

CHAPTER 4 – RESULTS AND DISCUSSIONS

4.1. Characterization of CBP as Kombucha Ingredients

4.1.1. Moisture Content and Total Phenolic Content of Ingredients

Moisture content and total phenolic content analysis were done on the raw materials such as earl grey black tea (BT), coffee silverskin (CS), coffee cascara (CA), and coffee pixel (PX). Phenolic content is closely tied to the antioxidant activity of kombucha (Jakubczyk, 2020), a health benefit that consumers often seek when consuming the functional drink, whereas moisture content was measured to determine whether the ingredients possess a similar total solid content for considerations in formulation. The results of both analyses are shown in Table 5 below.

Table 5 Moisture Content and Total Phenolic Content of Ingredients

Material	Moisture Content (%)	Total Phenolic Content (mg GAE/g)
BT	4.83 ^b ± 0.42	33.89 ^a ± 0.27
CS	4.22 ^b ± 0.13	18.16 ^b ± 0.32
CA	14.10 ^a ± 0.18	14.58 ^c ± 0.12
PX	13.81 ^a ± 0.50	20.00 ^b ± 0.60

Note: BT = earl grey black tea, CS = coffee silverskin, CA = coffee cascara, and PX = coffee pixel. Different letters indicate significant difference between the samples in between rows ($p < 0.05$)

As shown in Table 5, all the raw materials contained varying levels of moisture content, ranging from the lowest being $4.22 \pm 0.13\%$ in CS and the highest being $14.10 \pm 0.18\%$ in CA. No significant difference was found between BT and CS samples, where this finding was also similar with CA and PX values. The phenomenon occurred mainly due to the differing processes between BT and CBPs (Yang et al., 2024; Febrianto and Zhu, 2023; Java City, 2026). BT and CS were subjected to multiple steps of drying compared to CA and PX that

were only sun-dried (Yang et al., 2024; Febrianto and Zhu, 2023). The drying process in raw materials contributed to the moisture content and how easily they release free phenolic compounds, which directly affected TPC measurements (Bayram et al., 2024). The study reported an increased extractability of phenolic compounds in cornelian cherries dried using freeze-dryer and sun-drying compared to their fresh counterparts, mainly due to changes in the material's microstructure and formation of pores which facilitated the bound phenolics to be released more easily. Additionally, the higher moisture content in CA and PX could be an important consideration for long-term storage if these materials were utilized for production of beverages such as kombucha due to their potential of contaminating the product. In order to be considered microbiologically safe for storage, food materials are generally maintained to have moisture content below 10% (Zambrano et al., 2019). This aligns with the requirement of moisture content of black tea to be in between 2-9% (Dmowski and Ruszkowska, 2018), which indicates that both CA and PX may need to undergo further drying to be safely stored in the long-term compared to BT and CS. ®

Contrasting to moisture content findings, the highest total phenolic content (TPC) was found in BT at 33.89 ± 0.27 mg GAE/g (based on equalized dry weight of materials at 15 g/L), which was also significantly different from all CBPs ($p < 0.05$). This value fell within the range of a study by Aydemir et al. (2023), who reported a wide range of TPC values of black tea from various origins, starting from 17.8 ± 1.9 mg GAE/g to 57.1 ± 1.8 mg GAE/g. The aforementioned study used extracts obtained from pulverized dried tea, where it can be assumed there were no significant differences between the total solid content of tea with this research. It was possible that measurements of TPC of BT using wet weight as a base may exhibit lower values due to inhibition of bound phenolics by the lack of porous structure (Bayram et al., 2024). In contrast, a different study reported higher TPC values of BT samples at 180.09 mg GAE/g (Nadiah and Uthumporn, 2015). This large difference may be attributed to the different method of extraction, where the aforementioned study used extraction using ethanol. Extraction using ethanol was more effective due to higher solubility of phenolic compounds in less-polar organic solvents (Plaskova and Mlcek, 2023). Factors such as tea origin, harvest time, processing steps during manufacturing, fermentation and heating temperature of tea leaves may also contribute to the difference in TPC values compared to other studies (Aydemir et al., 2023; Nadiah and Uthumporn, 2015).

On the other hand, TPC of CBPs also varied, where CS and PX were not significantly different. Most of the TPC on the CBPs were due to the presence of CGA and flavonoids on both materials (Damato et al., 2026; Farah and Donangelo, 2006). CS and PX exhibited lower TPC compared to BT mainly due to degradation of phenolic compounds from high heat (Antony and Farid, 2022) and bound phenolic acids to structural polysaccharides within the cell wall (Bayram et al., 2024), respectively. Interestingly, CA showed the lowest TPC at 14.58 ± 0.12 mg GAE/g despite being of similar structure to PX. This may be attributed to PX consisting of defective coffee beans that may hold additional phenolic compounds within its structure, as compared to CA which only consists of the skin of the coffee cherry.

4.1.2. Antioxidant and Physicochemical Property Changes in CBP Kombucha Before and After Fermentation

Phenolic compounds are considered the major contributors to antioxidant properties, especially in kombucha, which primarily uses tea substrates (Jackubczyk, 2020). Determining the characteristics of antioxidant potential of each raw material during fermentation was done to observe the effects of CBPs on the functional benefits of kombucha in comparison to conventional tea kombucha. pH was measured to determine whether the kombuchas produced were successful or not, as pH is one of the essential factors in ensuring the safety of the product (Wang et al., 2023; Chong et al., 2024). SCOBY growth was observed as it played an important role in improving fermentation efficiency (Chong et al., 2024). Additionally, total soluble solids (TSS) and color values were measured to characterize the sensory attributes of the kombuchas. The changes in antioxidant and physicochemical properties of CBP kombucha are shown in Table 6 below.

Table 6 Changes in Antioxidant Properties of CBP Kombucha Before and After Fermentation

Sample		TPC (mg GAE/g)	RSA (mg AAE/L)
Tea (Before Fermentation)	Black Tea (BT)	$52.78^a \pm 0.05$	$1556.03^a \pm 16.10$
	Coffee Silverskin Tea (CST)	$14.59^c \pm 0.04$	$173.67^c \pm 4.42$
	Coffee Cascara Tea (CAT)	$9.56^d \pm 0.03$	$54.91^d \pm 1.90$
	Coffee Pixel Tea (PXT)	$16.11^b \pm 0.01$	$301.57^b \pm 10.42$

Kombucha (After Fermentation)	Black Tea Kombucha (KBT)	61.56 ^a ± 0.12	2999.56 ^a ± 21.46
	Coffee Silverskin Kombucha (KCS)	34.79 ^b ± 1.24	2240.40 ^b ± 2.21
	Coffee Cascara Kombucha (KCA)	29.38 ^c ± 0.71	2187.95 ^b ± 69.44
	Coffee Pixel Kombucha (KPX)	32.96 ^b ± 0.46	2215.63 ^b ± 14.52

Note: TPC = total phenolic content, RSA = radical scavenging activity, different letters indicate significant difference of samples in between rows within the same sample category (p<0.05)

All kombucha samples experienced a significant increase in antioxidant properties after 13 days of fermentation (Table 6). It was observed that all CBP tea showed lower TPC values compared to BT, however CBP kombucha showed comparable values after fermentation. Both CA and CS showed higher TPC increase compared to other ingredients. This indicated that while the accessible structure of CS itself facilitated the release of phenolic compounds, the fermentation process also enabled the breakdown of cell walls of more bulky structures in CA and PX. During the fermentation process, production of various enzymes including cellulases, glucosidases, and pectinases facilitated the breakdown of cell walls and polyphenolic structures which increased the TPC (Pereira, 2026). Interestingly, despite early grey black tea kombucha (KBT) showing the highest antioxidant activity compared to the CBPs, it exhibited the lowest increase in TPC when utilized in kombucha. This might be due to phenolic compounds in BT being more prone to degradation from enzymes produced by the SCOBY (Pereira, 2026). Additionally, the processing of BT itself may have contributed to the limited increase in phenolic content, where compounds in black tea are more soluble due to its damaged cell structure during the tea production process (Zhou et al., 2022). Despite conventional kombucha made from black tea has been known for its antioxidant properties due to its high phenolic content (412.25 ± 3.86 mg GAE/L) (Ivanišová et al., 2019), the higher increase of antioxidant properties in CBP kombucha during fermentation was found significantly higher, suggesting a possibility of a comparable or even higher antioxidant potential of CBP kombucha against tea kombucha.

Similar to the trend found in TPC analysis, all of the kombucha samples experienced a major increase in antioxidant activity. BT, despite showing the highest radical scavenging activity

(RSA) as tea, showed the lowest RSA increase compared to CBPs. This phenomenon indicated the increase in antioxidant activity was directly proportional to the increase of TPC of the samples, as phenolic compounds strongly contribute to antioxidant properties in various previous studies related to kombucha (Zhou et al. 2022; Kim et al., 2023; Jakubczyk et al., 2023). This finding also aligned with the results in a study done by Xuyen et al. (2023), where both TPC and RSA of kombucha made from coffee cascara progressively increased after 14 days of fermentation.

Table 7 Changes in Physicochemical Properties of CBP Kombucha Before and After Fermentation

Sample	pH		TSS (%Brix)		SCOBY Growth (g)			Color						ΔE
								L*		a*		b*		
	BF	AF	BF	AF	BF	AF	Δ	BF	AF	BF	AF	BF	AF	
Black Tea (KBT)	6.03	2.42	10.37	8.80				38.47	37.51	0.01	0.64	-2.36	-1.41	1.49
	$\pm$	$\pm$	$\pm$	$\pm$	90.00	154.00	64.00	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
	0.01	0.01	0.06	0.00				0.00	0.00	0.01	0.01	0.02	0.01	
Coffee Silverskin (KCS)	6.77	2.65	9.60	8.85				38.46	37.64	-0.06	0.69	-2.35	-1.35	1.50
	$\pm$	$\pm$	$\pm$	$\pm$	106.00	160.00	54.00	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
	0.01	0.01	0.10	0.07				0.01	0.01	0.00	0.01	0.02	0.02	
Coffee Cascara (KCA)	7.23	2.72	9.80	9.45				38.59	37.96	0.59	1.13	-2.07	-0.92	1.41
	$\pm$	$\pm$	$\pm$	$\pm$	99.00	151.00	52.00	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
	0.01	0.01	0.00	0.07				0.01	0.01	0.01	0.01	0.01	0.01	
Coffee Pixel (KPX)	7.06	2.58	10.27	8.80				38.65	38.52	0.82	1.23	-1.84	-0.56	1.35
	$\pm$	$\pm$	$\pm$	$\pm$	91.00	128.00	37.00	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
	0.00	0.00	0.06	0.00				0.01	0.01	0.01	0.02	0.02	0.01	

Note: TSS = total soluble solids, L* = lightness, a* = red-green, b* = yellow-blue, BF = before fermentation, AF = after fermentation

In regards to the physicochemical properties, all kombucha samples experienced a pH reduction, with the highest pH decrease found in KCA (Table 7). The decrease in pH can be attributed to both sugar availability and SCOBY composition, where the bacteria in SCOBY hydrolyzes sucrose into simple sugars before converting them into functional acids, such as gluconic acid and acetic acid (Dartora et al., 2023). It was also noteworthy that the pH of all CBP kombucha samples adhered to the standards of conventional kombucha between 2.3-3.8 (Kombucha Brewers International, 2023). Interestingly, despite experiencing the highest

decrease in pH, the SCOBY in KCA did not have the highest growth increase, instead KBT which had the lowest pH reduction, showed the highest SCOBY growth by 64 grams. This may suggest that SCOBY growth wasn't proportional to the decrease in pH as the sugar gets consumed. Alternatively, other factors, such as phenolic compounds, may contribute to the growth of SCOBY. In a roselle-based kombucha study done by Setyaningsih et al. (2025), it was found that a higher substrate mass promoted SCOBY growth by providing an acidic environment and nutrients from its bioactive compounds, where CGA was a dominant phenolic compound in roselle kombucha.

As for TSS, both KBT and KPX exhibited similar values and KCA showed the lowest reduction value at 0.35% Brix. Reduction of TSS during fermentation is commonly facilitated by the consumption of sugar by SCOBY as their carbon source (Rahmawati, 2025). This suggests there should be a strong interaction between pH, TSS, and SCOBY growth, where a higher TSS reduction value (due to SCOBY activity) leads to a proportional pH reduction. However, this did not align with the findings in Table 7, where KBT and KPX showed the highest TSS reduction, but KPX showed the lowest SCOBY growth. The SCOBY growth in KPX might be inhibited by the difficulty of its own structure to release phenolic compounds as mentioned previously.

On the other hand, LAB values of all kombucha samples showed a decrease in lightness ($-\Delta L^*$), shift towards redness ($+\Delta a^*$), and shift towards yellowness ($+\Delta b^*$). Figure 6 below shows the color difference between kombucha samples from before and after fermentation.



Fig. 6 Color Changes of CBP and BT Kombucha Before and After Fermentation

Note: (a) = kombucha samples before fermentation, (b) = kombucha samples after fermentation. Sample order in both figures positioned from left to right = KBT, KCS, KCA, KPX.

However, by observing the values expressed in Table 7, it became clear that color evaluation using a colorimeter in this research was not suitable. Figure 6(a) showed that all kombucha samples shifted into a lighter color (Figure 6b), directly contrasting with the findings of LAB values from the colorimeter. Additionally, color changes under the value of 1.0 are difficult to distinguish using the human eye (Ly et al., 2020). The poor alignment in LAB values and direct observation necessitates an alternative method to characterize the colors of the kombucha samples, which led to the shift in using a color intensity chart to characterize the CBP kombucha (Fig. 7).

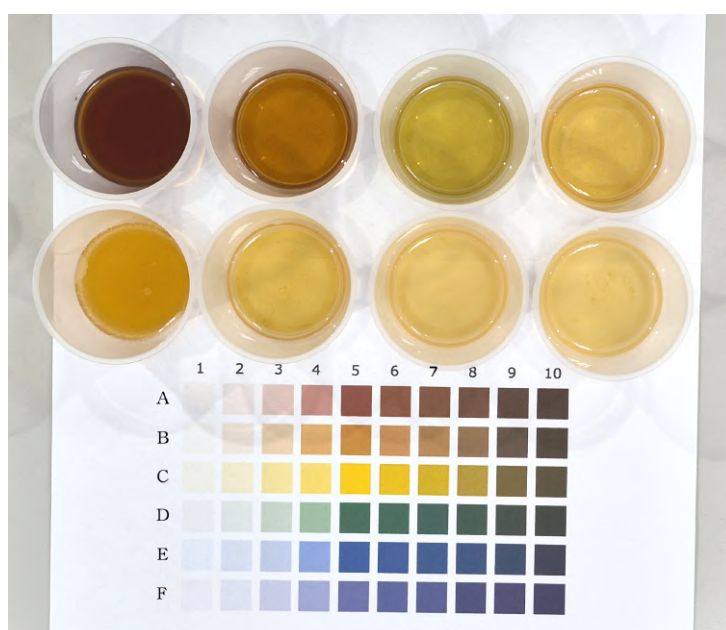


Fig. 7 Color Characteristics of CBP and BT Kombucha Based on Color Intensity

Note: top row = kombucha samples before fermentation, bottom row = kombucha samples after fermentation.

Sample order in both rows positioned from left to right = KBT, KCS, KCA, KPX.

In Figure 7, both CBP and BT kombucha from before and after fermentation were compared to a color intensity chart to determine each sample's color positioning. It was observable that most of the samples prior to fermentation (top row) had darker color values compared to samples after fermentation (bottom row). KBT had a color value between A5-A6, indicating a moderately dark brown color. CBP kombucha prior to fermentation had lighter color values compared to KBT, with KCS having the darkest value at B5. KCA had a more yellow-green color (value around C7-C8), whereas KPX had a yellow-orange color (value around C6). In contrast to the kombucha samples prior to fermentation, the post fermentation samples consistently showed light yellow-orange color values, where KBT had the darkest value at

B4. All CBP kombucha post fermentation had similar values between C3-C4. The phenomenon of lighter color values in post fermentation kombucha is attributed to microbial activity that caused color degradation. The soluble solids in the kombucha are consumed by microbes as a source of energy, resulting in a loss of color intensity (Azizan et al., 2025). Furthermore, Rahmawati (2025) also reported that the decrease in pH of kombucha could damage color-inducing phenolic compounds such as tannins and thearubigins, causing the solution's color to fade over time.

4.2. Optimization of Ingredients and Fermentation Duration of CBP Kombucha

The optimization of CBP kombucha revolved around determining the formulation with the most optimal responses (pH, acid value, SCOBY growth, and hedonic overall acceptance) from the 16 sets of experimental designs generated by the Design Expert 13.0® software. Physicochemical properties such as pH, acid value, and SCOBY growth relate to whether the CBP kombuchas could be deemed as successful according to an established standard of conventional tea kombucha, whereas hedonic evaluation measures the acceptability of CBP kombucha to consumers. Formulation and responses of each trial are shown in Table 8 below.

Table 8 Evaluation of Physicochemical and Sensorial Properties of 16 CBP Kombucha
Experimental Designs

Run	CA (g)	CS (g)	Sugar (g)	Fermentation Days	pH	Acid Value (%)	SCOBY Growth (g)	Hedonic Overall*
1	10.00	0	190.00	13	3.12 ± 0.03	0.37 ± 0.01	32.87	6.28 ^b
2	10.00	10.00	180.00	7	3.56 ± 0.04	0.19 ± 0.02	39.99	6.04 ^a
3	7.78	7.88	184.34	13	3.36 ± 0.01	0.31 ± 0.00	61.16	5.91 ^b
4	1.98	2.11	195.91	13	2.91 ± 0.03	0.34 ± 0.01	35.54	6.22 ^b
5	10.00	4.92	185.08	10	3.42 ± 0.04	0.20 ± 0.02	52.63	6.60 ^{ab}
6	0	10.00	190.00	13	3.22 ± 0.02	0.33 ± 0.01	64.89	5.54 ^b
7	2.16	7.71	190.13	7	3.36 ± 0.01	0.15 ± 0.00	28.53	6.67 ^a

8	0	4.86	195.14	10	3.23 ± 0.01	0.19 ± 0.01	32.39	6.74 ^{ab}
9	2.16	7.71	190.13	7	3.40 ± 0.03	0.16 ± 0.01	27.37	6.24 ^a
10	4.88	5.19	189.92	10	3.26 ± 0.02	0.26 ± 0.00	37.18	6.13 ^{ab}
11	7.89	2.16	189.95	7	3.30 ± 0.03	0.16 ± 0.01	19.58	6.87 ^a
12	5.00	10.00	185.00	10	3.44 ± 0.01	0.21 ± 0.00	35.91	5.57 ^{ab}
13	8.06	6.69	185.25	7	3.38 ± 0.02	0.17 ± 0.00	30.95	6.13 ^a
14	4.92	0	195.08	10	3.05 ± 0.01	0.26 ± 0.00	20.28	6.37 ^{ab}
15	7.78	7.88	184.34	13	3.23 ± 0.01	0.49 ± 0.01	54.17	5.67 ^b
16	0	10.00	190.00	10	3.33 ± 0.02	0.23 ± 0.00	46.49	5.83 ^{ab}

Note: *Score 1 represents “dislike extremely” and score 9 represents “like extremely”. Different letters indicate significant difference between the trials between groups of fermentation days ($p < 0.05$).

According to chemical standards published by Kombucha Brewers International’s code of practice, a kombucha is deemed successful when complying to a pH between 2.3-3.8 and titratable acidity between 0.27-2.03% (Kombucha Brewers International, 2023). This standard was used to ensure safety of the product from microbial contamination. Based on results in Table 8, pH of all the CBP kombucha fell within the range of the standards, which showed that all formulations suffered a pH reduction due to formation of organic acid during fermentation.

However, only trial 1, 3, 4, 6, 15, and control complied to the acid value standards, with trial 15 showing the highest titratable acidity at 0.49%. It was notable that the acid value of all trials from 7 and 10 day fermentation were below the minimum standards of 0.27%. Trial 1, 3, 4, 6, and 15 were all from the 13 day fermentation batch, suggesting that fermentation duration less than 13 days may result in an under-fermented CBP kombucha. The hedonic overall acceptance of CBP kombucha from 7 day fermentation was significantly higher than the 13 day fermentation ($p < 0.05$), while the 10 day fermentation batch was not significantly different to both groups ($p > 0.05$). The highest hedonic acceptance score was found in trial

11 (7 day fermentation) with a score of 6.87, whereas the lowest score was found in trial 6 (13 day fermentation) with a score of 5.54. This finding indicated that higher acid content may have negatively impacted the consumer acceptance of CBP kombucha (Appendix 5). According to Petty et al. (2020), preference of sugar (sucrose) in adults is significantly lower than children and adolescents. As a majority of panelists participating in this study were adolescents, this could contribute to the reason 7 day fermentation CBP kombucha achieved the highest hedonic acceptance score. Additionally, the fact that most of the panelists were not very familiar with or rarely consume kombucha, this may have contributed to a mismatch in the perception of a sensorically acceptable kombucha. Furthermore, it is possible that due to a majority of panelists participating in the sensory evaluation testing all batches of samples in one session, the data of the parameter was skewed. A possible method to lessen the burden of serving a large amount of samples is by conducting a screening evaluation prior to hedonic sensory evaluation, where Focus Group Discussion (FGD) is a possible option. On the other hand, the increase of acid value with longer fermentation duration also showed a proportional relationship towards SCOBY growth, where the highest growth was observed in trial 6, and the lowest in trial 11, both fermented for 13 and 7 days, respectively.

A correlation analysis was conducted to investigate the relationship between components (CA, CS, sugar) and the CBP kombucha parameters (Table 9). Values close to -1 or 1 indicate a significant correlation between variables (-1 indicates a negative correlation, whereas 1 indicates a positive correlation), whereas values close to 0 indicate a weak or lack of correlation. Only values >0.5 (both positive and negative) will be discussed as they were considered moderately significant enough to show correlation between variables (Newcastle University, 2025).

Table 9 Pearson Correlation of CBP Kombucha Variables to the Physicochemical and Sensorial Parameters

	Run	CA	CS	Sugar	Acid Value	pH	SCOBY Growth	Hedonic Overall
Run								
CA	-0.27							
CS	0.14	-0.24						

Sugar	0.11	-0.65	-0.59				
Acid							
Value	-0.06	0.12	-0.13	0.001			
pH	0.01	0.29	0.69	-0.78	-0.55		
SCOBY							
Growth	-0.22	0.01	0.54	-0.43	0.51	0.15	
Hedonic							
Overall	-0.17	0.09	-0.61	0.40	-0.52	-0.09	-0.62

Note: CA = coffee cascara, CS = coffee silverskin

Based on Table 9, acid value had a moderately strong correlation with pH, SCOBY growth, and hedonic acceptance ($r \geq 0.5$). A negative correlation value signified an inverse relationship between acid value and pH, suggesting that pH dropped due to formation of acetic acid (Xuyen et al., 2023). This trend also applied to the relationship between sugar and pH, as higher sugar content led to lower pH ($r \geq 0.7$). The increase of acid value also correlated to the increase of SCOBY growth as sugar consumption by the microbes resulted in the production of acetic acid, where this instead negatively impacted hedonic acceptance. The negative correlation of acid value and hedonic acceptance referred to the lower acceptance score due to increased acidity of kombucha. CS also had a moderate correlation with sugar, pH, and hedonic acceptance ($r \geq 0.5$). Higher CS content reduced sugar content by default to ensure the formulation still added up to 100%, similarly with CA. CS also impacted hedonic acceptance negatively due to more prominent bitter taste from the melanoidins. Interestingly, CS positively correlated with pH, which could be attributed to the high calcium and potassium content in the material that acted as chemical buffers that prevented the increase of titratable acidity that led to a higher pH (Franca et al., 2024; Thompson-Witrick et al., 2024). As CS was found to be the variable that affected the most responses (pH, SCOBY growth, and hedonic acceptance) compared to other ingredients, selection of CS as a parameter to be optimized became essential.

4.2.1. Selection of Variables and Solution for Optimization of CBP Kombucha

ANOVA modelling of parameters in CBP kombucha optimization was performed to ensure the final model could significantly explain the variance within the parameters (Table 10).

Parameters with significant models and insignificant lack-of-fit will be considered for further formula optimization as they indicate significant impact of the variations of the independent variables (CA, CS, and sugar) towards the responses.

Table 10 Analysis of Variance of Models for Physicochemical and Sensorial Analysis (pH, acid value, SCOBY growth, and overall hedonic)

Analysis	Dependent Variables / Responses			
	pH	Acid Value (%)	SCOBY Growth (g)	Hedonic Overall
<i>P-value</i>				
Model	0.0222 ^a	<0.0001 ^a	0.0124 ^a	0.0489 ^a
Lack of Fit	0.6400	0.9955	0.3293	0.8438
Fit Statistics				
R ²	0.9627	0.8072	0.9725	0.9430
Adjusted R ²	0.8603	0.7775	0.8971	0.7861
Predicted R ²	-0.5320	0.7028	-0.4821	0.1417
Adequate Precision	11.9885	10.4413	12.4955	7.9907

Note: ^a represents statistical significance ($p < 0.05$)

Table 10 showed that all four responses were significantly correlated to the independent variables ($p < 0.05$), which indicated the models were capable of explaining the variance of each variable. All responses also showed insignificance in the lack-of-fit ($p > 0.05$), which meant the models were fit to be applied for further optimization.

R² values closer to 1 indicated a higher accuracy of the models to predict the optimal formula through a regression model. All responses showed strong correlation with the factors. Adjusted R² and Predicted R² were found to be in reasonable agreement (difference less than 0.2) towards acid value. However, both pH and SCOBY growth showed a negative Predicted R² which implied that the overall mean might be a better predictor for the responses than the current model. Additionally, a large difference (>0.2) was observed between Adjusted R² and

Predicted R^2 in hedonic acceptance, which indicated a possible problem in the data, presence of outliers, or a large block effect. This may have been affected by the improper statistical analysis used on hedonic acceptance scores, where sensory data with multiple samples of this type rarely fit into normal distribution (Carabante and Prinyawiwatkul, 2018). As Design Expert® software uses ANOVA to analyze all the data inputted, this may have contributed to the reason behind the poor agreement between Adjusted R^2 and Predicted R^2 in hedonic acceptance.

Adequate Precision evaluated the signal-to-noise ratio, where ratio greater than 4 indicated the model can be used to navigate the design space. All four responses indicated adequate signals as their values were higher than 4, suggesting that they are suitable for further optimization.

As all variables were found to be fitting to be optimized based on the results of the ANOVA statistical analysis, goals of each independent and dependent variable were set according to the desired characteristics of the CBP kombucha end product (Table 11).

Table 11 Values of Independent and Dependent Variables for Optimization of CBP
Kombucha

Variable	Goal	Lower Limit	Upper Limit	Importance
pH	In range	2.3	3.8	+++
Acid Value (%)	In range	0.27	2.03	+++
SCOBY Growth (g)	Maximize	19.58	64.89	+++
Hedonic Overall	Maximize	5	6.87	+++

As shown in Table 11, both pH and acid value were adjusted to fit within the range of chemical standards established by Kombucha Brewers International (2.3-3.8 and 0.27-2.03%, respectively) (Kombucha Brewers International, 2023). SCOBY growth and hedonic overall acceptance were set to maximum, as high SCOBY growth promotes the efficiency of fermentation process in kombucha (Chong et al., 2024), and high hedonic acceptance indicated a strong consumer liking towards the product. The lower limit of hedonic

acceptance was set to 5 as it represents neutral preference (“neither like nor dislike”). The importance of all parameters were equalized considering all the variables intricately affected each other, as compared to prioritizing only one factor with the highest importance. Interactions of variables within kombucha are often complex, where one factor affects the other rather than being a standalone factor (e.g. pH-acid value, acid value-hedonic acceptance). Setting a certain variable with the highest importance may result in other factors not adhering to established criteria for optimization, especially when such parameters had inverse relationships (Table 9).

Several solutions were generated from the selected goals for each variable for CBP kombucha, where the most optimal formulation for producing a CBP kombucha that fit within the established chemical standards, predicted a high SCOBY growth (SG) and hedonic overall acceptance (HO) was prioritized for selection (Table 12).

Table 12 Solutions with Predicted Responses and Desirability Scores by Design Expert 13.0®

No	CA (g)	CS (g)	Sugar (g)	FD	AV (%)	pH	SG (g)	HO*	DES
1	10.000	8.920	181.080	13	0.368	3.476	63.432	6.289	0.790
2	10.000	10.000	180.000	13	0.368	3.523	64.030	6.258	0.786
3	0.000	5.475	194.525	13	0.368	3.043	53.894	6.242	0.686

Note: CA = coffee cascara, CS = coffee silverskin, FD = fermentation days, AV = acid value, SG = SCOBY growth, HO = hedonic overall acceptance, DES = desirability

*Score 1 represents “dislike extremely” and score 9 represents “like extremely”

From Table 12 above, solution 1 was chosen as the optimal formulation for CBP kombucha, where it included the use of 10.000 grams of CA, 8.920 grams of CS, 181.080 grams of sugar, and 13 days of fermentation. The selection of the solution took into account the highest desirability score (0 = least desirable, 1 = most desirable), as well as minimizing the usage of raw materials. Although no solutions managed to achieve a desirability score of ≥ 0.80 , where a value between 0.80 and 1.00 is generally considered excellent for optimization of formulation (Harrington, 1965), solution 1 achieved the closest value to that range. Furthermore, solution 1 and 2 had very similar predicted values on all parameters with solution 1 requiring less CS compared to solution 2, highlighting the optimization of raw

materials in the former solution. This detail becomes important especially in large-scale production where difference in ingredient volume can significantly affect production cost and product price in the market (Firmansyah and Damanik, 2024). Furthermore, selection of solution 1 also considered the inverse relationship between CS and hedonic acceptance based on the correlation analysis (Table 9), where a higher amount of CS resulted in a lower sensory acceptance.

In order to ensure the accuracy of the selected models for optimization, an additional verification was conducted. The selected CBP kombucha formulation was analyzed for its parameters (pH, acid value, SCOBY growth, and hedonic acceptance) through three replicates (Table 13). The model was considered valid when the experimental results fell within the 95% prediction interval (PI).

Table 13 Predicted and Validated Results of Selected Optimized CBP Kombucha

Response	Prediction by Design Expert	95% PI low	Experimental Verification	95% PI high
pH	3.4757	3.1844	3.4400 ± 0.0928	3.7671
Acid Value (%)	0.3680	0.2975	0.4456 ± 0.0113	0.4385
SCOBY Growth (g)	63.4319	42.2726	44.1900	84.5912
Hedonic Overall*	6.2889	5.3702	6.7100	7.2076

*Score 1 represents “dislike extremely” and score 9 represents “like extremely”

As observed from Table 13, most of the mean values from experimental verification fitted within the 95% prediction interval (PI), where pH, SCOBY growth, and hedonic acceptance successfully achieved values according to the selected criteria for optimization. However, the verified mean of acid value fell outside of the 95% PI, where this deviation may indicate either a potential problem with the model (the software’s prediction accuracy is <95% for acid value) or insufficient verification trials to confirm its validity. While the experimental data of acid value did not fit within the predicted range of the software, its value of 0.45 ±

0.01% was still in adherence to the established standards between 0.27-2.03% (Kombucha Brewers International, 2023).

4.2.2. Comparison of Optimized CBP Kombucha and Control

The selected optimized formulation of CBP kombucha was compared against a control kombucha made from black tea to investigate its positioning against a conventional kombucha (Table 14). Both kombucha samples were measured for their pH, acid value, SCOBY growth, and hedonic acceptance.

Table 14 Parameter Comparison Between Optimized CBP Kombucha and Control

Parameter	Control Kombucha (Black Tea)	Selected CBP Kombucha
pH	3.31 ^b ± 0.03	3.44 ^a ± 0.02
Acid Value (%)	0.86 ^a ± 0.03	0.45 ^b ± 0.01
SCOBY Growth (g)	85.05	44.19
Hedonic Overall	5.06 ^b	6.71 ^a

Note: Different letters indicate significant difference between the samples in between columns ($p < 0.05$)

In Table 14, both CBP and control kombucha successfully achieved a pH and acid value that adhered to the existing standards of conventional kombucha (Kombucha Brewers International, 2023). CBP kombucha showed a higher pH compared to black tea kombucha, possibly influenced by the presence of minerals that prevented a sharp decrease of pH during fermentation. On the other hand, black tea kombucha exhibited a higher acid value compared to CBP kombucha, where this indicated a higher perceived acidity of control kombucha. SCOBY growth of control kombucha was also found significantly higher than CBP kombucha, which aligned with the findings in the previous characterization stage (Table 7), where phenolic compounds were likely the main affectors (Setyaningsih et al., 2025). Interestingly, CBP kombucha achieved a higher hedonic acceptance score of 6.71 compared to black tea kombucha (5.06), highlighting the promising potential of CBP consisting of cascara and silverskin as non-conventional ingredients in kombucha production. Additionally, these CBPs are largely available from coffee processing chains, resulting in larger material volume and affordable price compared to black tea.