Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-026-04212-1

ORIGINAL PAPER

Modeling and process optimization of *Zoom-koom* beverage production using Box–Behnken design

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Received: 14 October 2025 / Accepted: 16 February 2026

Abstract

Zoom-koom is a traditional beverage derived from millet or sorghum grains through spontaneous lactic fermentation. It is an excellent source of nutrient and bioactive compounds. However, the processing conditions of *zoom-koom* have not been standardized and this leads to inconsistent product quality. The effects of three independent variables (blend ratio (millet: spices), steeping time and steeping temperature) on four major response variables-physicochemical properties, bioactive qualities such as DPPH scavenging activity and total phenolic compounds), microbial quality and sensory properties of the *zoom-koom* beverage were therefore studied using response surface methodology (Box Behnken design). In the laboratory processed *zoom-koom*, sodium benzoate was used as an anti-microbial agent to control the levels of the microbes which may ferment the product leading to a non-fermented product. Mathematical models and optimum levels of the response variables were generated. Blend ratio, steeping time and steeping temperature had significant effects ($P \leq 0.05$) on the response variables. The prediction of the desirability model, based on 95% confidence level in the range of the independent variables, gave optimal treatment conditions of blend ratio of 700:100 (millet: spices), steeping time of 6 h and a constant temperature of 35 °C. Based on the responses of the sensory panel, the desirability index obtained was between 0.741 and 1.000. The results were desirable, and the mathematical models developed could be used to predict the outcome of the response variables to a high degree of accuracy. *Zoom-koom* can be processed under optimized conditions, providing a unique taste, enhancing its bioactive activity, and ensuring its microbiological safety. *Zoom-koom* can be processed under optimized conditions to provide better sensory qualities, microbiological safety, enhanced proximate composition and bioactivity.

Keywords Zoom-koom · Response surface methodology · Physicochemical · Total phenolic compounds · DPPH Scavenging activity · Microbial quality · Sensory properties

Introduction

Zoom-koom is one of Ghana's traditional fermented beverages that is usually consumed in the Northern part of the country and in some parts of the capital city (Accra) especially Nima and Koforidua in the Eastern Region. The beverage which is usually consumed in a liquid form is also native to some West African countries such as Nigeria and Burkina Faso with varying methods of production based on the country of origin. *Zoom-koom* is commonly made from pearl millet, is gluten-free with low acid content, and importantly, easy to digest, and has low glycemic index [1, 2], making it an appropriate choice for people with diabetes and celiac disease [3]. The beverage contains protein (25.83–28.41% w/w), reducing sugars (32.1–36.9% w/w),

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Published online: 17 March 2026

calcium (392.0–435.0.0.0 mg/100 g DM, magnesium (59.0–65.0 mg/100 g DM) and iron (2.37–2.50 mg/100 g DM) [4]. In the Ghanaian culture, traditional beverages are important in social events. Branding of such beverages is now gaining some prominence [5]. There is therefore the need to improve the quality of traditional beverages through improved processing techniques. The attributes of a product influence its consumer demands [6]. Currently, no systematic study on the optimization of t he production processes and characteristics of *zoom-koom* has been carried out. *Zoom-koom* produced through spontaneous fermentation using different steeping times, temperatures and spices gave a product of inconsistent quality characteristics [7]. In the same study, traditional method of *zoom-koom* production gave a product of increased enterobacteria count which was unsafe for consumption. Inconsistent product quality and high enterobacteria count have the tendency to make quality standardization and commercialization challenging. Furthermore, increasing consumer education and consciousness on food quality and safety demands that *zoom-koom* of consistent and predictable quality should be produced. A thorough and systematic analysis of *zoom-koom* and its production process can help produce an optimized process that will ensure the manufacture of a product with consistent quality. Taking these considerations into account, this study aimed to optimize key processing parameters-blend ratio (millet: spices), steeping temperature, and steeping time-with respect to the beverage's physicochemical properties, bioactivity, microbial quality, and sensory attributes of *zoom-koom* beverage to meet the demands and acceptance of both domestic and international markets. Also, the proximate composition and the sensory qualities of the optimized *zoom-koom* were compared with the control.

Materials and methods

Apparatus

The following apparatuses were used for the experimental purposes: Eppendorf centrifuges and tubes (Thermo Fisher Scientifc, Waltham, USA) Analytical Balance Sartorius B20-Germany Genius analytical balance (Genius Electronic Company, Mumbai, India); falcon tubes (50 mL) (Thermo Fisher Scientifc, Waltham, USA), and micropipettes (P1000) (Gilson, Middleton, USA).

Table 1 Variables and their levels used in the Box-Behnken design

Variables	Symbols	Levels
	Coded	−1, 0, 1
Blend ratio (g) of steeped millet and spices	X1	700:50 700:100 700:150
Steeping time (hrs)	X2	2, 6, 12
Steeping temperature (°C)	X3	25, 35, 45

Reagents

The reagents (DPPH, Folin, Aluminium chloride, Sodium carbonate, Potato Dextrose agar, MacConkey agar, Mannitol Salt Agar and Violet Red Bile Glucose Agar) used for the study were purchased from Santa Cruz Biotechnology, Inc.10,410 Finnel St. Dallas- USA, TX 75,220 and Oxoid Ltd., Basingstoke, Hampshire, England.

Methods

Experimental design and data analysis

A 3 level 3 factor Box Behnken-design (BBD) using Minitab (Version 20, Minitab Inc., UK) was used to evaluate the effect of processing parameters such as the blend ratio of the millet and the spices, the steeping temperature and the steeping time on the physicochemical, microbiological, bioactivity and the sensory quality of the *zoom-koom* beverage. Three different levels of each independent variable were studied. The factors were chosen because they were noted to have greater influence on the overall acceptability of the *zoom-koom* beverage based on an in-person interview conducted with some processors in Ghana. The blend ratio of millet to spices of 700:50, 700:100 and 700:150 was chosen based on estimated quantity of the raw materials used by the various processors. The pearl type of millet was the main material, with ginger, cloves and black pepper in the ratios of 50: 25: 25 considered as the spices. The overall percentage of the spices (ginger, cloves and black pepper) blended with the millet were 7, 14 and 21% respectively. A total of 15 experiments (Different sets of combinations) were conducted for three independent variables (Table 1). The relation between the independent and dependent variables was illustrated by the following equation:

$$\mathrm{Y}_1 = \beta\mathbf{0} + \beta\mathbf{1} \times \mathbf{1} + \beta\mathbf{2} \times \mathbf{2} + \beta\mathbf{3} \times \mathbf{3} + \beta\mathbf{11} \times \mathbf{1} + \beta\mathbf{22} \times \mathbf{2} + \beta\mathbf{33} \times \mathbf{3} + \beta\mathbf{12} \times \mathbf{1} \times \mathbf{2} + \beta\mathbf{13} \times \mathbf{1} \times \mathbf{3} + \beta\mathbf{23} \times \mathbf{2} \times \mathbf{3} \tag{1}$$

Y_1 was the response; X_1, X_2 and X_3 were the independent variables; β_0 was the intercept; β_1, β_2 and β3 were linear coefficients; β_{11}, β_{22} and β_{33} were square coefficients; and β_{12}, β_{13} and β_{23} were interaction coefficients. The design was used to identify the combinations of process variables that provided the best quality characteristics for the following dependent variables: sensory acceptability, physicochemical properties, bioactive properties, and microbial quality of *zoom-koom*.

Mean ± SD was used to summarize physicochemical, bioactive properties, microbiological counts and sensory values. ANOVA was used to check the significant differences at an alpha level of 0.05.

Differences in mean values were assessed using a post hoc Tukey test using Minitab software Version 20.0. For the optimization, response surface regression techniques were used to analyse the experimental data. The runs were carried out in triplicate, and the mean results were reported. Mini tab software Version 20 was used for the data analysis. To be more precise, a strong correlation is defined by the model's coefficient of determination (R^2), which should be greater than 0.8000 for the model to be classified as predictive [8]. For the fitness of the model to be evaluated, the ANOVA was conducted at a confidence level of 95%.

Preparation of *zoom-koom*

One kilogram of pearl millet grains and the spices (ginger, cloves and black pepper in the ratio of 25: 25: 50 respectively) were washed (Fig. 1). The grains (1 kg) were steeped in 3 L of water and 5 g of sodium benzoate (5 g/1 kg grain weight in 3 L of water) to control microorganisms that may be present in the steeped sample [9]. The amount of sodium benzoate added was based on a preliminary test conducted in the laboratory where many of the fermentable organisms in the steeped water were controlled. Afterwards, the steeped grains were washed several times and blended using a multifunction robot blender (SHB-3088). The blend ratio used for pearl millet and spices was based on the experimental design in Table 2. Pearl millet slurry was then prepared from the blended product using water to millet ratio of 10:1. The extract from the slurry was filtered using a muslin cloth to obtain the wort. Sucrose (table sugar) 0.050 g/L was added to produce the final *zoom-koom.* The final *zoom-koom* product was then transferred to a glass bottle and stored at refrigeration temperature (5 °C) before further analysis. The *zoom-koom* produced was non-fermented because the sodium benzoate added controlled the growth of microorganisms that may ferment the millet.

Physicochemical analysis of *zoom-koom*

The physicochemical parameters of the *zoom koom* determined were pH, titratable acidity, brix, and colour. The pH

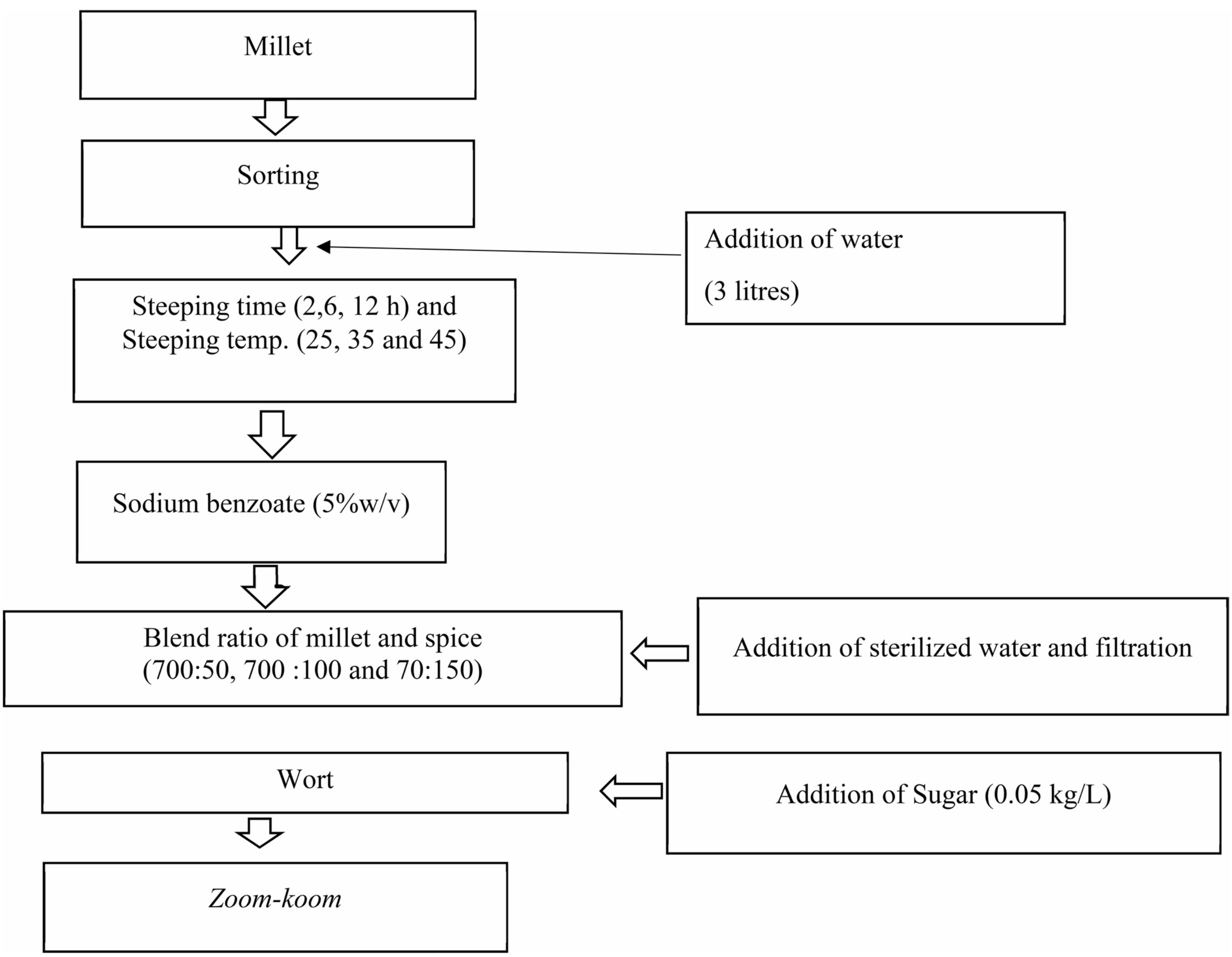


Fig. 1 Process flow showing Laboratory Processing of *zoom-koom.* [7]

Table 2 Box-Behnken Design matrix of variables (k = 3) for optimization of the *zoom-koom*

Runs	X_1	X_2	X_3
1	700:50	2	35
2	700:150	2	35
3	700:50	12	35
4	700:150	12	35
5	700:50	7	25
6	700:150	7	25
7	700:50	7	45
8	700:150	7	45
9	700:100	2	25
10	700:100	12	25
11	700:100	2	45
12	700:100	12	45
13	700:100	7	35
14	700:100	7	35
15	700:100	7	35

of the samples was evaluated using an Orion 2-star pH meter as described [10]. Sixty millilitre sample of *zoom-koom* was placed in a beaker and the pH recorded after calibration of the instrument according to the manufacturer's instructions. The titratable acidity, expressed as percent lactic acid, was calculated using the [10] method, which involved titrating 10 mL of sample against 0.1 N NaOH with 1% phenolphthalein (2–3 drops) as an indicator until a light pink color appeared (endpoint). Triplicate determinations per sample were made. The following formula was used to compute the titratable acidity:

$$\text{Titratable Acidity (\% Lactic acid)} = (V \times N \times 9.08)/(W \times 10) \quad (2)$$

Where V = titre value.

N = normality of the titrant.

W = sample weight.

9.08 = Equivalent weight of predominant acid (lactic acid).

Brix was determined using a digital refractometer (Hanna digital refractometer model HI 96801) at room temperature [10]. The colour of *zoom-koom* samples was determined using a colourimeter (Digital handheld colorimeter-FRU® 10QC160226). Samples of *zoom-koom* were poured into a plastic petri dish. Three different colour readings ($L^*a^*b^*$) were done on each sample and the mean of the CIE L* a* & b* colour value index calculated. Colour change (ΔE) was calculated using the formular:

$$\triangle E = \sqrt{(L_\circ{}^* - L^*) + (a_\circ{}^* - a^*) + (b_\circ{}^* - b^*)} \quad (3)$$

The control *zoom-koom* sample (sample bought from a processor) was the reference point, with colour parameters L* a* and b*. L is lightness, a is the positive value of red and negative value of green; b is the positive value of yellow and the negative value of blue [8].

Proximate composition of optimized *zoom-koom*

The proximate composition (moisture content, crude protein, crude fat, crude fibre, ash, carbohydrate and Energy) of optimized *zoom-koom* and the control was determined using the AOAC method [11].

Microbiological analysis of *zoom-koom*

Ten gramme of the sample was measured and homogenized in a sterile stomacher bag for 1 min. using a stomacher blender (Steward Stomacher blender 400 circulator) in 90 mL of 0.1% peptone water to suspend the microorganisms. The stock preparation was serially diluted from 10^{-1} to 10^{-9} and the appropriate dilution factor used [8]. All microbiological media used: Potato Dextrose agar for yeasts and moulds, MacConkey agar for *E. coli*, Mannitol Salt Agar for S*taphylococcus aureus* and Violet Red Bile Glucose Agar for *Enterobacteriaceae* (*E. coli*) were prepared following the instructions specified by the manufacturer. For the enumeration of total bacteria, yeasts and moulds, total coliform, *Escherichia coli* and *Staphylococcus aureus*; aliquot (0.1 mL) of appropriate dilutions was pipetted onto duplicate sterile agar plates using the spread plate technique. The MacConkey agar, Mannitol Salt Agar and Violet Red Bile Glucose Agar plates were incubated in an inverted position at 37°C for 24 h while Potato Dextrose agar plates were incubated at 25°C for 3–5 days. The agar plates of De Man Rogosa Sharpe agar were incubated anaerobically in an anaerobic jar at 37°C for 24 h [8].

Chemical analysis of *zoom-koom*

Total polyphenol determination

Using a slightly modified [12] method, the total phenol content of *zoom-koom* drink was estimated with gallic acid as the reference standard. Briefly, 1 mL aliquot of sample was mixed with 2 mL Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 4 mL sodium carbonate solution (7.5% w/v). The mixture was then incubated for 30 min. with intermittent shaking and absorbance values were read at a wavelength of 765 nm with a UV-VIS spectrophotometer (Ultraspec Pro). All measurements were performed in triplicate for each analysis. The total phenolic content was determined from the linear equation of standard gallic acid, and the result was expressed as mg/mL gallic acid equivalent (GAE).

Determination of antioxidant activity of *zoom-koom*

The DPPH assay was carried out according to [13] with slight modifications. The sample (1 mL) was combined with 3 mL of DPPH solution (4 mL of stock DPPH solution in 96 mL of 80% methanol) and maintained at room temperature for 30 min. A UV-Vis spectrophotometer was used to measure the absorbance of the mixture at 520 nm (Ultraspec Pro). As a blank, a mixture of 1 mL methanol and 3 mL DPPH solution was utilized.

$$\begin{aligned}&\%\, Inbition\\&= (A\, control - A\, sample)/(A\ \ control)\ \times\ 100\end{aligned} \tag{4}$$

where A control = absorbance of blank.

A sample = the absorbance of the extract and DPPH solution.

Consumer sensory acceptance of *zoom-koom*

The sensory acceptability of the 15 *zoom-koom* samples obtained as detailed in Table 2 was assessed. The study used the balanced incomplete block design described by Cochran and Cox (1957) and Montgomery (2001) with 15 treatments (samples), k = 5 (samples per judge), r = 7 (replicates), b = 21 (number of blocks/judge), = 2, and N = 120. Each panellist was allowed to assess five samples at a time (5 min. break time was observed in between the sampling) because it would be increasingly challenging for a single consumer to evaluate all 15 *zoom-koom* samples in one session without experiencing fatigue. A total number of 120 consumers consisting of 90females and 30males were recruited randomly from the University of Ghana Legon and used for the assessment. The panellists were given the *zoom-koom* served at a temperature 5 °C in disposable cups that were randomly generated with three-digit codes. Panellists were asked to evaluate the samples in sensory booths based on the taste, flavour, aftertaste, appearance, aroma, and overall acceptability using a 9-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely [8].

Verification of optimized conditions

To verify that the predictive models obtained were reliable for determining consumer acceptability of *zoom-koom*, samples from two process treatment combinations were selected from within the optimum region and compared with the control [Table 3 (S16)]. The control was a sample bought from a processor with blend ratio (millet: spices) of 1000: 500, steeping time of 14 h at a steeping temperature of 25 °C. The identity of the control was obtained from interview conducted with a processor. The three *zoom-koom* samples were then subjected to sensory evaluation by the same panel of judges to ascertain their consumer preference. The predicted and validated scores for sensory attributes were then generated for the optimum regions and the control to compare their sensory attributes.

Results and discussion

Influence of process variables on the physicochemical properties of the processed *zoom-koom*

Zoom-koom samples had a pH of 6.14 to 7.24 after steeping for 2 h, 5.70 to 6.31 after steeping for 7 h, and 5.77 to 6.87 after steeping for 12 h ((S2) Table 3). This demonstrates the slightly acidic to slightly neutral nature of the beverage. The control sample (sample bought from a processor) had a pH value of 4.95 and was more acidic than the laboratory processed *zoom-koom*. The low acidity value of the laboratory processed sample compared to the control may be due to the ability of the sodium benzoate (added during steeping) to control the microorganisms that might initiate fermentation process to increase the acidity of the beverage [9]. The regression model for pH was influenced significantly by the linear terms, blend ratio (P = 0.008), steeping time (P = 0.004), and steeping temperature (P = 0.039). Blend ratio (P = 0.004) and steeping time (P = 0.026) were the quadratic terms with significant (P < 0.05) contribution to the regression model. All the interaction terms did not exert significant effect on the model. The R squared and adj. R squared values were 0.9166 and 0.7665 respectively. Lack of fit for the regression model was not significant (P = 0.955), indicating that the model can appropriately predict the relevant response. The independent variables explained the model well and accounted for 91.66% of the variation in the pH of the *zoom-koom.* The R squared value provides an indication of the predictive capacity of the regression model [14] and hence indicating that the experimental (actual) values have a high degree of accuracy and are reliable owing to their proximity to the predicted values. Therefore, the model properly explained the response variable and can therefore be used to predict the pH of the *zoom-koom* sample. The quadratic model which explains the experimental data is seen in Eq. 5:

$$\boldsymbol{pH} = 5.70 -^{\circ} 0.0438X_1 -^{\circ} 0.134X_2 +^{\circ} 0.000236X1^2 +^{\circ}0.01485^{\circ}X_2^2 -^{\circ} 0.00279^{\circ}\mathrm{X}_3^2 -^{\circ} 0.000140\ X_1X_2 -^{\circ}0.000100^{\circ}X_1X_3 -^{\circ} 0.00265X_2X_3 \quad (5)$$

The contour plots generated from the model ((S1) Fig. 1a) showed that increasing the blend ratio from 700: 140 to 700: 150 (millet: spices) and the steeping time from 2 to 2.5 h resulted in increased pH. Increase in blend ratio from 700: 50 to 700: 53 and steeping time from 2 to 3 h also increased the pH value of the *zoom-koom*. Finally, increasing steeping time from 11.5 to 12 h and blend ratio from 700: 50 to 700: 52 also provoked higher pH value. This explanation is evident in the darkest green colour (Fig. 1a).

The total titratable acidity (TTA) of the laboratory processed *zoom-koom* samples steeped for 2 h ranged between 0.002 and 0.004 (% lactic acid), samples steeped for 7 h ranged from 0.002 to 0.008 (% lactic acid) and those steeped for 12 h ranged from 0.002 to 0.009 (% lactic acid) [Table 5 (S4)]. The TTA of the control sample, however, was found to be 0.01 (% lactic acid) which is quite high compared to the laboratory processed *zoom-koom.* In a study conducted by Soma et al. [15] and Tapsoba et al. [16] on *zoom-koom* produced with *Lactobacilllus fermentum* starter culture, the TTA varied between 0.25 and 1.89 (% lactic acid). Compared to the two previous studies mentioned above, the results of this study (0.002 to 0.009% lactic acid)) are lower, possibly because in the previous study, fermentation was done with a starter culture of *Lactobacillus species*, whereas in this study, fermentation occurred spontaneously, but sodium benzoate was used to control microbial growth. In other studies of indigenous fermented beverages, such as *gowe*, *kunun-zaki*, *chicha de jora*, *champus* and *mahewu*, low pH and high TTA values have been observed [17–25]. When the titratable acidity values were fitted into a response surface regression model, blend ratio, steeping time and steeping temperature exerted significant effect on the TTA, with P values of 0.030, 0.049, and 0.039 respectively. In addition, the quadratic effect of all the three independent factors, blend ratio ($P=0.025$), steeping time ($P=0.032$), and steeping temperature ($P=0.024$) on TTA was significant ($P<0.05$). The interaction effect which influenced the TTA significantly were the blend ratio and temperature, and steeping time and steeping temperature. The coefficient of determination, R^2 and the adjusted coefficient of determination, adjusted R^2 of the regression model were 0.7837 and 0.7343 respectively indicating that the independent variables could explain 78.37% of the model. Lack of fit of the model was not significant ($P=0.079$), therefore the model is a good predictor of the response. The regression equation for TTA is Eq. 6. The contour plot produced from the model (Fig. 1b) showed that increasing steeping time from 11.2 to 12 h and blend ratio from 700: 50 to 700: 60 increased TTA as seen in the darkest green region (Fig. 1b). Also, increasing the blend ratio from 700: 148 to 700: 150 and steeping time from 2 to 2.4 h also increased the TTA of the *zoom-koom*. Similarly, increasing the blend ratio from 700: 140 to 700: 150 and steeping time from 11.5 to 12 h also resulted in an increase in TTA as seen in the darkest green region of the contour plot.

$$\boldsymbol{TTA} = 0.0098\ -^{\circ} 0.000051\,^{\circ}X_1 -^{\circ} 0.00221^{\circ}X_2 -^{\circ}0.000076^{\circ}X_3 +^{\circ} 0.000001X1^2 +^{\circ} 0.000104^{\circ}X_2^2\ +^{\circ} 0.000002^{\circ}X_3^2 -^{\circ}0.000001^{\circ}X_1\ X_2 -^{\circ} 0.000003X_1X_3 +^{\circ} 0.000036^{\circ}X_2X_3 \quad (6)$$

Total soluble solids (TSS) is the degree of soluble solids content (simple sugars, dissolved proteins and other nitrogen compounds, etc.) in the wort [8]. The TSS of *zoom-koom* steeped for 2 h ranged from 0.53 to 0.74°Brix, while samples steeped for 7 h and 12 h ranged from 0.43 to 0.75 ° Brix, and 0.55 to 0.90° Brix respectively ((S3) Table 4). The control sample, however, gave a TSS of 6.54° Brix, which was comparatively higher than the laboratory processed sample. The contour plot [Figure 1c (S1)] produced from the model confirms that the TSS value was high at a low blend ratio (700: 50 to 700: 53) and lowest when steeping time ranged from 2 to 2.4 h and 11.2 to 12 h, respectively. The fitted regression model for TSS (Eq. 7), had an R-squared and an adjusted R-squared of 0.7300 and 0.6941 respectively, showing that 73.00% of the variation in the TSS model was due to the independent variables.

$$\boldsymbol{TSS}\ =\ 1.56 +^{\circ} 0.00092X1 +^{\circ} 0.0458^{\circ}X_2 +^{\circ} 0.1219^{\circ}X_3 +^{\circ}0.000021X_1^2 +^{\circ} 0.00358X_2^2 -^{\circ} 0.001304X_3^2 -^{\circ} 0.000220X_1 X_2 -^{\circ} 0.000150^{\circ}X_1X_3 -^{\circ} 0.00225\ X_2X_3 \quad (7)$$

Zoom-koom colour is a significant product property since it is one of the initial sensory parameters consumers notice about the beverage, therefore it influences its preference. The variation in unit operations, millet type, blend ratio of the steeped millet, steeping time and temperature are important factors which influence the colour of the beverage. The colour of the laboratory processed *zoom-koom* and the control was described by the L^*, a^* and b^*. For the laboratory processed *zoom-koom* lightness index L^*, ranged from 14.32 to 21.25, redness index, a^* ranged from 1.50 to 6.89, yellow index, b^* ranged from 6.70 to 11.23 compared to the control of $L*$ of 13.55; $a*$ of 4.19 and b* of 10.68 ((S3) Table 4). The colour parameters of the laboratory processed *zoom-koom* was comparable to that of the control. The model for $L*$ is seen in Eq. 8.

$$\boldsymbol{L*} = 42.7 -^{\circ} 0.131^{\circ} X_1 +^{\circ} 0.46^{\circ} X_2 -^{\circ} 1.195 X_3 +^{\circ} 0.000255^{\circ} X_1^2 -^{\circ} 0.0368 X_2^2 +^{\circ} 0.0137 X_3^2 +^{\circ} 0.00323^{\circ} X_1 X_2 +^{\circ} 0.00201 X_1 X_3 -^{\circ} 0.0061 X_2 X_3 \quad (8)$$

From the contour plot in Fig. 1d, increasing the blend ratio from 700: 140 to 700: 150 and increasing the steeping time from 6.2 to 12 h resulted in a higher L value of *zoom-koom* sample. Response surface regression modelling showed that the $L*$ value of samples was not influenced by the linear, quadratic and interaction effects of the independent variables. The low R-squared value of 0.4819 and adjusted R-squared value of 0.4221 indicates that there is less precision and hence the model cannot be fully relied upon in predicting the response ((S6) Table 7).

$$a* = -\ 6.3 +^{\circ} 0.1526 X1 +^{\circ} 0.899 X2 -^{\circ} 0.042 X3 -^{\circ} 0.000365 X_1^2 -^{\circ} 0.0508^{\circ} X_2^2 +^{\circ} 0.00245 X_3^2 -^{\circ} 0.00103 X_{12}^{\circ} 0.00151^{\circ} X_1\ X_3 -^{\circ} 0.0011^{\circ} X_2 X_3 \quad (9)$$

$$b* = -6.8 +^{\circ} 0.215^{\circ} X_1 +^{\circ} 1.82^{\circ} X_2 -^{\circ} 0.021 X3\ 0.000280^{\circ} X1^2 -^{\circ} 0.0985\ X_2^2 +^{\circ} 0.0059^{\circ} X_3^2 -^{\circ} 0.00002^{\circ} X_1 X_2 -^{\circ} 0.00369^{\circ} X_1 X_3 -^{\circ} 0.0111^{\circ} X_2 X_3 \quad (10)$$

The response surface regression models for a* and $b*$ values revealed that the linear, quadratic and interaction effect of blend ratio, steeping time, and steeping temperature were not significant ((S7 & 8) Tables 8 and 9). The R-squared and adjusted R squared values of a* were 0.7103 and 0.6867 respectively (Table 8). The respective R-squared and adjusted R squared values of $b*$ were 0.7095 and 0.6867 ((S8) Table 9). The relatively low adjusted R squared values of a* and $b*$ is an indication that the predictive power of the model is low and cannot be relied upon in predicting the response. The models (Eqs. 9 and 10) were used to generate contour plots (Fig. 1e and f) which showed that the *zoom-koom* produced from pearl millet steeped for 4 to 10.2 h at a millet: spices ratio of 700: 85 to 700:150 had higher $a*$ values at a constant steeping temperature of 35 °C. At a constant steeping temperature of 35 °C, *zoom-koom* produced by steeping millet for 5 to 9 h at a millet: spice ratio of 700: 110 to 700:150 also had higher $b*$ values. This shows that even though blend ratio and steeping time changes increased a* and $b*$ indices their contribution to the response variables was not significant (Tables 8 and 9). The linear, quadratic and interaction terms of the model for ΔE did not contribute significantly to the response ((S9) Table 10). The R-squared and an adjusted R- squared values of ΔE were 0.5240 and 0.5114 respectively. This shows that only 52.40% of the variation in ΔE can be explained by the model, therefore its predictive power is low and cannot be relied upon to predict the response. The contour plot ((S1) Fig. 1g) generated from the model (Eq. 11) showed that increasing blend ratio and steeping time at constant steeping temperature of 35 °C resulted in increased ΔE of the *zoom-koom* samples. However, this increment was not significant (Table 10). Difference in colour of the beverages is attributable to the quantities of ingredients such as the black pepper, cloves and ginger used in the beverage formulations [26]. Differences in blending ratio of ingredients, steeping time and steeping temperature, etc. may generally account for the quality variation between the laboratory processed *zoom-koom* and the control.

$$\triangle E = 33.3 +^{\circ} 0.011 X_1 +^{\circ} 1.45 X_2 -^{\circ} 1.07 X_3 +^{\circ} 0.000056 X_1^2 -^{\circ} 0.0913 X_2^2 +^{\circ} 0.0153\ X_3^2 +^{\circ} 0.00276^{\circ} X_1\ X_2 -^{\circ} 0.00037 X_1 X_3 -^{\circ} 0.0105^{\circ} X_2 X_3 \quad (11)$$

Influence of process variables on the microbiological quality of processed *zoom-koom*

Zoom-koom samples steeped for 2 h were found to have an total plate count (TPC) of 2.83 to 3.45 log cfu/mL, whereas those steeped for 7 h ranged from 1.30 to 3.22 log cfu/mL and those steeped for 12 h ranged from 1.70 to 3.18 log cfu/mL, compared to the control sample with a count of 4.44 log cfu/mL, showing a significant difference between the laboratory processed and the control *zoom-koom* samples. [(Table 11 (S10)]. Yeasts and moulds count of *zoom-koom* ranged from 0.66 to 2.11 log cfu/mL, for those steeped for 2 h whilst those steeped for 7 h ranged from 1.01 to 2.42 log cfu/mL and those steeped for 12 h also ranged from 1.18 to 3.45 log cfu/mL, compared to the control sample of a mean value of 3.95 log cfu/mL, showing a significant difference between the laboratory processed and the control *zoom-koom* samples. *S. aureus* count of *zoom-koom* also ranged from 2.15 to 2.18 log cfu/mL for those steeped for 2 h whilst those steeped for 7 h ranged between 0.66 and 2.49 log cfu/mL and those steeped for 12 h ranged from 2.32 to 3.24 log cfu/mL compared to the control sample of 4.75 log cfu/mL, showing a significant difference between the laboratory processed and the control *zoom-koom* samples. It has been observed that the main microorganisms in other indigenous beverages such as *gowe*, *kunun-zaki*, *chicha de jora* and *mahewu* are yeasts and moulds [17, 18]. Yeasts convert sugars into ethanol, carbon dioxide and other useful metabolites. They also produce volatile organic compounds such as alcohols, esters, aldehydes and ketones which may influence the sensory properties of the fermented cereal product and help to decrease mould growth and spore formation [27]. Total coliform and *E. coli* were also not detected in all fifteen (15) laboratory-processed *zoom-koom* which are within the satisfactory limit of 4 log cfu/mL of the Ghana Standard Authority (GSA) microbiological standard for ready-to-eat foods. However, total coliform and *E. coli* were detected in the control sample with a mean value of 4.07 and 4.04 log cfu/mL respectively which are above the satisfactory limit of 4 log cfu/mL of the Ghana Standard Authority (GSA) microbiological standard for ready-to-eat foods, signifying potential health hazards posed by the control sample. Non-detection of total coliform and *E. coli* and the absence of pathogenic bacteria such as *S. aureus* in some of the laboratory processed *zoom-koom* may be attributed to the addition of sodium benzoate to the pearl millet steeped in water. It has been reported in a previous study that steeping of millet in water alone for 24 h led to substantial reduction in coliform count [7].

A regression model output (Eqs. 13, 14, and 15) and contour plots ((S1) Fig. 2a, b and c) was produced to display the influence of process variables on the microbiological quality of laboratory produced *zoom-koom.* There was no regression output for *E. coli* and total coliform since all the fifteen (15) *zoom-koom* experimental samples had no counts. The interaction effects of blend ratio and steeping time as well as the quadratic effects of the steeping temperature and (millet and spices) was significantly influenced the model. The process variables significantly influenced total or aerobic plate count of *zoom-koom* and the model, with 0.8215 and 0.7903 as R- square and adjusted R- squared values respectively [Figure 2a (S1) & Eq. (18)], and it demonstrate that the model was acceptable and could predict the responses. As shown in the contour plot in Fig. 2a, increasing the blend ratio from 700: 130 to 700: 150 at a steeping time of 2 to 2.6 h at constant temperature of 35 °C, decreased the aerobic plate counts. The linear, quadratic, and interaction terms of both yeast and moulds and *Staphylococcus aureus* count did not influence the response significantly ((S11) Tables 11 and 12), but their R squared values of 0.8098 and 0.7996 respectively indicates that 80.98% and 79.96% respectively of the response is explained by the independent variables. The models of the *S. aureus* count and yeast and moulds counts were used to generate contour plots (Fig. 2a and b) showing that increasing blend ratio from 700: 50 to 700: 55 and increasing the steeping time from 11.2 to 12 h at constant steeping temperature of 35 °C significantly decreased the *S. aureus* count. Also, an increase in blend ratio from 700: 55 to 700: 150 and steeping time from 9 to 12 h resulted in decreased yeast and mould counts.

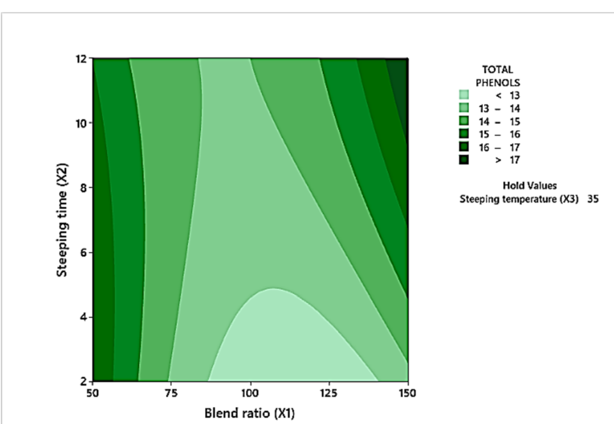
$$\begin{aligned}\mathbf{APC} &= 8.23 -^{\circ} 0.0415\mathrm{X}_1 -^{\circ} 0.061\mathrm{X}_2 -^{\circ} 0.196\mathrm{X}_3 \\ &+^{\circ}0.000142\mathrm{X}_1^2 + 0.02984\,\mathrm{X}_2^2 +^{\circ} 0.00256^{\circ}\mathrm{X}_3^2 \\ &-^{\circ}0.000857^{\circ}\mathrm{X}_1\mathrm{X}_2 +^{\circ} 0.000505\mathrm{X}_1\mathrm{X}_3 -^{\circ} 0.00891\mathrm{X}_2\mathrm{X}_3\end{aligned} \tag{12}$$

$$\begin{aligned}\mathbf{Yeast\&Molds} &= 9.94 -^{\circ} 0.0100X_1 -^{\circ} 0.612X_2 -^{\circ} 0.319X_3 \\ &-^{\circ}0.000080X_1^2 -^{\circ} 0.0027X_2^2 +^{\circ} 0.00272X_3 +^{\circ} 0.00198X_1X_2 \\ &+^{\circ}0.000174X_1X_3 +^{\circ} 0.01580X_2X_3\end{aligned} \tag{13}$$

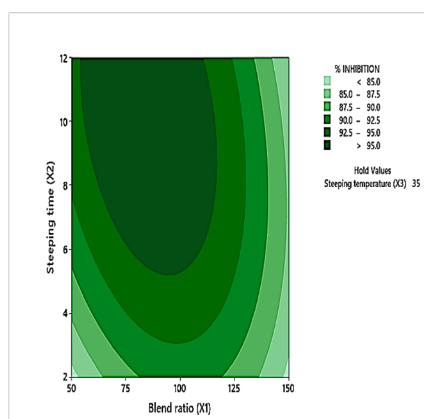
$$\begin{aligned}S.\,aureus &= 9.98 -^{\circ} 0.0328X_1 -^{\circ} 0.725X_2 -^{\circ} 0.194X3 \\ &-^{\circ}0.000004X_1^2 +^{\circ} 0.0460\,X_2^2 -^{\circ} 0.00102^{\circ}X_3^2 -^{\circ} 0.00314X_1X_2 \\ &+^{\circ}0.001195X_1X_3 +^{\circ} 0.01195X_2X_3\end{aligned} \tag{14}$$

Influence of process variables on bioactive properties of processed *zoom-koom*

Total phenolic compounds for *zoom-koom* steeped for 2 h, ranged from 11.54 to 15.36 mg/mL, whilst those steeped for 7 h ranged from 12.83 to 15.52 mg/mL and those steeped for 12 h ranged from 11.79 to 21.03 mg/mL compared to the control of 10.55 mg/mL ((S19) Table 20). The present phenolic compounds values are extremely lower than



a: Contour plots of Total phenolic compounds of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



b: Contour plots of DPPH Scavenging activity (%Inhibition) of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

Fig. 2 **a**:Contour plots of Total phenolic compounds of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2). **b**: Contour plots of DPPH Scavenging activity (%Inhibition) of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2)

310.00 mg/mL reported in a previous study [28]. The variety of millet used, and the processing conditions variation may account for these differences. Antioxidant activity (% inhibition) ranged from 82.36 to 96.01%, for those steeped for 2 h, whilst those steeped for 7 h ranged from 85.91 to 97.54%, and those steeped for 12 h were in the range 88.32 to 96.82% compared to the control of 80.33% (Table 20). DPPH scavenging activity was also reported in a non-alcoholic cereal beverage [28]. The data shows a significant difference between the laboratory processed *zoom-koom* and the control. Antioxidants function as reducing agents, ultimately eliminating free radical intermediates and inhibiting further oxidation [27]. Some phenolic compounds are known to exhibit antioxidant properties. It is evident that the addition of the spices (ginger, cloves and black pepper) provoked the increase in phenolic content of the *zoom-koom* samples. The differences in the ratio of spices also accounted for the differences in the bioactivity of the laboratory processed and the control *zoom-koom* samples. A surface regression model output was used to display the influence of process variables on the bioactive quality of laboratory-produced *zoom-koom* (Eqs. 16 and 17). The quadratic effect of the blend ratio ($P=0.025$) and steeping temperature ($P=0.041$) had significant effect on the total phenolic compounds of the *zoom-koom.* The model for total phenolic compounds had R-squared and adjusted R-squared values of 0.8570 and 0.8039 respectively and the *P*-value of lack of fit being 0.065 ((S20)Table 21). Therefore, the total phenolic compound data can fit the model and describe the response appropriately. From the contour plot in Fig. 2a, increasing the blend ratio from 700: 140 to 700: 150 and the steeping time from 9 to 12 h increased the total phenolic compounds. The steeping time ($P=0.039$), steeping temperature ($P=0.047$) and the quadratic effect of blend ratio ($P=0.005$) influenced the antioxidant activity of the *zoom-koom* samples significantly and the respective R-squared and adjusted R-squared values were 0.8980 and 0.8444 with *P* value for lack of fit of 0.101 (Table 22). The antioxidant activity data could fit and describe the model well. The model of the antioxidant activity was used to generate contour plots (Fig. 2b) and showed that at constant steeping temperature of 35 °C, increasing blend ratio from 700: 55 to 700:50 and steeping time from 6 to 12 h significantly increased the antioxidant activity of the samples from 85% to 95%.

$$\boldsymbol{TPC} = 1.5 -^\circ 0.2485X_1 -^\circ 0.590X_2 +^\circ 1.509^\circ X_3 +^\circ 0.001183X_1^2 -^\circ 0.0116X_2^2 -^\circ 0.02161X_3^2 +^\circ 0.00519X_1X_2 -^\circ 0.00088X_1X_3 +^\circ 0.0114X_2X_3 \quad (15)$$

$$\%\boldsymbol{DPPH} = 65.6 +^\circ 0.451X1 +^\circ 3.13^\circ X2 -^\circ 0.23X3 -^\circ 0.002672X12 -^\circ 0.1044X22 +^\circ 0.0009X32 -^\circ 0.00960X1X2 +^\circ 0.00297^\circ X1X3 -^\circ 0.0050X2X3 \quad (16)$$

Consumer acceptability of processed *zoom-koom*

Zoom-koom samples were scored using a 9-point hedonic for the most relevant sensory attributes (appearance, taste, aroma, flavour, mouthfeel, aftertaste, overall acceptability). From the results in Table 15 (S14), the overall likeness ranged between 7 and 8. The scores for appearance was 7 and the scores for aroma ranged between 7 and 8. Equally, the scores for flavour varied from 6 to 7, and scores for both mouthfeel and aftertaste ranged from 6 to 7. The overall liking also ranged from 7 to 8. The score ranges of all the attributes from the consumer's assessment of the *zoom-koom* showed it was liked. The influence of process variables on the attribute liking scores of the samples were modelled using response surface regression and the coefficients of the explanatory variables with equivalent R-squared values are presented in Table 16 (S15).

Modelling of the sensory attributes of laboratory produced *zoom-koom*

Response surface regression models were used to fit the data for the appearance, aroma, flavour, mouthfeel, aftertaste, and overall acceptability scores [Table 16 (S15)]. Some responses (flavour, mouthfeel, aftertaste, and overall acceptability) had high coefficients of determination (R^2), which means that a significant amount of variability in those responses was explained by the data. The low R-square values for appearance and aroma signals that the model cannot be very reliable in the prediction of these sensory responses. The blend ratio, steeping time and steeping temperature could not explain the variation in appearance and aroma. Other extraneous factors may account for the variability in the response variables. The inhibition of fermentation by the sodium benzoate might have led to the production of *zoom-koom* of appearance and aroma quite different from the one produced through spontaneous fermentation. The models for the various sensory attributes were used to generate contour plots [(Fig. 4a and f) S1]. The contour plots show that increasing the blend ratio from 700: 85 to 700: 110 and steeping time from 2 to 2.2 h increased the score for appearance of the *zoom-koom* samples from 6.75 to 7.05 [(Fig. 4a) S1] The low R-squared [Table 16 (S15)] value for appearance signals that the model cannot be very reliable to predict the appearance of the *zoom-koom*. The contour plots for aroma show that increasing the blend ratio from 700: 76 to 700: 120 and steeping time from 8.2 to 12 h increased the score for aroma of the *zoom-koom* from 6.85 to 7.10 [(Fig. 4b) S1]. The low R-squared [Table 16 (S15)] value for aromas also demonstrates that the model cannot be very reliable under this condition. The contour plots for flavour showed that increasing blend ratio from 700:52 to 700: 110

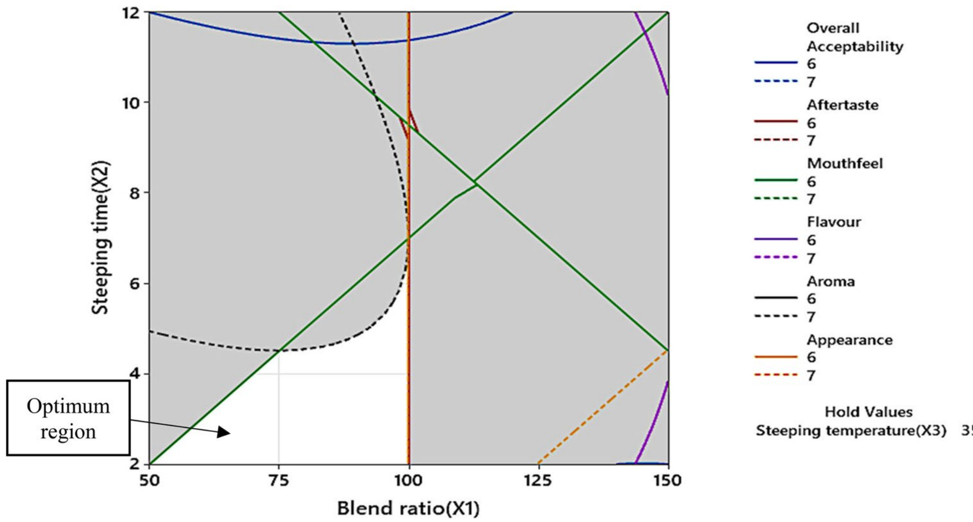


Fig. 3 Overlaid contour plot of appearance, aroma, flavour, mouthfeel, aftertaste and overall acceptability of *zoom-koom*

and steeping time from 2 to 8 h increased the score for flavour of the *zoom-koom* samples from 6.3 to 6.8 [(Fig. 4c) S1]. The contour plots for mouthfeel show that increasing blend ratio from 700: 115 to 700: 120 and a slight increase of steeping time from 2 to 2.4 h increased the mouthfeel score of the *zoom-koom* samples from 6.20 to 6.36 [(Fig. 4d) S1]. Also, an increase in the blend ratio from 700: 70 to 700: 120 and steeping time from 9 to 12 h increased the flavour score of the *zoom-koom* samples. The contour plots for aftertaste show that slightly increasing both the blend ratio from 700: 50 to 700: 60 and steeping time from 2 to 2.8 h increased the aftertaste of the *zoom-koom* samples from 6.20 to 6.40 [(Fig. 4e) S1]. The high R-squared [Table 16 (S15)] value for the aftertaste signifies that the model can be reliable in the prediction of this response. The contour plots for overall acceptability show that decreasing the spices from 700: 55 to 700: 80 and slightly increasing the steeping time from 2 to 4 h increased the overall acceptability of the *zoom-koom* samples from 6.40 to 6.80 (Fig. 4f).

Summary of all the models

The values determined for each of the regression coefficients in the coded units [Tables 17, 5, 18 and 6 (S2), 12,14 (S7),16–17, 19, 20–24] show that some of the study factors have significant linear effects, as they correspond to a P-value less than 0.05. The coefficient with the greatest weight, determined by its absolute value, was X_2 (Steeping time), and for which some had positive effect and others negative effect. The X_2 (steeping time) coefficient is followed by X_3 (steeping temperature) and X_1 (Blend ratio), where some again exhibited positive and negative effects, and finally the interaction coefficients $X_{2\times 3}$ (Steeping time × steeping temperature) and $X_{1\times 2}$ (Blend ratio × steeping time), which also had positive and negative effects. An interaction coefficient with a positive sign represents a synergistic effect, whereas a negative sign indicates an antagonistic interaction between the factors involved [29]. Regarding the quadratic terms, only the X_3^2 (steep. temperature × steep. temperature) factor corresponded to significant effects with a *P*-value, which is indicative of a parabolic trend in the response variable [30]. Therefore, the presence of at least one value for the time factor to maximize the *zoom-koom* is anticipated. Finally, the coefficients of the terms $X_2{}^2$ (steeping time × steeping time), X_1^2 (Blend ratio × Blend ratio) and $X_{1\times 3}$ (Blend ratio × steeping temperature time) also exhibited some significant and non-significant effects with $P<0.05$ and $P>0.05$ respectively.

Selection of optimum conditions

The contour plots of all the sensory attributes for *zoom-koom* were overlaid on the same axis, using.

Minitab (Version 20, Minitab Inc., UK). The optimum region for the process treatment variables was the portion where all the criteria of the sensory attributes were satisfied (Fig. 3). The sensory criteria used were based on the mean scores which gave very acceptable sensory attributes ranging from like moderately to like extremely. Accordingly, the constraints for the acceptable criteria for the sensory attributes ranged from scores of 7 (like moderately) to 9 (like extremely). The overlaid contour plot for all the sensory attributes is illustrated in (Fig. 3). From the plot, the process treatment combinations that provided the optimum region for all the sensory qualities for *zoom-koom's* contour plots were superimposed on the same axis, and where all the sensory attribute criteria met, was chosen as the optimal location for the process treatment variables.

Verification of optimized conditions

The results from the verification study [Table 19 (S17)] showed that the appearance, taste, aroma, mouthfeel, aftertaste and acceptability scores for the samples within the optimum region (Optimized 1 and Optimized 2) were significantly different from the control. The verification findings also showed that for each sample, the observed and predicted scores agreed well. This gives a confirmation of the accuracy and predictive reliability of the model. Similar findings in which observed and predicted values were in close agreement were reported in a previous study (Aviles-Rivera et al., 2025).

Proximate composition of optimized *zoom-koom vs. control sample*

To compare the nutrient profile of the optimized samples and the control sample, proximate analysis, which is an important tool for the assessment of the composition of food and food products [28] was conducted. Table 20 shows the composition of the optimized *zoom-koom* (Optimized 1 and Optimized 2) and the control sample. Moisture content of food influences its shelf life. The result of the moisture content was high for both the optimized and control sample (sample bought from a processor). However, even though the difference between the control and Optimized 1 was not significant, the difference between the control and Optimized 2 was significant ($P<0.05$). The variation may be due to the differences in steeping time and steeping temperature of the samples. Thus, the high moisture content means the *zoom-koom* beverage may be more susceptible to microbial attack particularly fungi and mould. The moisture content in this study, is similar to that (82.0±0.15 to 90.70±0.15%) [28] obtained for *kunu-zaki*, which is also a cereal non-alcoholic beverage. The high moisture content is characteristic of liquid-based beverages. The crude protein of the control and the optimized *zoom-koom* ranged from 3.29 to 3.73%. There was no significant difference in crude protein content between the control and optimized 1, but the difference between Optimized 2 and the control was significant ($P<0.05$). Ofudje et al. [31] reported that the protein content of *Kunu-zaki*, a non-alcoholic local beverage was in the range of 2.18 to 8.40% and is comparable to the present results. The percentage of fat in the *zoom-koom* varied from 1.12 to 1.35%. These values were higher than 0.39% for millet beverage [28] but lower than 5.5% for *kunu* drink [32]. Ash content represents the total amount of minerals present in the sample. The ash value for the *zoom-koom* ranged from 2.40 to 3.06%. These values were comparable to 2.40% reported for *Obiolor*, a non-alcoholic cereal beverage in Nigeria [28]. Crude fibre and total solids content ranged from 0.61 to 0.82% and 9.90 to 10.31%, respectively with optimised 2 being the most superior. The crude fibre content of the *zoom-koom* was higher than 0.30% obtained in a previous study [28]. The carbohydrate and energy value for the *zoom-koom* ranged from 1.54 to 2.46% and 28.39 to 32.88 KJ/g respectively. A previously reported carbohydrate value of 8.90% for a non-alcoholic cereal beverage [28] was quite high compared to those obtained in the present study. The energy values were also very low compared to the value reported by [33]. The amount of accessible carbohydrate in the beverage could serve as a source of energy for consumers [28].

Conclusion

In this study, statistically based experimental designs (Box-Behnken design) was used to characterize and optimize the *zoom-koom* production process. The effect of steeping temperature, steeping time and blend ratio of the millet and the spices on the bioactivity, physicochemical, microbial quality and sensory properties was investigated. The design proved to be a valuable tool for optimizing the production of the *zoom-koom* beverages. This study also showed that *zoom-koom* produced using different processing variables exhibited high antioxidant activity, high total phenolic composition, and high microbial quality and was therefore suitable for consumption. The results obtained indicated that among all the independent variables, steeping temperature had the highest effect on all the response variables. The desirability index obtained in this work with respect to the independent variables considered ranged from 0.700 to 1.000. The prediction of the desirability model, based on 95% confidence in the range of the independent variables, that gave optimal treatment conditions were blend ratio of 700: 100, steeping time of 6 h and a constant temperature of 35 °C. Comparing the results of proximate analysis of optimized *zoom-koom* and control it was revealed that optimized *zoom-koom* (Optimized 2) was superior to the control. In addition, sensory analysis data also showed that optimized samples were judged to be of higher quality than control samples in nearly all quality attributes. Future studies can explore the use of starter cultures under the optimal conditions to obtain more uniform products with consistent quality. The limitations of the study are that the validity of the optimum conditions is for the specific range of variables studied, and beyond this range predictions may be unreliable; and the laboratory conditions under which the experiments on optimization of the *zoom-koom* took place is more controlled and as such the results obtained may not be reproducible under large scale conditions where there is a high level of variability.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11694-026-04212-1.

Acknowledgements I am grateful to the Sensory Laboratory, Microbiological Research Laboratory at UG, Ghana and the Chemistry Laboratory of the Department of Food and Postharvest Technology, Koforidua Technical University, Ghana for all the analysis.

Author contributions Conceptualization, I.I., N.A.A and E.T.M.; Methodology: E.T.M., I.I., B.A.D., F.K.S and J.O.; Supervision: B.A.D., J.O and I.I., Visualization: B.A.D., J.O., I.I., and F.K.S Writing—original draft: E.T.M., Writing—review and editing: E.T.M., I.I., B.A.D., N.A.A., and J.O. All authors have read and agreed to the published version of the manuscript.

Funding This work was self-funded.

Data Availability Data is contained within the article.

Declarations

Institutional review board statement The participation of the consumers was voluntary. Sensory evaluation was performed in different sessions.

Conflict of interest The authors declare no conflict of interest.

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