \pm 0.41\%$) and lowest in Sample S6 ($1.14 \pm 0.05\%$). The predicted sample (S4) had an average fat content ($1.92 \pm 0.00\%$), which would likely contribute positively to mouthfeel without creating an overly rich texture. The carbohydrate content of most commercial beverages is usually high [25]. This is because they are tailored to give a quick energy boost, which appeals greatly to consumers [26]. Resultantly, Sample S6 (commercial sample) had the highest carbohydrate content ($8.97 \pm 0.50\%$), while Sample S2 had the lowest ($1.31 \pm 0.05\%$).

Table 4: Formulation and Processing Factors of the Selected Samples

SAMPLE/FACTORS	S1	S2	S3	S4	S5	S6
Volume of water (ml)	350	300	250	287	250	250
Stabilizer (Xanthan gum) (g)	1.5	1	1.5	1.6	--	--
Preservative (sodium benzoate) (g)	0.15	0.1	0.15	0.1	--	--
Volume of CO ₂ (g)	2	2.5	2	2.5	--	--

S1: Sample 3 from RSM overall Sensory score; S2: Sample 19 from RSM overall Sensory score; S3C: Sample 30 from RSM overall Sensory score; S4: Predicted sample formulation (Design Expert); S5: Control; S6: Commercial sample; --: Not included

Table 5: Proximate Composition of Selected *Soykunun-Zaki* Samples

PARAMETER	S1	S2	S3	S4	S5	S6	SEM	LSD
Moisture Content	90.02 ± 0.01^b	91.93 ± 0.04^a	86.05 ± 0.04^d	86.69 ± 0.04^d	87.46 ± 0.01^c	86.24 ± 0.05^d	88.16	*
Protein	2.43 ± 0.05^d	2.62 ± 0.01^b	2.49 ± 0.05^c	2.73 ± 0.01^a	2.72 ± 0.05^a	2.13 ± 0.01^e	2.51	*
Ash	2.10 ± 0.01^a	2.09 ± 0.05^a	2.08 ± 0.05^a	1.84 ± 0.00^b	1.17 ± 0.05^c	1.02 ± 0.00^e	1.17	*
Fat	2.41 ± 0.41^a	2.05 ± 0.01^a	1.87 ± 0.01^a	1.92 ± 0.00^a	1.83 ± 0.05^a	1.14 ± 0.05^b	1.87	*
Carbohydrate	3.13 ± 0.33^c	1.31 ± 0.05^d	7.21 ± 0.70^b	7.25 ± 0.90^b	7.25 ± 0.44^b	8.97 ± 0.50^a	5.79	*

Key: means on the same row with the same superscript are significant $P \leq 0.05$. S1: Sample 3 from RSM overall Sensory score; S2: Sample 19 from RSM overall Sensory score; S3C: Sample 30 from RSM overall Sensory score; S4: Predicted sample formulation (Design Expert); S5: Control; S6: Commercial sample

Antioxidant Properties

The antioxidant activity of the carbonated *Soykunun-zaki* samples was evaluated using DPPH radical-scavenging activity and ferric reducing antioxidant power (FRAP), and the results are presented in Figures 6 and 7, respectively. Significant differences ($p \leq 0.05$) were observed among the samples at the different concentrations evaluated.

The DPPH radical-scavenging activity of the samples decreased progressively with dilution. At 5% concentration, the DPPH inhibition values ranged from 45.64 to 47.52%, while at 0.63% concentration, the values ranged from 0.22 to 3.88%.

Sample S2 recorded the highest DPPH inhibition across the concentrations evaluated, with values of 47.52, 24.87, 11.04, and 3.88% at 5, 2.5, 1.25, and 0.63% concentrations, respectively. At 0.63% concentration, Sample S3 and Sample S6 recorded the lowest DPPH inhibition values of 0.22 and 0.67%, respectively. The FRAP results showed a trend similar to that observed for DPPH activity. At 5% concentration, FRAP values ranged from 35.14 to 58.88, with Sample S2 recording the highest reducing power (58.88) and Sample S6 recording the lowest (35.14). The FRAP values decreased with decreasing sample concentration across the evaluated samples.

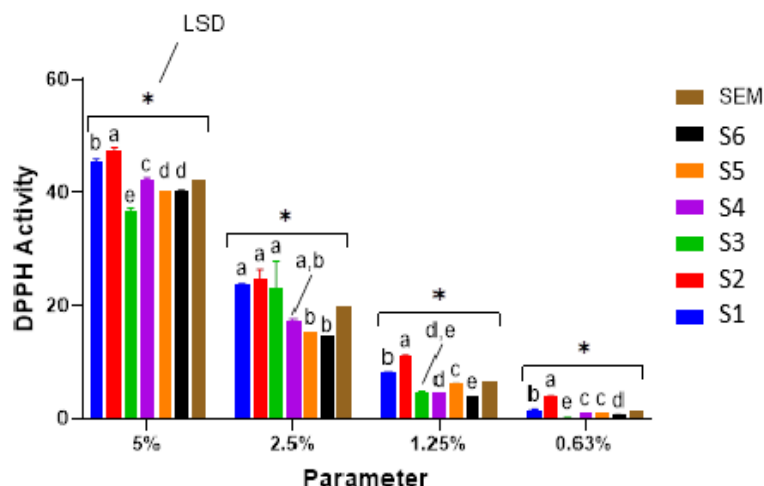


Figure 7. DPPH radical-scavenging activity of carbonated Soykunun-zaki samples.

Key: Means with the same superscript within a concentration level are not significantly different at $p \leq 0.05$. S1: Sample 3 from RSM overall Sensory score; S2: Sample 19 from RSM overall Sensory score; S3C: Sample 30 from RSM overall Sensory score; S4: Predicted sample formulation (Design Expert); S5: Control; S6: Commercial sample

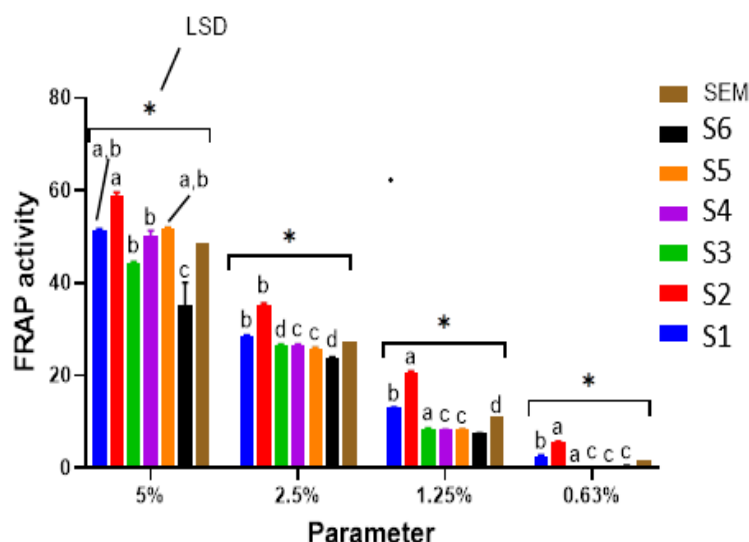


Figure 8. Ferric Reducing Antioxidant Power of Carbonated Soykunun-Zaki Samples.

Key: Means with the same superscript within a concentration level are not significantly different at $p \leq 0.05$. S1: Sample 3 from RSM overall Sensory score; S2: Sample 19 from RSM overall Sensory score; S3C: Sample 30 from RSM overall Sensory score; S4: Predicted sample formulation (Design Expert); S5: Control; S6: Commercial sample

DISCUSSION

The improvement in the sensory properties of carbonated Soykunun-zaki with increasing xanthan gum concentration shows the important role of stabilizers in beverage quality. The effect of Xanthan gum is achieved through thickening of the continuous phase, reducing sedimentation, and enhancing product stability. Xanthan gum increased the viscosity of the beverage, thereby improving its body, mouthfeel, and overall sensory acceptance. This is also in accordance with Stokes' law [21], as xanthan gum functions as a hydrophilic polysaccharide. This mechanism promotes a more homogeneous beverage system and improves physical

stability throughout storage. The present findings agree with those of Koksoy and Kilic and Liu et al. [17,18], who reported that stabilizers improved viscosity and prevented phase separation in yoghurt (ayran) and other beverage systems.

Taste was also significantly influenced by preservative concentration, carbon dioxide volume, and their interaction. This may be attributed to the formation of carbonic acid following the dissolution of carbon dioxide, which enhances flavor perception and provides the characteristic refreshing sensation associated with carbonated beverages. Similar observations have been reported in

carbonated beverage systems, where carbonation improves flavor perception while appropriate preservative levels help maintain sensory stability [19]. The RSM of Color showed that acceptability of *Soykunun-zaki* improved with increasing carbon dioxide volume and decreasing preservative concentrations. Carbon dioxide has a preservative nature, which may have helped in maintaining color quality. This occurs by reducing oxidative changes during beverage processing and storage. Similar trends were reported in beverage studies, where carbonation improved visual appeal and overall consumer preference [20].

In addition to enhancing the sensory properties, xanthan gum contributed positively to mouthfeel and texture, resulting in a smoother and more uniform beverage. This effect is common for similar hydrocolloid-stabilized beverages [22]. The sensory evaluation further indicated that formulation variables influenced the aroma of the beverage. The samples with incorporated soybeans were observed to have maintained desirable aroma characteristics. This could be attributed to the volatile aroma compounds found in soybeans. These compounds are known to possess flavor-binding properties [23]. Although soymilk is often associated with a characteristic beany flavor resulting from lipoxygenase activity, this undesirable aroma was minimized through thermal treatments, including blanching and pasteurization, which effectively inactivated the lipoxygenase enzyme before incorporation into the beverage [24,25]. This processing approach helped preserve the traditional aroma of *kunun-zaki* while simultaneously improving its nutritional value through soybean fortification.

The moisture contents of the carbonated *Soykunun-zaki* samples were within the typical range reported for beverage products (85–98%), indicating that the formulations possessed the desirable fluid characteristics expected of non-alcoholic beverages. High moisture content is a common feature of cereal-based beverages and contributes to their refreshing nature, ease of consumption, and palatability [27]. Similar moisture ranges have been reported for soybean-fortified *kunun-zaki* and related cereal beverages [2, 28], suggesting that the incorporation of soymilk, stabilizer, preservative, and carbon dioxide did not adversely affect the beverage matrix. The relatively higher protein contents observed among the optimized *Soykunun-zaki* formulations demonstrate the nutritional contribution of soybean fortification to the beverage (2.43 – 2.73% for samples S1- S6). This trend is like a study on a similar beverage, where a higher protein content was recorded for an optimized

sample fortified with soymilk. The findings showed that the protein content of Soymilk Beverage was 4.88% and increased for Pearl Millet Soymilk Beverage to 5.21% [29]. Soybean is an excellent source of high-quality protein and contains essential amino acids, particularly lysine, which complements the amino acid profile of cereal proteins such as pearl millet [30]. Consequently, the incorporation of soymilk enhanced the nutritional quality of the beverage while maintaining desirable sensory characteristics. These findings are consistent with previous findings where soybean supplementation significantly improves the protein content and nutritional value of cereal-based beverages [31, 32]. Although the optimized formulation recorded the highest protein content, the difference between the optimized and control formulations was minimal, suggesting that optimization enhanced nutritional quality through soybean incorporation. In contrast, the comparatively lower protein content of the commercial beverage indicates differences in formulation and highlights the nutritional advantage of the soybean-fortified formulations developed in this study.

Ash content provides an estimate of the total mineral composition of food products and is therefore an important indicator of nutritional quality. The ash content ranged from 1.02% to 2.10%. Samples S1, S2, and S3 have similar and higher ash content (around 2.10%), indicating a higher mineral content, while Sample F has the lowest ash content (1.02%). Most of the soybean-fortified formulations had higher ash contents compared with the control. This suggests improved mineral composition such as calcium, phosphorus, magnesium, potassium, and iron resulting from soybean incorporation. Furthermore, such fortified beverages will support normal physiological functions, bone development, and metabolic activities for consumers [33, 34]. Similarly, because Soybean is naturally rich in unsaturated fatty acids and essential lipids, most of the optimized *Soykunun-zaki* formulations had higher fat content. However, the fat percentage was found to be 2.15% in Soymilk Beverage, while 2.56% in the optimized beverage [29]. Lower values recorded for total fat during fermentation may be attributed to the breakdown of fat molecules during this process [35]. Overall, fat content may help the beverage by contributing to required dietary energy and facilitating the absorption of fat-soluble vitamins [36]. In addition, the acceptable mouthfeel observed in the optimized formulation may also be attributed to the combined effects of soybean lipids and xanthan gum. While lipids contribute to creaminess and lubrication, xanthan gum improves viscosity and texture by stabilizing

the beverage matrix. Similar observations have been reported in yoghurt and other hydrocolloid-stabilized beverages, where appropriate fat levels in combination with stabilizers improved texture, mouthfeel, and overall consumer acceptance without adversely affecting product quality [37].

The carbohydrate content of the formulations reflects the major contribution of pearl millet, which constitutes the main ingredient in *Soykunun-zaki*. Soybean supplementation generally increases the protein proportion of cereal-based foods, which may consequently influence the relative carbohydrate content of the final product. Hence, Sample S6 (commercial sample) had the highest carbohydrate content ($8.97 \pm 0.50\%$), while Sample S2 had the lowest ($1.31 \pm 0.05\%$). This trend has been reported in soybean-fortified cereal beverages, where improvements in protein composition were accompanied by corresponding changes in carbohydrate levels [38, 39]. As previously reported, the carbohydrate content was slightly higher in beverages with a combination of Pearl Millet and Soymilk (4.03%) than in Soymilk Beverage (3.08%) only [29, 40]. However, the optimized formulations demonstrated that improvements in nutritional composition can be achieved while maintaining acceptable sensory quality. The nutritional improvements associated with soybean fortification, together with the enhanced sensory acceptability of the optimized formulation, highlight the potential of carbonated *Soykunun-zaki* as a nutritious alternative to conventional non-alcoholic beverages.

The antioxidant activity observed in the carbonated *Soykunun-zaki* samples indicates that the beverage contains constituents capable of participating in both radical-scavenging and reducing reactions. Sample S2 consistently exhibited the highest DPPH activity, suggesting that its formulation was associated with greater retention or availability of antioxidant constituents compared with the other samples. This may be related to differences in the formulation of the beverage, particularly the levels of stabilizer and carbonation. Hydrocolloids such as xanthan gum can modify the physical structure of beverage systems and may influence the retention and accessibility of compounds within the food matrix. Increased viscosity may also reduce oxygen diffusion and consequently contribute to the preservation of oxidation-sensitive compounds [41]. The presence of antioxidant activity in *Soykunun-zaki* may also be associated with the raw materials used in its preparation. Soybean contains phytochemicals such as isoflavones, phenolic acids, and flavonoids that have been reported to possess radical-scavenging properties [42]. Similarly,

millet contains phenolic constituents, including hydroxycinnamic acid derivatives such as ferulic and *p*-coumaric acids, which may contribute to its antioxidant potential [43]. Thus, incorporation of soybean into the millet-based beverage may contribute not only to its protein and overall nutritional composition but also to its antioxidant characteristics.

The FRAP results followed a pattern like that observed for DPPH, with Sample S2 exhibiting the highest reducing power and Sample S6 the lowest at 5% concentration. The agreement between the two assays is relevant because DPPH and FRAP assess antioxidant activity through different mechanisms. DPPH primarily reflects the ability of antioxidant constituents to donate hydrogen atoms to stabilize free radicals, whereas FRAP measures the electron-donating capacity of constituents through the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions [44]. The similar trends observed in both assays therefore provide complementary evidence of the antioxidant potential of the beverage samples. The higher FRAP value observed for Sample S2 may similarly be associated with differences in formulation and the presence and availability of antioxidant constituents within the beverage matrix. The protective effect of stabilizers may also be relevant because hydrocolloids can alter the microenvironment of food constituents and reduce oxygen diffusion within the matrix [45, 46]. Such effects could contribute to the retention of oxidation-sensitive compounds during processing and storage [47].

Overall, the DPPH and FRAP results show that carbonated *Soykunun-zaki* has measurable in vitro antioxidant capacity. However, the significance varies among formulations and concentrations. The antioxidant characteristics of a combination of soybean and millet suggest that the carbonated beverage has potential as a value-added indigenous beverage with both nutritional and functional attributes. Therefore, further characterization of the individual antioxidant constituents would be useful for establishing the specific compounds responsible for the observed activity.

LIMITATIONS OF THE STUDY

This study was limited to the optimization of selected formulation variables and sensory evaluation using a panel of 50 participants. Other factors that may influence product quality, such as long-term consumer acceptance across different population groups, packaging materials, storage under varying environmental conditions, and large-scale industrial production, were not investigated. The antioxidant evaluation was based on in vitro DPPH

radical-scavenging and FRAP assays, which provide an indication of antioxidant capacity but do not directly establish the bioavailability or physiological effects of the antioxidant constituents after consumption. In addition, individual antioxidant compounds responsible for the observed activities were not quantified or characterized. Further studies involving identification and quantification of specific phenolic compounds, isoflavones, and other antioxidant constituents, as well as assessment of their stability during storage and bioavailability, are therefore recommended. Future studies should evaluate these factors and validate the optimized formulation under commercial production conditions.

CONCLUSION

The study showed that RSM can effectively optimize the formulation of *Soykunun-Zaki* beverage and improve its sensory properties. The addition of soymilk improved nutritional composition, particularly the protein content, with the predicted Sample S4 showing the best overall nutritional and sensory quality. The beverage also demonstrated antioxidant activity through DPPH radical-scavenging and ferric-reducing abilities, indicating potential functional benefits. Overall, RSM can be used to develop a nutritious, stable, and commercially acceptable carbonated *Soykunun-Zaki*, although further research is needed to confirm its microbial stability.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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