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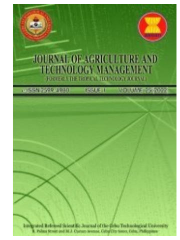

Original article

Development and quality assessment of apple *(Malus pumila var. domestica)* and pineapple *(Ananas comosus L. var. queen)* flavored kombucha

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ABSTRACT

A response surface methodology (RSM) was used for the determination of the best point combination of apple (*Malus domestica*) and pineapple (*Ananas comosus*) pulp to produce an acceptable fermented tea (kombucha) beverage. This study specifically aims to develop kombucha with tropical flavors to improve its sensory acceptability and physico-chemical properties. Kombucha was produced using black oolong leaves and fermented for eight (8) days. Apple and pineapple pulp were added in 3%, 6%, and 9% w/v each arranged in a Central Composite Design (CCD) with a quadratic model. Sensory acceptability attributes that were determined are color, appearance, aroma, sourness, sweetness, mouthfeel, and general acceptability. Physico-chemical tests include pH total soluble solids (TSS), titratable acidity (TA) and L*, a*, and b* color values. The effects of the sensory and physico-chemical parameters were studied by employing a second-order central composite design. The coefficient of determination, R^2 for sensory analysis, ranged from 0.5-0.7 and 0.72- 0.98 for the physico-chemical tests. Analysis of the regression coefficients showed that the different levels of apple and pineapple pulp did not affect the sensory acceptability of kombucha. However, pineapple levels significantly affected the TA, L* & a* values as they exerted a highly significant influence ($p < 0.001$) on these parameters. Based on the contour plots overlay, best formulation levels for the fruits are 7.3% apple pulp and 9.2% pineapple pulp.

KEYWORDS: *Fermentation, Kombucha, RSM, Sensory*

1 INTRODUCTION

The beverage industry, especially functional beverages, is a vibrant, growing market. In the last decade, the intake of foods with beneficial health and phytomedicine benefits has increasingly gained popularity. Some main groups of functional ingredients are probiotics which are present in fermented products such as kombucha. Kombucha is a functional beverage from fermented tea originating from Central and Eastern Asia. It is usually prepared through the aerobic and static fermentation of sucrose-sweetened black, green, or blue (Oolong) tea with a symbiotic culture of bacteria and yeast (SCOBY) (Dutta & Paul,2019; Martinez Leal et al., 2018). The recipe for making Kombucha varies in detail, such as the amount of tea or added sugar and starter culture, and the time and temperature required for the fermentation. However, the primary process for kombucha processing is similar.

The taste of kombucha is mildly sweet, sour, and refreshing, and it is well-received by consumers around the globe (Teoh et al., 2004). In several investigations, Kombucha has been shown to have antibacterial, antioxidant, anticancer, antidiabetic, and anti-inflammatory properties (Villarreal-soto et al., 2018). Kombucha beverages are claimed to have several curative properties, such as improving general health, increasing longevity, and improving gastrointestinal disorders (Dufresne & Farnworth 2000; Jayabalan et al. 2007).

The main ingredients in traditional kombucha are black tea and table sugar (sucrose). Many studies were carried out to change the substrates by incorporating fruit juices to boost the biological activities and improve the flavor profile (Akbarirad et al., 2017; Yavari et al., 2018). Because tropical fruit juices are high in phenolic compounds, they have the potential to boost the number of phenolic compounds in fruit-based kombucha as well as its biological activity. Being a tropical country, the Philippines has an abundance of natural resources. However, few locally available plants are being thoroughly utilized for their full potential, wherein some of these underutilized crops are a source of food and nutritive compounds (Balangcod & Vallejo, 2011)

Pineapple and apple are tropical fruits that are rich in phytochemicals including flavonoids, phenolic acids, and carotenoids (Septembre-Malaterr, 2016). These compounds have been shown to have antioxidant, anti-inflammatory, and anticancer properties (Sharma et al., 2017). The fermentation process produces beneficial

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compounds including vitamins, minerals, amino acids, and antioxidants. These compounds can help to improve gut health, boost immunity, and increase energy levels (Martínez Leal et al., 2018). Pineapple and apple make excellent additions to fermented tea. Their sweetness may balance the sourness of the fermentation, and their phytochemical content provides numerous health benefits. Therefore, this study investigated the use of tropical flavors that are acceptable in the production and developed kombucha to create a beverage that is rich in flavor and biological compounds. To the best of the authors' knowledge, no studies were carried out to use apple and pineapple flavors.

2 MATERIALS AND METHODS

Procurement of Raw Materials

Black tea (Oolong tea) was purchased in a Chinese herbal store in Cebu City, Philippines. Apple (*Malus pumila var. domestica*) and pineapple (*Ananas comosus L. var. queen*), and other ingredients were procured at the local market. Kombucha SCOBY and previous kombucha starter were purchased from local kombucha brewers produced from the fermentation of unflavored kombucha.

Formulation and Production Kombucha

First Fermentation

Kombucha is processed following the method cited by Nummer (2013) with some modifications. Black tea (oolong tea) was prepared at a concentration of 12 g/L with water at 75°C for 2 min. Subsequently, the tea leaves were removed by stainless steel sieves. It was added with 5.28% w/v of sucrose. After cooling down to room temperature, the solution was poured into a glass jar and was inoculated with 2.7% (w/v) or 27.73 g/L of starter culture and large-sized, 25 cm, SCOBY. The fermentation was controlled at 27°C for 4 days or until biofilm was visible.

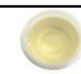
Second Fermentation

Kombucha was transferred into 1 L flip-top bottles and was added with different apple and pineapple levels as a flavoring. This was kept at room temperature (27°C) for 4 days. Afterwards, kombucha was filtered and transferred into 300 mL sterilized glass bottles and was kept in the refrigerator until use. Table 1 shows the different apple and pineapple pulp levels and the resulting kombucha color and appearance.

Sensory Evaluation

The kombucha was evaluated through sensory evaluation to determine whether the product possesses the desirable sensory attributes. The sensory qualities considered include color, appearance, aroma, sourness, sweetness, flavor, mouthfeel, and overall acceptability. It was evaluated using a combination of quality description as perceived by the consumer and a 9-point hedonic scale, with 1 as "dislike extremely" to 9 as "like extremely", for the acceptability of the product. An Incomplete Block Design (IBD), as laid out by Cochran and Cox (1957), was used during the presentation of the treatments. The set plan of t=13, k=4, r=4, b=13, E=.81, Type IV where t refers to the number of treatments, k the number of samples presented to the panelist, r to the number of replications based on the plan IBD, b the number of blocks and E the efficiency factor. The plan was repeated four times to get 24 evaluations per treatment and requires 72 panelists.

Table 1. Treatment combinations of the experiment

Trt	Code for Independent Variables		Apple % w/v	Pineapple % w/v	Kombucha
1	-1	0	3	6	
2	0	-1	6	3	
3	+1	0	9	6	
4	0	0	6	6	
5	0	+1	6	9	
6	0	0	6	6	
7	+1	-1	9	3	
8	0	0	6	6	
9	-1	+1	3	9	
10	+1	+1	9	9	
11	-1	-1	3	3	
12	0	0	6	6	
13	0	0	6	6	

Physico-chemical Analysis

pH and total soluble solids (TSS)

For pH, a 15 mL sample was placed in a plastic cup and was measured using a pH meter (Atago DPH2, Japan). To measure the TSS, 1-2 drops of the sample were placed in a hand refractometer (Atago PAL-2,

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Tokyo, Japan). The pH and TSS values were obtained using the mean of three readings from triplicates.

Titratable acidity

Titratable acidity (TA) was determined by acid-base titration using phenolphthalein (0.1%) as the indicator according to the AOAC method (AOAC 947.05, 2005). Previously standardized 0.1 M NaOH (Scharlab, Spain) was used to titrate against 10 mL of kombucha, and mixed with 10 mL of distilled water. Four to five (4-5) drops of phenolphthalein solution (1%) were added to the mixture and swirled to mix. The mixture was titrated against standardized 0.1 M NaOH solution until the first persistent (30 s) pink color was noted, and the volume of titer used was recorded. Calculated titratable acidity was expressed in grams of acetic acid per liter of the sample (equation 1). The titratable acidity was measured in duplicate, and the experiment was conducted twice (AOAC 947.05, 2005).

$$\text{\% Acetic Acid} = \frac{\text{Volume of NaOH used (ml)} \times 0.006}{\text{sample weight (g)}} \times 100 \quad (1)$$

Molecular weight of acetic acid= 60 g/mol
1 mL 0.1 M NaOH= 0.006 g acetic acid
1 ML of test sample= 1 g of sample

Color Intensity Determination

The evaluation of the surface color of kombucha was analyzed using a hand-held colorimeter (3NH, China), in reflectance mode and in the CIE L*, a*, and b* color scale through subjecting five to ten mL of each sample to the instrument.

Experimental Design and Data Analysis

RSM was used in designing this experiment. Statistica Software version 8 was used to generate the experimental designs, statistical analysis, and regression model (ECHIP, 1995). The central composite design (CCD) was employed with a quadratic model (Box & Draper, 1987).

The independent variables were apple juice levels (x) (3%, 6% & 9%) and pineapple juice levels (y) (3%, 6%, 9%), which were previously determined using preliminary studies. Each independent variable had three levels: 1, 0, and +1. A total of thirteen combinations (including five replicates at the center point with each value coded as 0) were chosen in random order according to CCD configuration for two factors (Rastogi & Rashimi, 1999). The coded and actual values of the two independent variables, together with the responses, were shown in Table 1. The response functions (z) were sensory acceptability responses (color, aroma, flavor, sourness, sweetness, mouthfeel, general acceptability) and physico-chemical responses (pH, total soluble solids (TSS), titratable acidity (TA), and colorimetric values). Using the equation below, these values were related to the coded variables (x & y) by a second-degree polynomial.

$$z = b_0 + b_1x + b_2y + b_{11}x^2 + b_{12}xy + b_{22}y^2 \quad (2)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22} (quadratic effects), and b_{12} (interaction effects). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic, and interaction terms were determined. The significance of all terms in the polynomial was judged statistically by computing the F-value at a probability (p) of 0.001, 0.01, or 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models.

Statistical analysis

The result of the experiment was subjected to Response Surface Regression (RSREG) Analysis using the SAS statistical computer software to determine the effects of the independent variables on the sensory attributes and physico-chemical analysis. A response contour plot was made for all analyses to clarify the different effects of the factor variables on the studied responses. Analysis of variance for the regression coefficients and parameter estimates was done to describe the regression models for each attribute.

Determination of the Best Formulation

Contour plots which are graphical presentations of contour models were generated by the computer STATISTICA (version 6.0, Statsoft, Inc. 1984-1995) to demonstrate the different effects of factor variables on each response studied. For each sensory attribute acceptability, a cut-off acceptable sensory score within the experimental range was set to establish the region above which the product is considered as best formulations. This was done to determine the best formulations' limiting attributes and physico-chemical properties.

3 RESULTS AND DISCUSSIONS

The statistical analysis indicates that the proposed model was adequate, possessing no significant lack of fit and with satisfactory values of the R^2 for all the responses. The R^2 values for sensory acceptability and physico-chemical responses ranged from 0.5-0.7 and 0.72- 0.98 (Table 4). The closer the value of R^2 to unity, the better the empirical models fit the actual data. Conversely, the smaller the value of $R^{2,}$ the less relevant the dependent variables in the model have in explaining the behavior of variations (Little & Hills, 1978; Mendenhall, 1975). All regression models' probability (p) values in the intercept were less than 0.05, with no lack-of fit.

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Sensory Acceptability Tests

Table 2 and Table 3 presents the quality description and mean acceptability ratings, respectively, of the sensory attributes of kombucha as affected by different levels of apple and pineapple flavorants. Apple and pineapple pulp were added to kombucha in 3%, 6%, and 9%. Table 4 presents the parameter estimates of sensory attributes for each dependent variable with their coefficients of determination (R^2).

Color

The color and appearance of 13 treatments of kombucha are illustrated in Table 1. Color of kombucha produced ranges from light yellow to yellow. As shown in Table 4, the color of kombucha is not significant in linear and quadratic effects. This could be due to the similar yellowish color of apple and pineapple fruits. Only the intercept between the two factors is significant which can be illustrated by the flat contour in the graph (Figure 1a).

Table 2. Quality description of the sensory attributes of Kombucha as influenced by the levels of apple and pineapple as flavorants

	Apple % w/v	Pineapple % w/v	Color	Appearance	Aroma	Sourness	Sweetness	Flavor	Mouthfeel
1	3	6	Yellow	Clear	Very perceptible A	Slightly sour	Slightly Sweet	Very perceptible A	Smooth
2	6	3	Light Yellow	Slightly Turbid	Very perceptible A	Slightly sour	Slightly Sweet	Very perceptible A	Smooth
3	9	6	Brownish Yellow	Slightly Turbid	Well blended PA & A	Moderately	Slightly Sweet	Well blended PA & A	Fizzy
4	6	6	Yellow	Slightly Turbid	Well blended PA & A	Slightly sour	Slightly Sweet	Well blended PA & A	Fizzy
5	6	9	Yellow	Slightly Turbid	Well blended PA & A	Moderately	Slightly Sweet	Well blended PA & A	Fizzy
6	6	6	Yellow	Slightly Turbid	Well blended PA & A	Slightly sour	Slightly Sweet	Well blended PA & A	Fizzy
7	9	3	Brownish Yellow	Slightly Turbid	Very perceptible A	Slightly sour	Slightly Sweet	Very perceptible A	Smooth
8	6	6	Yellow	Clear	Well blended PA & A	Slightly sour	Slightly Sweet	Well blended PA & A	Smooth
9	3	9	Yellow	Slightly Turbid	Very perceptible A	Slightly sour	Slightly Sweet	Very perceptible A	Smooth
10	9	9	Brownish Yellow	Slightly Turbid	Very perceptible A	Moderately sour	Slightly Sweet	Very perceptible A	Fizzy
11	3	3	Light Yellow	Clear	Well blended PA & A	Slightly sour	Slightly Sweet	Well blended PA & A	Smooth
12	6	6	Yellow	Slightly Turbid	Well blended PA & A	Slightly sour	Slightly Sweet	Well blended PA & A	Fizzy
13	6	6	Yellow	Slightly Turbid	Well blended PA & A	Slightly sour	Slightly Sweet	Well blended PA & A	Fizzy

*Legend: PA- Pineapple, A- Apple

Table 3. Mean acceptability ratings of the sensory attributes of Kombucha as influenced by the levels of apple and pineapple used as flavorants

	Apple % w/v	Pineapple % w/v	Color	Appearance	Aroma	Sourness	Sweetness	Flavor	Mouthfeel
1	3	6	6.47	6.78	6.75	6.68	6.75	6.72	7.06
2	6	3	7.00	6.91	7.06	6.75	6.66	6.84	7.06
3	9	6	6.84	6.50	6.69	6.25	6.88	6.84	7.13
4	6	6	6.63	6.34	6.75	6.75	6.72	6.69	6.47
5	6	9	6.81	7.13	7.00	7.19	7.41	7.28	7.00
6	6	6	6.63	6.34	6.75	6.75	6.72	6.69	6.47
7	9	3	6.97	6.75	6.94	6.88	6.91	7.16	6.72
8	6	6	6.72	6.20	6.75	6.69	6.75	6.69	6.52
9	3	9	6.88	6.72	6.72	6.78	6.63	6.78	6.81
10	9	9	6.69	6.47	6.69	6.47	6.50	6.66	6.75
11	3	3	6.53	6.38	6.38	6.50	6.84	6.59	6.69
12	6	6	6.57	6.41	6.69	6.81	6.72	6.78	6.52
13	6	6	6.65	6.47	6.71	6.75	6.75	6.92	6.65

No. of Panelists= 64

Range of acceptability score: 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, 1- dislike extremely

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Table 4. Parameter estimates of Sensory Attributes for Kombucha

	Color^{ns}	Appearanc^{ns}	Aroma^{ns}	Sourness^{ns}	Sweetnes^{ns}	Flavor^{ns}	Mouthfeel^{ns}	General Acceptability^{ns}
Intercept	6.248307**	5.967094**	5.432200**	5.682236**	6.101590**	5.886742**	6.614988**	6.985498**
Apple (g)	0.026313	0.036565	0.047286	0.050073	0.025571	0.033491	-0.000246	0.006965
Apple (g)^2	-0.000101	-0.000223	-0.000286	-0.000338	-0.000187	-0.000158	0.000017	0.000042
Pineapple (g)	-0.014601	-0.014442	-0.002394	-0.013867	0.000683	-0.005044	0.008171	-0.000102
Pineapple (g)^2	0.000203	0.000220	0.000104	0.000226	0.000026	0.000143	-0.000051	0.000091
Apple (g)*Pineapple (g)	-0.000174	-0.000174	-0.000165	-0.000191	-0.000052	-0.000191	-0.000026	-0.000208
R^2	0.613853	0.611226	0.408453	0.58434	0.649316	0.500879	0.669075	0.708309

Appearance

The turbidity in kombucha is attributed to the amount of cellulose and fibrous matter from the SCOBY being released to the kombucha broth (Amarasinghe et al., 2018). Turbidity ranged from clear to light turbid. However, there were no significant differences on the acceptability of kombucha appearance between treatments. The contour plot of appearance sensory attribute can be seen in Figure 1b. From the graph, it is known that to gain consumer preference level at 7 (like moderately), the kombucha should be processed using the combination of apple and pineapple in accordance with the red shaded value on the graph. High color & appearance acceptability can be observed at 4-10% levels of apple and 2-3% and 9-10% pineapple, which is apparent in the red & dark red regions of the contour plot. Equations 3 & 4 show the influence of variables on color and appearance, respectively.

(3) COLOR = 6.2431+0.025*x-0.0125*y-9.2593E-5*x*x-0.0002*x*y+0.0002*y*y

(4) APPEARANCE = 5.9618+0.0352*x-0.0123*y-0.0002*x*x-0.0002*x*y+0.0002*y*y

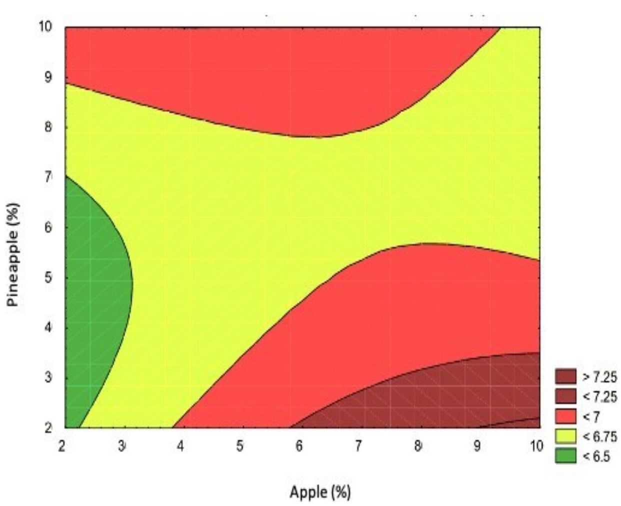

Figure 1a. Contour Plot for Color

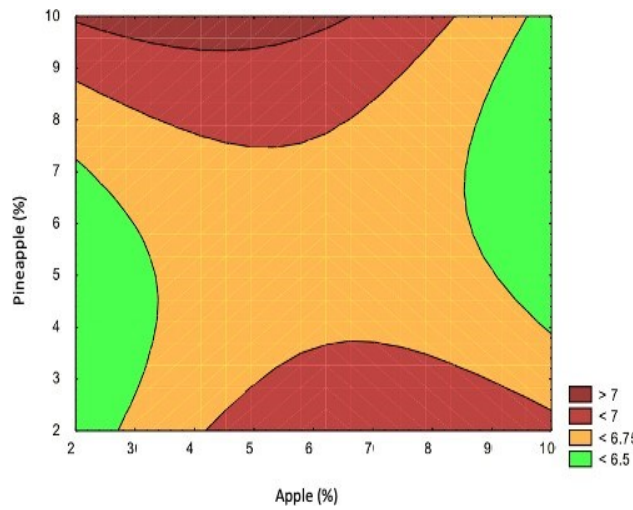

Figure 1b. Contour Plot for Appearance

Aroma

Aroma compounds can be detected in ppb concentrations and are detected by olfactory nerve endings in the nose. The brain processes information from these senses to give an integrated flavor experience (Sharma et al., 2014). Aroma quality descriptors for the produced kombucha are described as "well blended apple and pineapple for aroma" (Table 2). According to Akbarirad et al. (2017), the aroma of apple kombucha is produced by short-chain organic acids and more organic acids are produced as fermentation time increases. More organic acids increase the aroma of kombucha but decrease its taste acceptability (Zubaidah et al., 2018). Despite the possible presence of acids that affect kombucha aroma, there were no significant differences on the acceptability of kombucha aroma between treatments.

Taste

Taste is the detection of non-volatile compounds (in concentration of parts per hundred). These correspond to

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the perception of kombucha's predominant sweet and sour tastes. Taste quality descriptor for the produced kombucha is described as “slightly to moderately sour for sourness” and “slightly sweet” for sweetness. The taste acceptability regression effects of kombucha are not significant in both linear and quadratic (Table 4), thus different flavorants have no effect on sweetness and sourness acceptability. Apart from citric acid in fruits, the taste of kombucha also depends on the ratio of added sugar and acid produced (acetic acid) (Zubaidah et al., 2018). In accordance with the findings of Akbarirad et al. (2017) and Reis (1994), the taste of kombucha at the end of fermentation was evaluated as having a moderate vinegar-like flavor.

Flavor

The second fermentation (flavoring stage) aims to increase the characteristic flavor of tea and gas formation. However, the amount of flavoring added during this stage is controlled to prevent excessive fermentation and carbonation. After a certain amount of carbon dioxide is generated, which increases the pressure; there is a risk of rupturing the glass bottle or packaging. In order to minimize this hazard, the products need to be refrigerated to slow down the second fermentation. Thus, 10% to 20% flavoring is suggested for second fermentation of kombucha (Volkas, 2018). Furthermore, according to Liamkaew et al. (2016), 15% apple juice added in kombucha produces a significant amount of polyphenols and acetic acid content. Levels of apple and pineapple fruit combination are predicted to coincide with these values to prevent excessive carbonation and achieve high levels of polyphenols.

(5) $AROMA = 5.4236+0.0451*x+0.001*y-0.0003*x*x-0.0002*x*y+7.5231E-5*y*y$

(6) $SOURNESS = 5.6822+0.0501*x-0.0139*y-0.0003*x*x-0.0002*x*y+0.0002*y*y$

(7) $SWEETNESS = 6.1076+0.0271*x-0.0017*y-0.0002*x*x-5.2083E-5*x*y+4.6296E-5*y*y$

(8) $FLAVOR = 5.8889+0.034*x-0.0059*y-0.0002*x*x-0.0002*x*y+0.0002*y*y$

Fig. 2 a, b, c & d shows the contour map for the effect of the independent variables on the aroma, taste (sourness & sweetness), and flavor. The taste and flavor regression effects of kombucha are not significant in both linear and quadratic (Table 4). Processing of kombucha with 4-9% apple and at different levels of pineapple yielded moderately likable attributes (7 at the hedonic scale) for aroma, taste, and flavor sensory attributes. However, it can be observed that acceptability started to decrease when apple levels reached 9-10%. These results can be attributed to the similar flavor profile of apple and pineapple. The total number of volatile components, such as alcohols (Mehinagic et al., 2006), esters, and ketones in a given apple, is cultivar specific and depends on its enzymatic activity. The aroma of apples is mainly due to several volatile compounds present in their peel and flesh (Holland et al., 2005). Similarly, the main volatile compounds found in pineapple fruit are esters, followed by terpenes, ketones, and aldehydes (He et al., 2007).

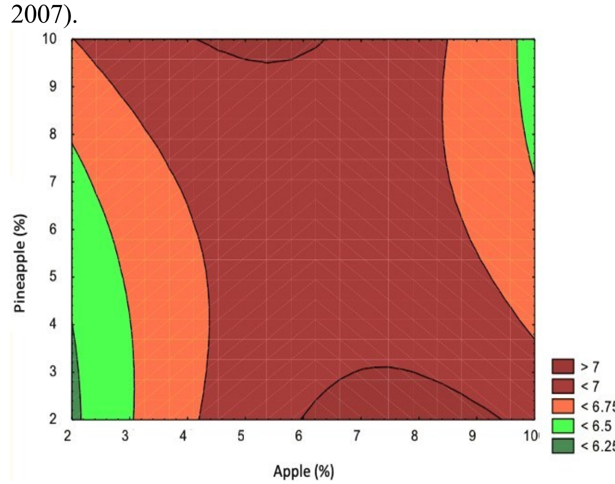

Figure 2a. Contour Plot for Aroma

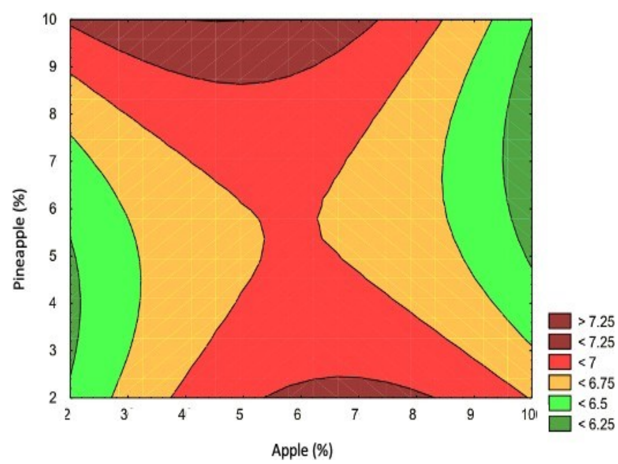

Figure 2b. Contour Plot for Sourness

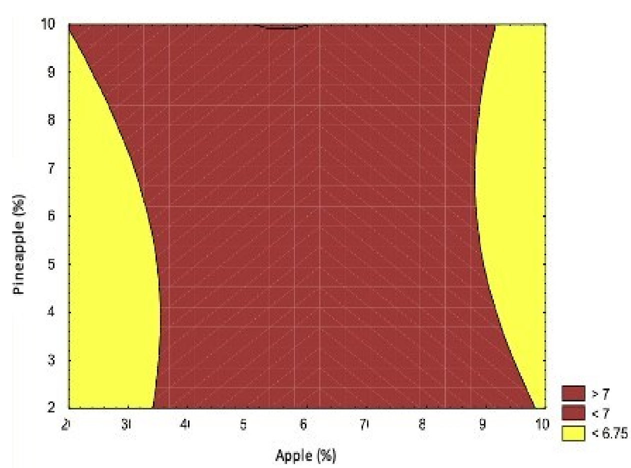

Figure 2a. Contour Plot for Sweetness

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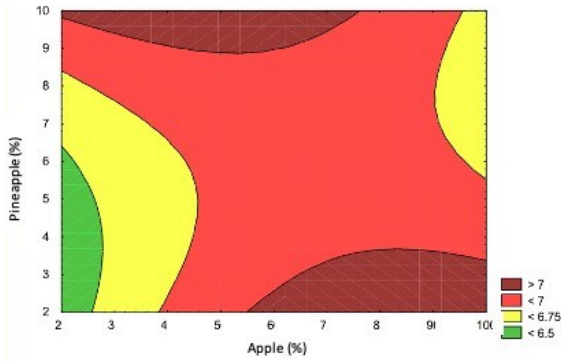

Figure 2a. Contour Plot for Flavor

Mouthfeel

The contour plot for kombucha mouthfeel is shown in Figure 3. It is evident that acceptability is the same (like moderately- 6.75) at all levels of apple and pineapple flavors. Equation 9 shows the effect of different variables on the mouthfeel of kombucha.

(9) $\text{MOUTHFEEL} = 6.6146-0.0003*x+0.0083*y+1.7361E-5*x*x-2.6042E-5*x*y-5.2083E-5*y*y$

Mouthfeel is another sensory element to be considered in kombucha production. The type of tea used in kombucha (e.g., green, black, or yellow) processing also affects the product's mouthfeel, clarity, and smoothness (Gramza-Michałowska, et al. 2016). Many of the flavor and mouthfeel characteristics of kombucha, such as astringency, could be explained by the presence of compounds such as piperidides, isobutyl amides, and esters (Obst et al., 2017). The mouthfeel of apple-pineapple kombucha is described as smooth with no astringency and light fizziness which is liked moderately by sensory panelists (Table 2). Despite the known presence of astringent polyphenols in kombucha, the product is not described as astringent. Some studies indicate that the presence of sugars in kombucha inhibits the sensation of astringency (Lyman and Green, 1990). Considering that apple and pineapple are good sources of sugar (fructose), the sugar content in kombucha may reduce the astringency.

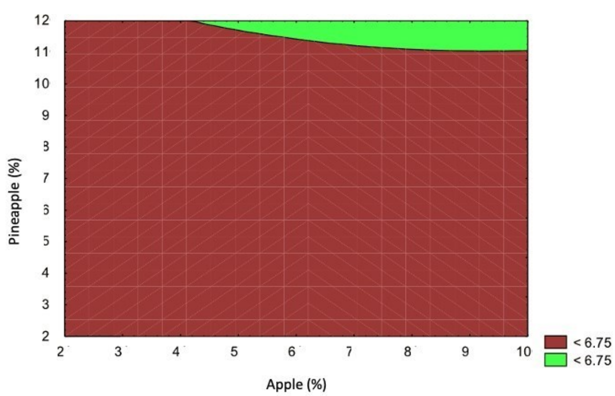

Figure 3. Contour plot for Mouthfeel

General Acceptability

General acceptability is an overall response given by panelists considering all sensory product features. The best value given by the panelist was 7.5, corresponding to an acceptability between "like moderately" and "like very much". Product development using Response Surface Method (RSM) provides an opportunity to improve the quality of products so that the acceptance level of the panelists increases. In this case, the acceptance level of panelists can be enhanced using a higher proportion of pineapple pulp. This is illustrated in the plot for the overall acceptability of kombucha (Figure 4). It can be observed that high general acceptability is evident at high levels of pineapple and apple pulp added to kombucha. However, the same with other sensory attributes, regression effects in both linear and quadratic are not significant (Table 4). Equation 10 shows the effect of different variables on the general acceptability of kombucha.

(10) $\text{OVER-ALL ACCEPTABILITY} = 6.9826+0.0063*x+0.001*y+4.6296E-5*x*x-0.0002*x*y+8.1019E-5*y*y$

Fermented tea microbiota from these fruit flavors use this as a carbon source and, in the fermentation, process produce bioactive products with unique, pro-health properties (Moreno-Jimenez et al., 2018; Vazquez-Cabral et al., 2017). However, the sensory properties of kombucha vary depending on the composition of the raw material and added flavors such as fruits. In the case of apple-pineapple flavored kombucha, sensory qualities and general acceptability are not influenced by different levels of apple, and pineapple pulp added.

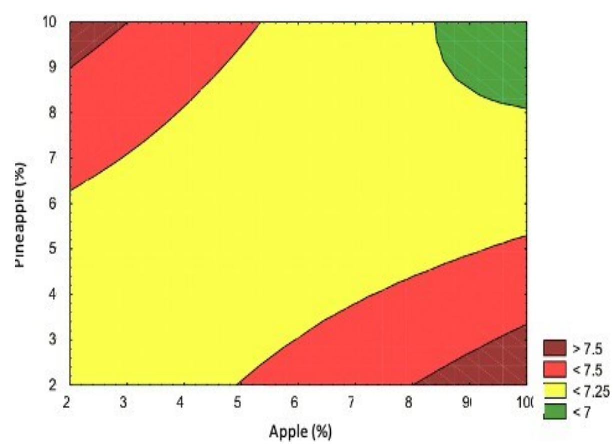

Figure 4. Contour Plot for General Acceptability

Table 5 presents the parameter estimates of the sensory attributes of kombucha as influenced by the levels of apple and pineapple in the formulation.

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Table 5. Physico-chemical characteristics of kombucha as influenced by the levels of apple and pineapple as flavorants

	Apple % w/v	**Pineapple % w/v**	**pH**	**TSS**	**TA**
1	3	6	3.60	6.93	0.17
2	6	3	3.67	7.07	0.25
3	9	6	3.73	7.03	0.26
4	6	6	3.63	6.87	0.20
5	6	9	3.70	7.20	0.26
6	6	6	3.63	6.87	0.20
7	9	3	3.67	7.20	0.33
8	6	6	3.63	6.87	0.20
9	3	9	3.67	6.57	0.20
10	9	9	3.67	3.67	3.67
11	3	3	3.63	6.73	0.24
12	6	6	3.63	6.87	0.20
13	6	6	3.63	6.87	0.20

The effect of different levels of apple and pineapple flavors on sensory attributes and physico-chemical tests are reported (Table 5) by the coefficient of the second-order polynomials. Response contour plots were used to illustrate the effect of the variables on the responses. Equations 10, 11 and 12 show the effect of different variables to pH, TSS, and TA of kombucha.

(10) $pH = 3.6222+0.0011*x-0.0017*y+2.1023E-17*x*x+1.8173E-17*x*y+1.8519E-5*y*y$

(11) $TSS = 6.4333+0.0206*x-0.0063*y-0.0001*x*x+1.8519E-5*x*y+3.7037E-5*y*y$

(12) $TA= 0.3175+0.0012*x-0.0056*y+7.1574E-6*x*x-1.3542E-5*x*y+4.838E-5*y*y$

The kombucha's pH, TSS, and TA are 3.66, 6.59 °Brix and 0.62% acetic acid, respectively (Table 6). The evaluation of the physico-chemical properties of kombucha is vital since it influences some sensory properties perceived by consumers. Consumers may judge the drink mostly based on the sourness, acidity, sweetness, and color.

pH and TSS were found to be not significant in all regression effects. Titratable Acidity was mainly a function of the pineapple levels with a positive linear effect ($p < 0.001$) and a positive quadratic effect ($p < 0.05$) in both apple and pineapple interactions. pH, TSS, and TA contour maps are shown in Figures 5 a, b, and c. The physicochemical properties indicated increased acid levels, which might decrease the consumer appeal of the fermented beverage.

The pH endpoint of >2.5 is advised for safe kombucha intake; thus apple-pineapple kombucha is considered safe to consume. It is accepted that the pH of kombucha should fall between pH 2.5 and 3.5, TSS of 6-10°Brix (Jayabalan et al., 2014; Nummer et al., 2013; Khosravi et al., 2018), and TA of less than 1% are evident in most commercially available kombucha (Kombucha Kamp, 2022). The findings show a statistically significant increase in acidity of samples as fruit pulp levels increased, both linearly and in quadratic effects. This implies a high fermentation rate for treatments having a larger quantity of fermentable sugars (primarily fructose and glucose) consumed by bacteria, thus generating organic acids, particularly acetic acid (Khosravi et al. 2018). Further studies are necessary to test the accumulation of organic acids, nucleic acids, and toxicity of kombucha during an extended period of fermentation.

Table 6. Parameter Estimates of Physico-chemical Attributes of Kombucha

Intercept	pH	TSS	TA	Color values L	a	b
Intercept	3.666667	6.59999	0.628013	7.301667	0.503333	51.13185
Apple (g)	0.001111	0.020556	0.001247	-0.025194	0.000528	-1.02075
Apple (g)^2	0.000000	-0.000130	0.000007	0.000572	0.002847*	0.00619
Pineapple (g)	-0.001667	-0.006296	-0.005618**	-0.072000	-0.128028	-1.03339
Pineapple (g)^2	0.000019	0.000037	0.000048**	0.000969**	0.003464**	0.00607
Apple (g)*Pineapple (g)	0.000000	0.000019	-0.000014*	-0.000776**	-0.005328**	0.00801
R^2	0.877350	0.720271	0.910654	0.931265	0.893432	0.984224

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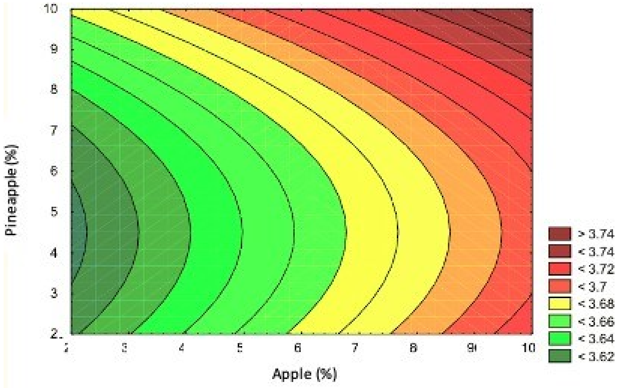

Figure 5a. Contour Plot for pH

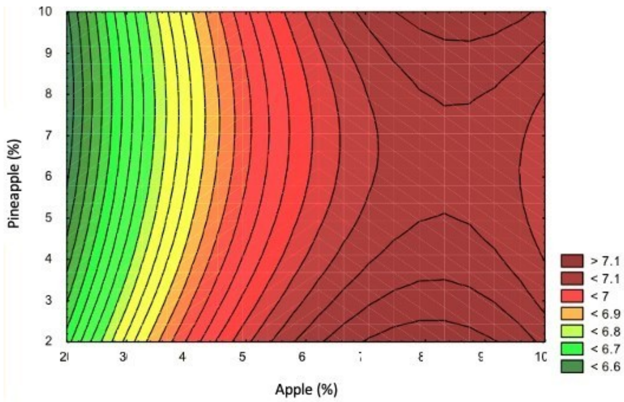

Figure 5b. Contour Plot for Total Soluble Solids (TSS)

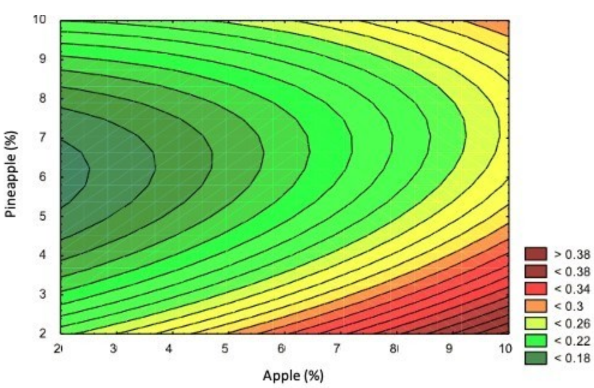

Figure 5c. Contour Plot for Titratable Acidity (TA)

b. Color Intensity

For the color values, only L (lightness) and a (green–red opponent values) were significant in linear and quadratic effects and interaction of the pineapple and apple. The lightness value, *L**, defines black at 0 and white at 100, thus the L value of 7.3 represents a dark shade. Furthermore, low values of a* depict towards green color, and high positive values of b* depict yellow color. However, b (blue-yellow opponents) was not significant in all regression. The contour map for L*, a*, and b* color values are shown in Figures 6 a, b, and c.

The significant changes in L values of fruit pulps were attributed to enzymatic browning (Lagnika et al., 2017). Together with fruit enzymes, microorganisms cause the degradation of carbohydrates (Pereira et al., 2011; Hounhoigan et al., 2014; Kamarul et al., 2016) and thus change the color in kombucha. Equations 13, 14, and 15 depict the effects of the different variables in L* , a*, and b* values.

(13) $L = 7.3017-0.0252*x-0.072*y+0.0006*x*x-0.0008*x*y+0.001*y*y$

(14) $a = 0.5033+0.0005*x-0.128*y+0.0028*x*x-0.0053*x*y+0.0035*y*y$

(15) $b = 51.1319-1.0208*x-1.0334*y+0.0062*x*x+0.008*x*y+0.0061*y*y$

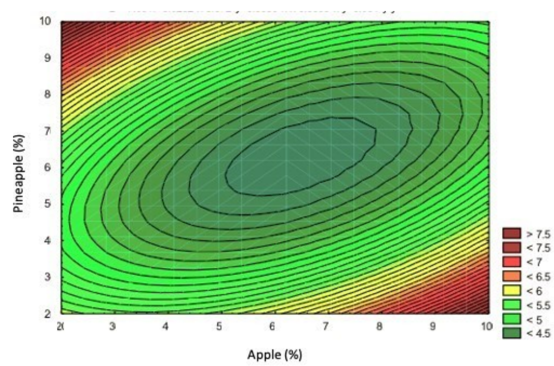

Figure 6a. Contour Plot for L*

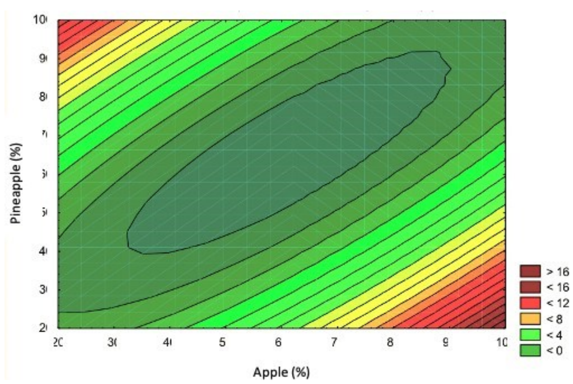

Figure 6b. Contour Plot for a*

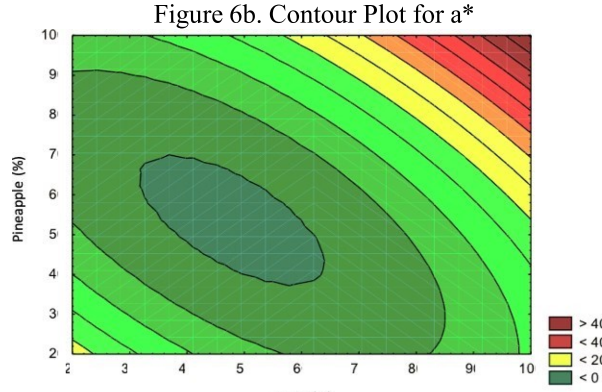

Figure 6c. Contour Plot for b*

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Overlay for Best Point Formulation

The overlay response of apple-pineapple flavored kombucha can be seen in Figure 7. The figure showed the overlay of 7 sensory attributes (color, appearance, aroma, sourness, sweetness, flavor, mouthfeel, and general acceptability) and physico-chemical attributes (pH, TSS, TA). The overlays were determined by the expected value of the panelist's preference which is a value of 6.75 and general acceptability of 7, both corresponding to "like moderately"). Values for pH, TSS and TA are 3.66, 6.59 ºBrix and 0.62% acetic acid, respectively. The result of this overlay suggested that the acceptable product can be generated by using the scatter values in the pink shaded region.

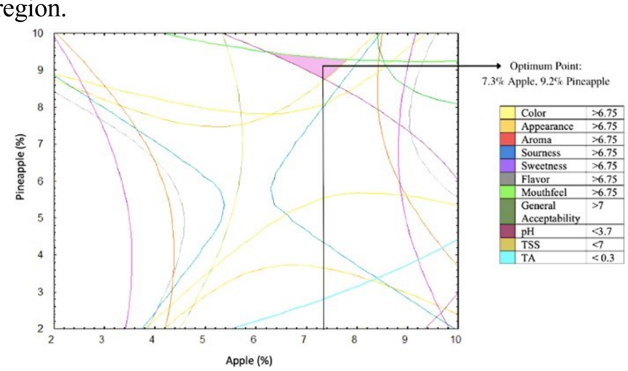

Figure 7. Most acceptable region (pink shaded region) for apple and pineapple flavored kombucha

The amount of apple and pineapple pulp can be generated by putting a point in the pink shaded area and then drawing a line to the x-axis and y-axis. Hence, from these experiments, the suggested amount of apple and pineapple levels are 7.3% and 9.2%, respectively.

4 CONCLUSIONS

The best formulation for apple and pineapple pulp as a flavoring for kombucha was determined. The best combination point was 7.3% apple pulp and 9.2% pineapple pulp added during kombucha's second fermentation (flavoring stage). This formulation would yield a 6.75 acceptability score corresponding to "like moderately" for sensory attributes and a general acceptability of 7, which corresponds to "like moderately". pH value is 3.66 with 6.59 ºBrix and 0.62% acetic acid. Further studies are needed such as comprehensive consumer evaluation, nutritional analysis for shelf-life testing, and the accumulation of acid and other physico-chemical changes as fermentation and product storage progresses.

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