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Development of a Water Kefir Beverage Made From Jackfruit Processing By-product and Its Potential Probiotic Property

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Water kefir is a fermented probiotic drink produced by the fermentation of dried sugary fruits and sugar using water kefir grains. It is characterized by its fizzy and slightly acidic and alcoholic taste. Instead of using fresh or dried fruits and sugar as raw materials in fermentation, this study aims to explore the utilization of jackfruit concentrate, a by-product of dehydrated jackfruit production. The beverage formulation was performed through a 3 x 3 factorial design with independent variables: jackfruit concentrate (25–75%v/v) and kefir grains (5–15%w/v) levels. Through response surface methodology (RSM), the kefir fermented jackfruit beverage (JFB) formulation was optimized with 75%v/v jackfruit concentrate and 9.7475%w/v kefir grains that could give optimal values of product acceptability score of 7 in the nine-point Hedonic scale, total soluble solids (TSS) of 10.0019, and pH of 4.2864. Regression analysis shows it cannot predict the microbial count of the product; however, the microbial viabilities of all the treatments range from 7.55 x 10^6 to 3.44 x 10^7 CFU/mL, which suggests that the formulated beverage has a good probiotic potential. Acceptability studies also suggest that the product has good market acceptability in terms color, aroma, taste, mouthfeel, and general acceptability.

Keywords: by-product processing, fermentation, jackfruit, lactic acid bacteria (LAB), probiotic, water kefir

INTRODUCTION

Water kefir is a fermented probiotic drink produced by the fermentation of dried sugary fruits and sugar using water kefir grains. It is characterized by its fizzy, slightly acidic, and alcoholic taste. This beverage is a good alternative for people who are lactose intolerant. According to Lynch *et al.* (2021), this is similar to but distinct from milk or dairy kefir, which is produced typically by bovine milk using milk kefir grains. The gelatinous grains of water kefir are a symbiotic mixture of bacteria and yeast embedded in a primarily polysaccharide matrix (Martinez-Torres *et al.* 2017). This beverage is made by placing the grains in the sucrose medium and fermentation between 21–30 °C for 1–4 d. It is typically produced on a home scale under non- or minimally-aseptic conditions (Horisberger 1969). The grains can be recovered from the fermented liquid and can be reused for another fermentation cycle (Lynch *et al.* 2021).

Sucrose is used as a sugar source in the traditional production of this beverage (Tuncay *et al.* 2019). Table sugar or brown sugar is commonly used as a carbon source, and fruits or dried fruits are added as a nitrogen source (Lynch *et al.* 2021). In a study by Reiß (1990),

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figs produced the optimum fermentation compared to other fruits. The substitution of other dried fruits (raisins, dates, and plums) also modified the fermentation and rate of production of lactic acid and acetic acid. Therefore, the fermentation process of water kefir would vary depending on factors such as substrate, sugar content, temperature, and many more. It is by this concept that this study was undertaken.

Nowadays, there is an increasing interest in the fermentation of water kefir grains with other fruit substrates such as red pitaya and apple (Bueno *et al.* 2021), pear (Hampton *et al.* 2021), olive (Darvishzadeh *et al.* 2021), coconut (Alves *et al.* 2021; Dwiloka *et al.* 2020), and pineapple (Maldonado *et al.* 2020). Therefore, the aim of this study was to explore the utilization of jackfruit concentrate, which is a by-product of the osmotic dehydration of jackfruit pulp for 3 h. It is prepared following the method of Rahman *et al.* (2012) with some modifications on the concentration of sugar used (20–25 °B). Like simple water kefir fermentation prerequisites, jackfruit concentrate possesses good substrate characteristics. It is a rich mixture of sugar and jackfruit extracts that could sustain the growth of microorganisms involved in the fermentation. In fact, according to Wichienchot *et al.* (2011), jackfruit appears to have a high potential for commercial prebiotic development because of its high concentrations of indigestible polysaccharides in the skin, pulp, and seed. This claim can be supported by the study of Othman *et al.* (2012), wherein the supplementation of jackfruit puree improves the viability of the LAB *Lactobacillus acidophilus* FTDC 1295.

Considering the context presented above, this study aims to investigate the usability of jackfruit concentrate on the physico-chemical characteristic, microbial quality, and sensory acceptability of the water kefir JFB. Therefore, in this study, the main physico-chemical and microbial changes in water kefir production and sensory acceptability of the JFB will be determined.

MATERIALS AND METHODS

Preliminary Experiment

A preliminary test was conducted to check whether the experimental set-up would allow the water kefir starter culture to ferment the diluted jackfruit concentrate. Other fermentation parameters such as sugar content, fermentation time, and storage were also studied before conducting the experiment.

Materials

Jackfruit concentrate was collected from the dehydrated jackfruit processing of the Department of Food Science and Technology (DFST), Visayas State University, Visca, Baybay City, Leyte, Philippines. It is prepared following the method done by Rahman *et al.* (2012) with some modifications on the concentration of sugar used (20–25 °B). Osmotic dehydration of jackfruit pulp was done for 3 h. The concentrate was collected and stored.

Water kefir grains were purchased from Banawa, Cebu City, Philippines.

Experimental Design

A 3 x 3 factorial experimental design in two blocks or replicates with three levels of kefir grains (5, 10, and 15%w/w) and three levels of jackfruit concentrate (25, 50, and 75%w/w) were used in the study.

Product Preparation

Jackfruit concentrate collected and used in the experiment has a TSS ranging from 20–25 °B. The concentrate was then added with tap water to dilute based on the required concentration following the experimental design and pasteurized at 70 °C for 30 min. The initial TSS of solutions were 10.5–15 °B, respectively. Solutions were then cooled prior to the incorporation of kefir grains. Grains were drained from the previous solution and added to the mixture. The mixture was aerobically fermented for 24 h at room temperature following fermentation set-up done in a common household, which uses glass jars (mason jars) that are covered with cheesecloth and tied to prevent insects from contaminating the beverage. After fermentation, grains were drained, and the beverage was then subjected to physico-chemical analysis, microbial examination, and sensory evaluation. Figure 1 shows the process flow of the water kefir fermented JFB production.

Microbial Examination

The number of viable cells in the product was determined using the standard pour plating method. It was carried out by plating inoculants in MRS agar medium in three replications. The plates were incubated at 37 °C for 24–48 h. The calculation of colony-forming units (CFU) was done using the formula below (Equation 1):

$$\frac{CFU}{ml} = \frac{average\ colonies\ counted\ x\ dilution\ factor}{volume\ plated} \quad (1)$$

Physico-chemical Analysis

The parameters taken for the physicochemical analysis were the TSS, titratable acidity (% TA), and pH of the different beverages.

TSS. The TSS was measured using a calibrated ATAGO hand refractometer. Calibration was done by placing a

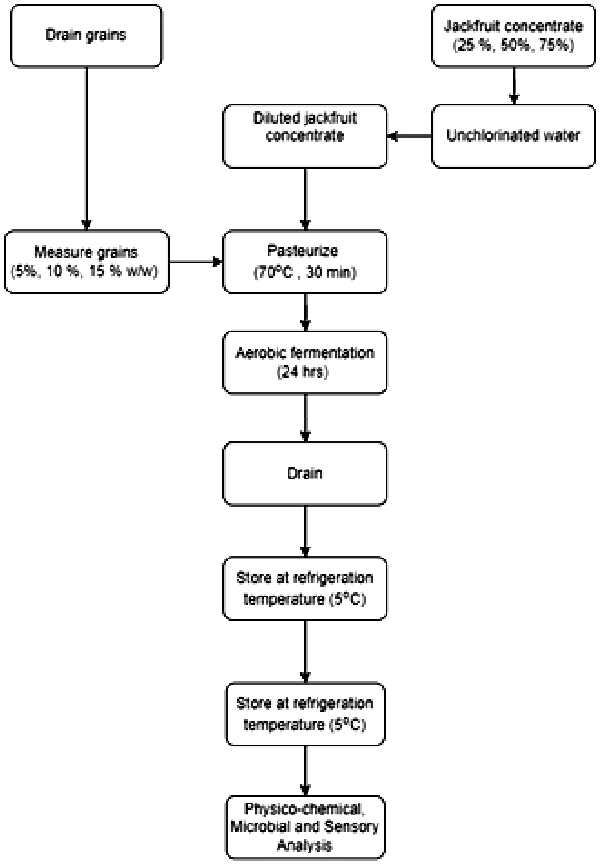

Figure 1. Process flow of fermented jackfruit beverage production.

drop of distilled water on the prism. After calibration, a drop of the sample was placed on the prism, and the TSS value was read and taken immediately. There were four replications for each sample. The mean of the four readings was used in reporting the data.

% TA. The % TA was determined by employing the standard titration method using a standardized 0.1N NaOH solution. Five (5) mL of the sample was diluted with 25 mL of distilled water in an Erlenmeyer flask. Then, 2–3 drops of 1% phenolphthalein indicator were added. It was then titrated with the standardized 0.1 N NaOH solution until a stable faint pink color was observed.

Percent lactic acid was calculated using the formula below (Equation 2):

$$\%TA = \frac{volume\ of\ NaOH\ x\ meq\ of\ prevailing\ acid}{volume\ of\ sample}\ x\ 100 \quad (2)$$

pH. The pH was monitored using a pH hydro tester by HM digital. pH buffers were used to calibrate the pH meter. About 20 mL of the samples were placed in small cups, and readings were taken and gathered in four replications. The mean of the readings was used in reporting the data.

Sensory Evaluation

Sensory evaluation using quality scoring and a nine-point Hedonic rating scale was carried out using a laboratory taste panel composed of 48 panelists. They were asked to evaluate the color, aroma, taste, mouthfeel, and perception of the product's acceptability. Samples were presented to the panelist following an incomplete block design, as laid out by Cochran and Cox (1957) given the seat plan t = 9, k = 6, r = 8, b = 12, and E = 0.94, Type II; t refers to the number of treatments, k the number of samples that were presented to the panelist, r is the number of replications, b is the number of blocks, and E is the efficiency factor.

Statical Analysis

RSM was used to determine the effects of the experimental variables and their interactive relationship with the response variables. All responses were equally weighted and using analysis of variance Fisher test value (ANOVA F test), response variables were analyzed to identify the significant factors and accuracy of the suggested model. Subsequently, the P-value ($p \leq 0.05$) and the coefficient of determination R^2 were used to express the quality of the fit of the polynomial model. The calculations were performed using Statistica® version 14.0 by TIBCO.

RESULTS AND DISCUSSION

Traditionally, water kefir fermented beverages can be prepared by combining water, (dried) fruits, and sugar (Gulitz *et al.* 2011; Laureys and de Vuyst 2017). With the known health-promoting benefits of water kefir (Pendón *et al.* 2021), in this study, we are exploring a new material that could be a potential substrate for water kefir fermentation. Jackfruit concentrate is a by-product of osmotic dehydration of pulps for dehydrated jackfruit processing. Instead of discarding this material, this research utilizes it as a source of flavor and sugar for water kefir preparation. Initial TSS and pH (10.5–15 °B and 5.0, respectively) of the raw material is a good prerequisite for water kefir fermentation in reference to other studies conducted that involve other fruit extracts and materials (Alves *et al.* 2021; Darvishzadeh *et al.* 2021; Hampton *et al.* 2021; Maldonado *et al.* 2020).

The kefir fermented JFB was subjected to analysis to assess its physico-chemical and microbial quality and acceptability to consumers.

Physico-chemical and Microbial Analysis of Fermented Jackfruit Beverage

According to the results (Table 1), the TSS of the product ranged from 3.85–11.90 °B after fermentation. As the levels of jackfruit concentrate increase, the TSS of the beverage

Table 1. Physico-chemical and microbial analysis of fermented jackfruit beverage.

Jackfruit concentrate (%w/w)	Kefir grains (%w/w)	TSS before fermentation	TSS 24 h after fermentation	pH before fermentation	pH 24 h after fermentation	% TA 24 h after fermentation	Mean microbial count viable CFU/mL
25	5	10.5	5.15	5	4	0.243	7.55 x 10^6
25	10	10.5	4.4	5	3.9	0.279	1.31 x 10^7
25	15	10.5	3.85	5	3.8	0.315	1.19 x 10^7
50	5	13	9.25	5	4.3	0.302	1.06 x 10^7
50	10	13	7.95	5	4.1	0.315	3.60 x 10^7
50	15	13	7.75	5	4.05	0.369	5.45 x 10^7
75	5	15	11.9	5	4.4	0.324	1.06 x 10^7
75	10	15	11.2	5	4.35	0.324	2.60 x 10^7
75	15	15	10	5	4.05	0.414	3.44 x 10^7

also increases. The increasing trend of the TSS value of the beverage before fermentation can be attributed to the jackfruit concentrate containing high sugar (20–25 °B before dilution with water), thus increasing the product's TSS value. TSS value of the product after fermentation decreases as the levels of kefir grains increase. This can be explained by the fermentation of sugar by the bacteria and yeast found in the kefir grains. The primary sugar found in a jackfruit concentrate is sucrose. In traditional water kefir production, sucrose is used as a common sugar source (Tuncay *et al.* 2019). It can be observed in Figure 2a that as levels of jackfruit increase and levels of kefir grains decrease, the TSS value of the product increases. Higher levels of kefir grain could reduce TSS because an increase in the microbial population could mean an increase in the rate of fermentation of the beverage.

The initial pH of all the treatments was 5.0. Hence, it can be presumed that the increase in kefir grains contributes to lowering pH. On the other hand, the value of the initial

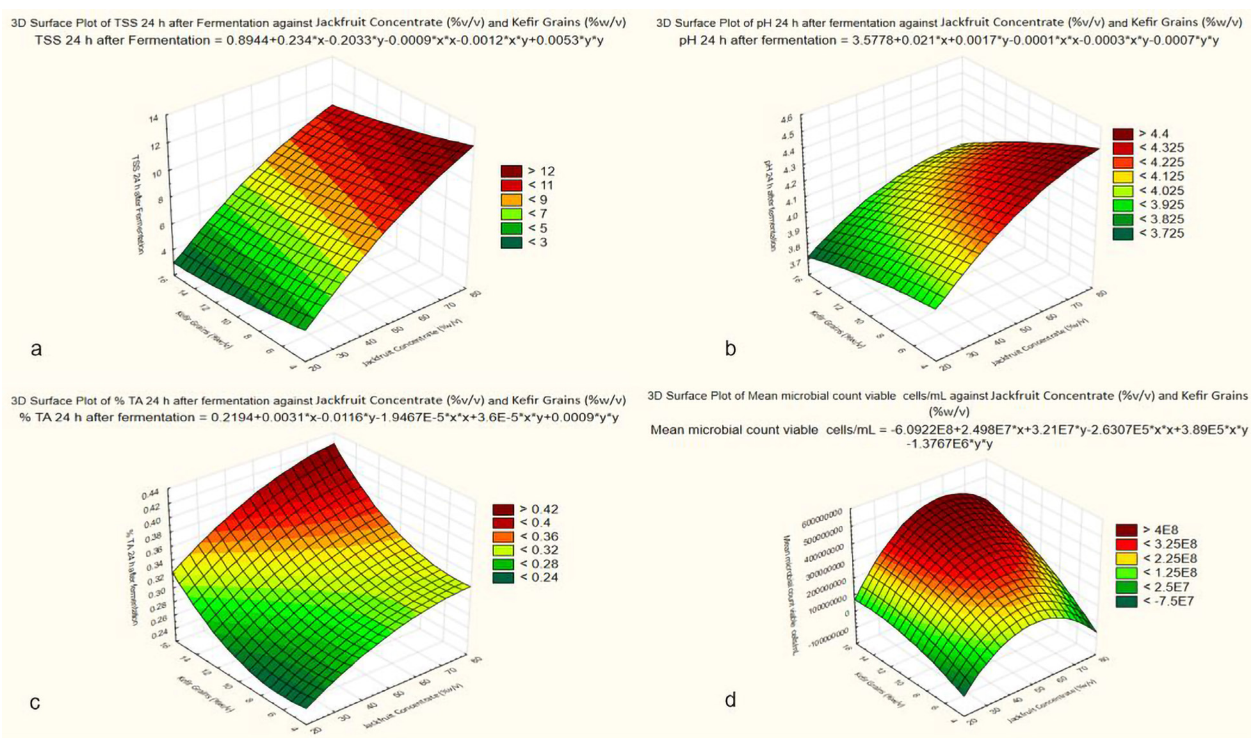

Figure 2. 3D contour plots showing the combined effect of level of kefir grains and jackfruit concentrate on product: [a] TSS; [b] pH; [c] % TA; [d] microbial count.

and final pH (Table 1) of the beverage is in accordance with the experiments conducted by Laureys and de Vuyst (2017) and Tuncay *et al.* (2019).

The initial % TA of the product ranged from 0.0207–0.288% and 0.243–0.414% after fermentation (Table 1; Figure 2c). There is an increase in % TA values as the kefir grains and jackfruit concentrate levels increase. The decrease in pH and increase in % TA are primarily attributed to the increased production of lactic acid and acetic in the beverage through fermentation of sugars. According to Otles and Cagindi (2003), kefir grains cause the fermentation of sugars, resulting in numerous components in the beverage – including lactic acid, acetic acid, CO_2, alcohol (ethyl alcohol), and aromatic compounds production.

Another way to assess if the fermentation of kefir in JFB is successful is through the proliferation of the microbial population. In connection to this, another concern is the presence of preservatives such as sodium metabisulfite in the JFB that might inhibit the growth of the microorganisms. Sodium metabisulfite is used to prevent the browning of the dehydrated product. However, Restaino *et al.* (1982) stated that the inhibitory effect of preservatives varies depending on the preservative concentration. So, to avoid this, dilution of the JFB is a recommended step not just to dilute the sugar concentration but also the preservative. According to the Codex Alimentarius Commission (2018) and the Canadian Food Inspection Agency (CFIA 2019), to exert a beneficial impact on health, the recommended number of viable cells in fermented products must be 1.0 x 10^9CFU of one or more of the eligible probiotic microorganism(s) per serving size of a product. Most probiotic drinks available in the market are available in 100–200 mL servings. According to Table 1, the microbial viability of the beverage ranged from 7.55 x 10^6 to 3.44 x 10^7 CFU/mL throughout the treatment. Therefore, a minimum number of 7 x 10^9 live probiotics per 100 mL serving size of fermented JFB can potentially deliver health benefits to the target host, thus making it a potential probiotic drink.

ANOVA revealed that only jackfruit concentrate level has a linear effect on TSS. All other parameters are not significantly affected by the factors. Regression analysis shows a highly significant effect on TSS and a significant effect on pH and % TA change. Regression analysis also shows that the level of jackfruit concentrate and kefir grains cannot predict the microbial count of the JFB. Only the level of jackfruit concentrate contributes a significant effect to the TSS of the product.

Sensory Qualities of Different Fermented Jackfruit Beverage

The color description of the product range from "very weak yellow" to "moderate yellow" as perceived by 79% of the panelists. The actual appearance of the JFB was shown in Figure 3. The yellow coloration of the JFB is attributed to the color of the raw material. This yellow color is contributed by the carotenoid pigment in jackfruit. In a study of de Faria *et al.* (2009), 18 carotenoids were successfully identified in jackfruit pulp. Surface plots for color acceptability show that increasing the jackfruit concentrate and the kefir grains level would give a color acceptability score of "7 – like moderately" on the nine-point Hedonic scale (Figure 4a).

The product's aroma was perceived by 79.5% of the panelist as "slightly perceptible jackfruit aroma" to "perceptible jackfruit aroma." This can be explained by the strong characteristic aroma of jackfruit, thus making it a good product flavoring. In a study by Maia *et al.* (2004), the major components identified in the aroma concentrate of identified jackfruit varieties were isopentyl isovalerate, butyl isovalerate, butyl acetate, ethyl isovalerate, and 2-methylbutyl acetate. The surface plot (Figure 4b) for aroma acceptability shows that an increase in the level of jackfruit concentrate starting from 30%v/v and in any levels of kefir grains would give aroma acceptability not less than 7 in the nine-point Hedonic scale.

The taste of the fermented jackfruit concentrate ranged from "moderately sweet" to "very sweet," as perceived by 83.85% of the panelist. The surface plot for taste

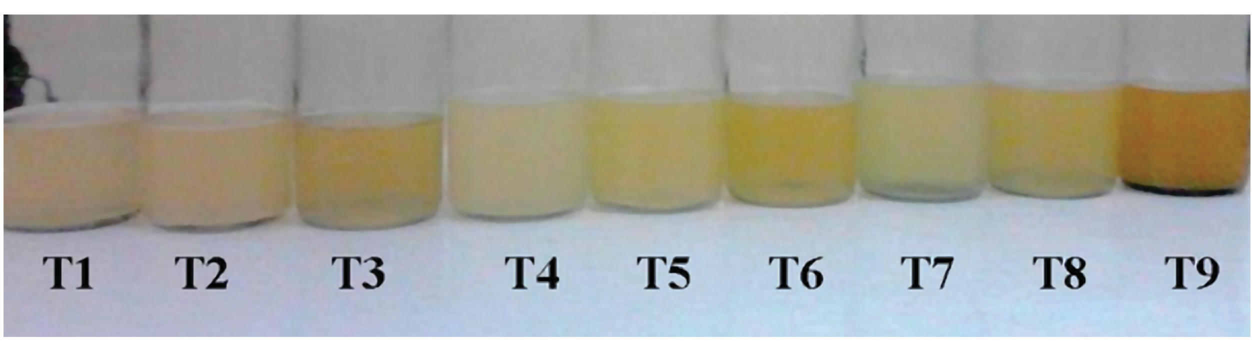

Figure 3. Fermented jackfruit beverage as influenced by different levels of jackfruit concentrate and kefir grains.

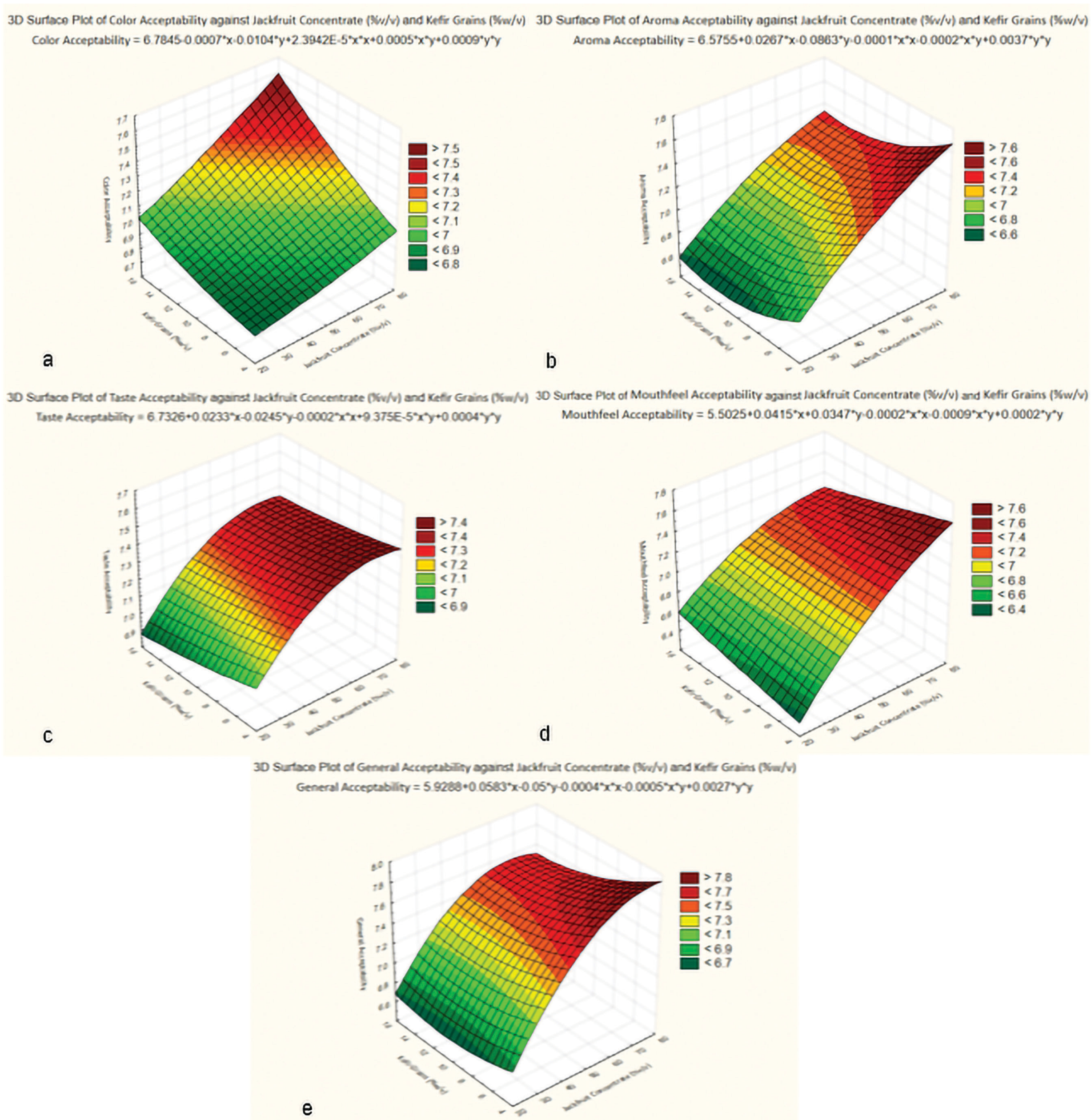

Figure 4. 3D contour plots showing the combined effect of level of kefir grains and jackfruit concentrate on product acceptability: [a] color; [b] aroma; [c] taste; [d] mouthfeel; [e] general acceptability.

acceptability shows that an increase in the level of jackfruit concentrate starting at 30%v/v at any levels of kefir grains would give a taste acceptability score of 7 on the nine-point Hedonic scale (Figure 4c).

The mouthfeel description of the product is "watery," as perceived by 96.70% of the panelists. The mouthfeel acceptability values of kefir fermented JFB ranged from 6.59–7.47. The surface plot for viscosity acceptability shows that a level of jackfruit concentrate of not less than 45% (w/w) at any levels of kefir grains would give consistency acceptability of not less than 7 in the nine-point Hedonic scale (Figure 4d).

The general acceptability score ranged from 6.66–7.88. The surface plot for general acceptability (Figure 4e) shows that a level of jackfruit concentrate of more than 60% (w/w) and kefir grains of less than 7.0% (w/w) would give the optimum general acceptability of "like moderately."

Table 2 presents the ANOVA in each response. Table 3 presents the linear and quadratic equations and the R^2 values of each

Table 2. Summary of ANOVA for physico-chemical characteristics and microbial quality of fermented jackfruit beverage after 24-h fermentation.

Parameter	TSS	pH	% TA change	Microbial count
Regression	190.46**	14.04*	14.96*	3.58
Linear	27.80*	3.29	2.30	2.20
Jackfruit concentrate (%v/v)	51.43*	6.53	2.63	4.30
Kefir grains (%w/v)	1.55	0.00	1.49	0.28
Square	4.67	1.03	2.53	2.85
Jackfruit concentrate (%v/v) * jackfruit concentrate (%v/v)	8.85	1.93	1.20	5.47
Kefir grains (%w/v) * kefir grains (%w/v)	0.49	0.12	3.85	0.24
Interaction				
Jackfruit concentrate (%v/v) * kefir grains (%w/v)	1.24	1.22	0.33	0.96

**Highly significant at p-value ≤ 0.005; *significant at p-value ≤ 0.05

Table 3. Summary ANOVA F test for acceptability of fermented jackfruit beverage.

Parameter	Color acceptability	Aroma acceptability	Taste acceptability	Mouthfeel acceptability	General acceptability
Regression	2.43	6.30	0.80	8.20	10.07*
Linear	5.78	15.19*	1.80	19.15*	22.53*
Jackfruit concentrate (%v/v)	5.83	27.52*	3.26	38.15*	42.42*
Kefir grains (%w/v)	5.72	2.86	0.33	0.15	2.65
Square	0.03	0.50	0.19	0.44	2.33
Jackfruit concentrate (%v/v) * jackfruit concentrate %v/v)	0.02	0.33	0.38	0.87	4.28
Kefir grains (%w/v) * kefir grains (%w/v)	0.04	0.66	0.00	0.00	0.37
Interaction					
Jackfruit concentrate %v/v) * kefir grains (%w/v)	0.54	0.11	0.01	1.81	0.64

**Highly significant at p-value ≤ 0.005; *significant at p-value ≤ 0.05

Table 4. Regression equation coefficients for the response of kefir fermented jackfruit beverage response models.

Effects	Responses									
	Color acceptability		Aroma acceptability		Taste acceptability		Mouthfeel acceptability		General acceptability	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
Constant	7.04789		7.04701		7.31597		7.09105		7.44965	
Linear										
Jackfruit concentrate (%v/v)	0.167	0.095	0.34755	0.013*	0.17969	0.169	0.4022	0.009*	0.41667	0.007*
Kefir grains (%w/v)	0.16543	0.097	–0.11198	0.19	–0.05729	0.605	–0.02488	0.728	–0.10417	0.202
Quadratic										
Jackfruit concentrate (%v/v) * jackfruit concentrate (%v/v)	0.01496	0.908	–0.0663	0.604	–0.10677	0.579	–0.10532	0.419	–0.22917	0.13
Kefir grains (%w/v) * kefir grains (%w/v)	0.0234	0.858	0.09354	0.475	0.01042	0.956	0.00405	0.974	0.06771	0.584

Table 4. continuation . . .

Effects	Responses									
	Color acceptability		Aroma acceptability		Taste acceptability		Mouthfeel acceptability		General acceptability	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
Constant	7.04789		7.04701		7.31597		7.09105		7.44965	
Interaction										
Jackfruit concentrate (%v/v) * Kefir grains (%w/v)	0.062	0.517	–0.02734	0.758	0.01172	0.929	–0.1072	0.271	–0.0625	0.483
R-sq	0.8018		0.9130		0.5707		0.9318		0.9438	
R-sq (Kondakova *et al.*)	0.4716		0.7680		0.0000		0.8181		0.8501	

Efffects are statistically significant at *p*-value** ≤ 0.005; *p*-value* ≤ 0.05

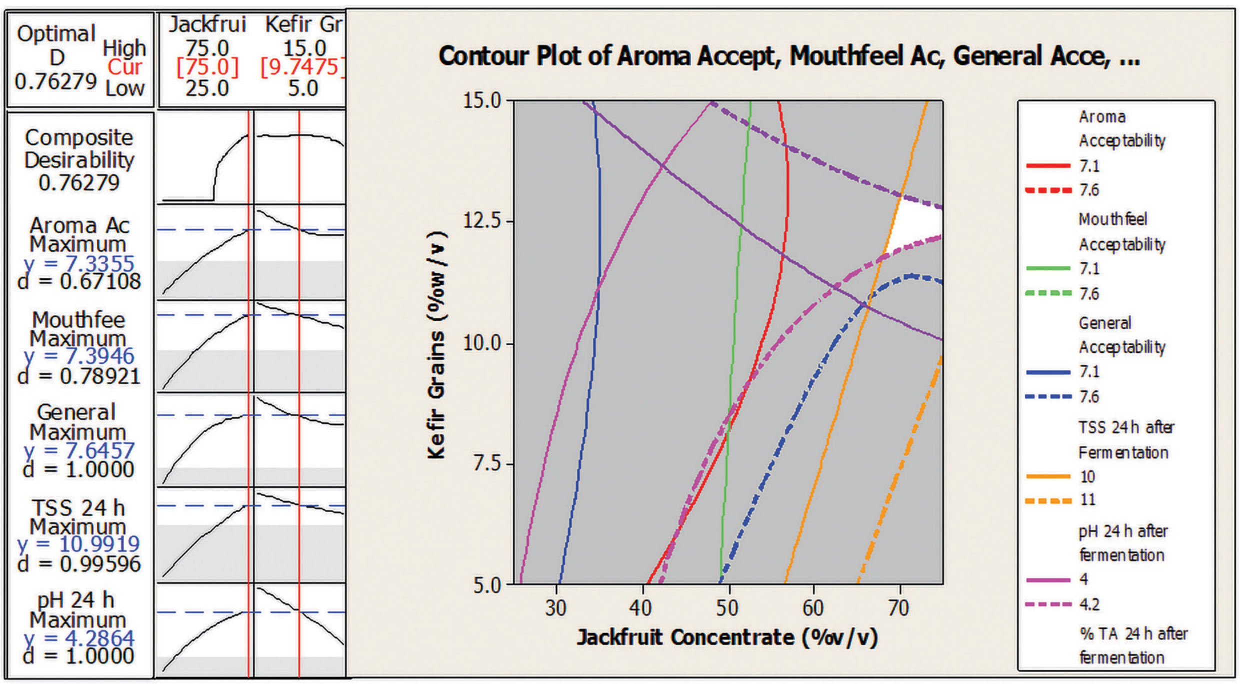

Figure 5. Optimum value of independent variables and overlaid contour plots of significant parameters.

parameter. As shown, the levels of jackfruit concentrate have only a linear significant effect on aroma, mouthfeel, and general acceptability. Even with an insignificant F value ($p > 0.05$), the high coefficients of determination (R^2 values) of the linear and quadratic polynomial models of color, aroma, mouthfeel, and general acceptability indicate that experimental data fit the equation effectively.

Optimization of Beverage Levels

Using the RSM response optimizer (Figure 5), the overall optimum region was at 75%v/v JFB and 9.7475%w/v kefir grains with respect to parameters that significantly affect the product's physico-chemical and microbial quality and acceptability. According to the results, predicted response values under the optimum conditions for aroma, mouthfeel, and general acceptability were 7.3355, 7.3946, and 7.6457, which fall under the "like very much" score of the nine-point Hedonic scale. The predicted response for TSS and pH were 10.9919 and 4.2864, respectively. Regression coefficients of the developed linear models exhibited significant ($p \leq 0.05$) relationships between the dependent variables and corresponding responses of the final fermented JFB. The optimal value of 0.76729 was selected for the desirability function.

CONCLUSION

Using jackfruit concentrate as a substrate for water kefir fermentation is an economical way to develop a new beverage with enhanced flavors and potential nutritional benefits. In addition, a new product is developed using a by-product that has less economic value. Through RSM, the formulation of the beverage is optimized with 75%v/v JFB and 9.7475%w/v kefir grains that could give optimal values of product acceptability score of 7 on the nine-point Hedonic scale, a TSS of 10.0019, and pH of 4.2864. Regression analysis shows it cannot predict the microbial count of the product; however, the microbial viabilities of all the treatments ranged from 7.55 x 10^6 to 3.44 x 10^7 CFU/mL, which suggests that the formulated beverage has a good probiotic potential. Acceptability studies also indicate that the product has good market potential.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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