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*by Santoso Filiana*

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## 2 Application of *Stenochlaena palustris* in Black Tea and Coffee Beverages Targeting Consumers with Sugar Concern

Filiana Santoso<sup>1</sup>, Natasya Oktavianti<sup>1</sup>, Febbyandi I. Pandiangan<sup>1</sup>, Yanetri A. Nion<sup>2</sup>,  
Maria D.P.T. Gunawan Puteri<sup>1,\*</sup>

<sup>3</sup>  
<sup>1</sup>Department of Food Technology, Faculty of Life Sciences and Technology, Swiss Gerni<sup>5</sup> University, Indonesia

<sup>2</sup>Study Program of Agrotechnology, Faculty of Agriculture, Universitas Palangka Raya, Indonesia

\*Corresponding author: [maria.gunawanputeri@sgu.ac.id](mailto:maria.gunawanputeri@sgu.ac.id)

### ABSTRACT

*Kelakai* is an endemic fern found abundantly in Central Kalimantan. Regardless of its high availability, low economical value, and potential health benefits (natural source of iron, folic acid, antioxidant, antidiabetic, etc.), the utilization of the plant as a food ingredient is still limited. Black tea and coffee are very popular in Indonesia. Replacing sugar with artificial sweeteners is an alternative choice for reducing sugar consumption in sugar-sweetened beverages. The aims of this study were to study the effect of *kelakai* and milk addition in black tea and coffee beverages based on sensory characteristics, and to develop a formulation of *kelakai* beverages with high consumer acceptance. The sensory observation showed that coffee was better in masking putrid aroma and milk helped to reduce bitterness, astringency, and distinct aroma from the plant. Milk coffee was chosen to be optimized with further adjustment in sweetness (0.02%) and thickness (0.15%) levels. A maximum *kelakai* concentration that was still acceptable was evaluated using hedonic and forced ranking tests (n=53). The formulation with the highest consumer acceptance was milk coffee with ratios of *kelakai* : coffee : milk = 8:4:3 (v/v/v), sucralose 0.02% (w/v), sodium benzoate 0.04% (w/v), and CMC 0.15% (w/v). The sample was rated between slightly to moderately liked. It was ranked as the most preferred sample in the forced ranking test.

**Keywords:** Black tea, Coffee, Kalimantan local product, RTD beverages, Sensory evaluation, *Stenochlaena palustris*, Sugar concern.

### 3 1. INTRODUCTION

*Stenochlaena palustris* (*kelakai*) is an endemic fern found abundantly in Central Kalimantan and is very adaptable since it has the ability to grow and survive in various environmental conditions. This endemic plant can be found growing in peat swamps [1], mangroves or areas near water sources [2]. Furthermore, the plant can also grow and survive in post-fire peatland [2], deserted areas [3], and in urban areas where it was believed to be heavily contaminated with toxic metals [4]. However, *kelakai* is considered a weed in palm and rubber plantations [3]. Additionally, *kelakai* is also resistant to disease and pest [5], resulting in a less maintenance cost.

Traditionally, the endemic plant has been used to treat anemia, anti-aging, and to increase milk production in breastfeeding mothers by the Dayak Community [6]. *Kelakai* has been discovered to possess high iron content,

which was 2 times higher than spinach [7] and strong antioxidant activity [8], which was 3 times higher than gallic acid [9] and comparable with ascorbic acid [10]. Besides that, *kelakai* was also reported to exhibit antidiabetic properties due to its  $\alpha$ -glucosidase inhibitory activity [9], [11], [12], i.e. it could reduce the blood sugar level. It was observed that antidiabetic activity of *kelakai* was higher than the standard AGI agents used [9], [11]. Some researchers also discovered other bioactivities in *kelakai* such as anti-obesity [13], antimicrobial [14]–[21], anticholinesterase [22], and anti-cancer [23], [24].

Aside from its good survivability, low economical value, and potential health benefits, the utilization of *kelakai* in food and/or beverages is still limited and has not been properly studied yet. In Kalimantan, *kelakai* plants are commonly consumed as vegetable dishes using its young fronds, *kelakai* chips, or served as *kelakai* tea.

**Table 1.** Formulations of black tea and coffee samples with *kelakai*

Sample code	Ingredients (300 mL)					
	<i>Kelakai</i> Extract (mL)	Black Tea Extract (mL)	Coffee Extract (mL)	Water (mL)	Sucralose (gr)	Sodium Benzoate (gr)
K1T1	120	120	-	60	0.03	0.12
K2T1	160	80	-			
K3T1	180	60	-			
K1C1	120	-	120			
K2C1	160	-	80			
K3C1	180	-	60			

The most common beverages in Indonesia are tea and coffee. They have been consumed by Indonesian people, either in the morning or as a company during mealtime. Both tea and coffee are available in forms of ready-to-drink (RTD) beverages. According to Sangaji (2020), RTD tea is ranked 2<sup>nd</sup> in terms of popularity in Indonesia. On the other hand, there is also an increasing demand for RTD coffee beverages as can be seen in the growing number of coffee outlets in Indonesia. However, RTD beverages are usually high in sugar content or known as sugar-sweetened beverages (SSB).

Based on the survey conducted by Kementerian Kesehatan Republik Indonesia (2019b), it was found that 61.3% of the Indonesian consumers consumed SSB more than once per day. Higher consumption of SSB can lead to high sugar consumption, triggering a rise in insulin level, thus developing insulin resistance and causing hyperglycemia. Insulin is a hormone that is responsible for controlling blood sugar level. There are some diseases reported related to blood sugar level and the most common are obesity [27]–[30], coronary heart disease [31] and type 2 diabetes [32]. In recent years more and more Indonesians have a higher health awareness hence they want to reduce their sugar consumption. There are two most common ways that people choose. Some reduce their sugar consumption by eliminating the addition of sugar in their food and/or beverages. Others substitute the table sugar with artificial sweeteners to still have the sweet taste.

This research was conducted to develop beverages using the underutilized *kelakai* plants in popular common beverages, namely black tea and coffee. To meet the consumers' preference of sweet taste in black tea and coffee, table sugar was replaced with a safe artificial sweetener, thus, it can target consumers with sugar concern. Moreover, the application of *kelakai* in popular beverages as local product souvenirs from Kalimantan hopefully would increase the economic value of this endemic plant.

## 2. MATERIALS AND METHOD

### 2.1. Preparation of *S. palustris* Extract

The endemic ferns used were in the form of dried *kelakai* powder which was harvested 2 years ago from peat land of 5x5 m<sup>2</sup> in Tumbang Nusa (2°26'10.5"S, 114°10'24.8"E), Central Kalimantan in rainy season. The *kelakai* powder has already gone through pre-treatment that was conducted by Teji (2019) and stored in an aluminum pouch at low temperature. Additionally, the dried *kelakai* powder used both young and mature fronds.

The dried *kelakai* powder was extracted using the method conducted by Mahadika (2017) with few modifications. The extraction was done at 88°C for an hour using a slow cooker (Mitzui MZ-506SC) using water solvent. The ratio of dried *kelakai* and water (Aqua®) used in this extraction method was 1:20 (w/v). After the sample was extracted, it was vacuum filtered.

### 2.2. Preparation of Black Tea Extract

The black tea leaves (Goalpara) were extracted by following the method done by Uchida *et al.* (2013). The extraction was done by steeping black tea leaves using 80°C hot water for 3 minutes and the ratio of dried tea leaves to water was 1:25 (w/v).

### 2.3. Preparation of Coffee Extract

The coffee coarse grounds (Sidikalang Robusta, in Kopro market) were extracted using hot brew method conducted by Fuller & Rao (2017). The coffee grounds were extracted with water at 98°C for 6 minutes using a French press (Ikea) and with ratio of coffee grounds to water was 1:10 (w/v). Furthermore, the brewed coffee was filtered using filter paper.

### 2.4. Formulation of Beverages

The samples were prepared by mixing all the ingredients, while prioritizing hygiene and safety. In the first step of analyzing the effect of *kelakai* addition, there were four types of beverages prepared. They were black

tea with *kelakai*, black tea with *kelakai* and milk, coffee with *kelakai*, and coffee with *kelakai* and milk (Tables 1 and 2.)

**Table 2.** Formulations of black tea and coffee samples with *kelakai* and milk

Sample code	Ingredients (300 mL)					
	<i>Kelakai</i> Extract (mL)	Black Tea Extract (mL)	Coffee Extract (mL)	Full Cream Milk (mL)	Sucralose (gr)	Sodium Benzoate (gr)
K1T1M	120	120	-	60	0.03	0.12
K2T1M	160	80	-			
K3T1M	180	60	-			
K1C1M	120	-	120			
K2C1M	160	-	80			
K3C1M	180	-	60			

The sample code “K” indicated *kelakai*, “T” indicated black tea, “C” indicated coffee, and “M” indicated full cream milk (Ultra Milk®). Sucralose and sodium benzoate (Koepoe-koepoe®) concentrations used were 0.01% and 0.04% (w/v), respectively. All ingredients were formulated with a volume of 300 mL. Then, the sensory characteristics of the applied beverages were observed by a trained panelist, including its taste, color, aroma, and trigeminal sensation. The trained panelist used in this research has been screened, selected and trained to do sensory evaluations.

In the second step, there was an elimination to choose suitable type of beverage to be developed. The sample used in focus group discussion (FGD) was “K3C1M” with a ratio of *kelakai* extract, coffee extract and full cream milk of 3:1:1 (v/v/v). The formulation used for FGD can be seen in Table 3.

Prior to FGD, there was an observation in comparing the sample with the existing milk coffee beverages (Kopiko Lucky Day, Ichitan Thai Milk Coffee, ABC Kopi Susu) to determine which attributes to be re-adjusted.

The variables were sweetener and thickener concentrations. The thickener used in this research was

CMC (Koepoe-koepoe®). The sample code “S1” indicated 0.015% sucralose, “S2” indicated 0.02% sucralose, “T1” indicated 0.1% CMC, and “T2” indicated 0.15% CMC. The percentage ratios are based on weight per volume.

Furthermore, the last stage of formulation for conducting sensory evaluation used sweetener and thickener concentrations of 0.02% and 0.15% (w/v), respectively. Three different ratios of *kelakai* to coffee extracts samples were evaluated and the formulation can be seen in Table 4.

**2.5. Alpha-glucosidase Inhibitor Assay**

The alpha-glucosidase inhibitory activity was conducted following the procedure from Gunawan-Puteri & Kawabata (2010) with a slight modification. The Tris-HCl solution used 121.15 mg/ml of tris(hydroxymethyl)aminomethane (Merck, Germany) in distilled water at pH 7. Sucrose and maltose substrate solutions were prepared using 21.90 mg/ml sucrose (Kanto Chemical) in aqueous and 2.88 mg/ml of maltose monohydrate (Merck, Germany) in aqueous.

**Table 3.** Formulations of coffee with *kelakai*, milk and varying concentrations of sweetener and thickener

Sample code (K3C1M)	Ingredients (300 mL)					
	<i>Kelakai</i> Extract (mL)	Coffee Extract (mL)	Full Cream Milk (mL)	Sodium Benzoate (gr)	Sucralose (gr)	CMC (gr)
T1S1	180	60	60	0.12	0.045	0.3
T1S2					0.06	0.3
T2S1					0.045	0.45
T2S2					0.06	0.45

**Table 4.** Formulations of coffee samples added with milk and varying ratios of *kelakai* to coffee

Sample code (T2S2)	Ingredients (300 mL)					
	Kelakai Extract (mL)	Coffee Extract (mL)	Full Cream Milk (mL)	Sodium Benzoate (gr)	Sucralose (gr)	CMC (gr)
K2C1M	160	80	60	0.12	0.06	0.45
K3C1M	180	60				
K4C1M	192	48				

**Table 5.** Alpha-glucosidase inhibitor assay sample, enzyme, and reagent proportion in reaction mixtures

Reagent	Sucrose Inhibition Assay				Maltose Inhibition Assay			
	S	SB	C(+)	CB	S	SB	C(+)	CB
Sample	25	25	-	-	25	25	-	-
Distilled water	-	-	25	25	-	-	25	25
Sucrose substrate solution	125	125	125	125	-	-	-	-
Maltose substrate solution	-	-	-	-	125	125	125	125
Enzyme Solution	100	-	100	-	100	-	100	-
Potassium phosphate buffer 0.1 M at pH 7	-	100	-	100	-	100	-	100
Tris-HCl solution	750	750	750	750	750	750	750	750

For enzyme preparation 2 mL of EDTA (Merck, Germany) 5mM was used to dissolve 0.1 gram of intestinal acetone powder (Sigma-Aldrich, Germany). The enzyme solution was homogenised using cold mortar and then centrifuged for 1 hour at 11000 rpm at 4 °C. The supernatant was collected and preserved on ice, and it was identified as rat intestinal glucosidase with maltose and sucrose hydrolysis activity. The measurements were conducted in triplicate, except for the sample of coffee with *kelakai* and milk that was done in duplo. 25 µl sample solution was put into a 2 ml microtube for sample and sample blank tubes. 25 µl of distilled water was added to the control and control blank. Then 125 µl of sucrose substrate solution and 125 µl of maltose monohydrate substrate solution was added into each tube and agitated with the vortex. The mixtures were pre-incubated in the water bath for 5 minutes at 37°C. Next, 100 µl of the enzyme solution was added to sample and control tubes, whereas the blanks were added with 100 µl potassium phosphate buffer (Merck, Germany) 0.1 M at pH 6.9. The mixture was then incubated at 37 °C for 20 minutes for the maltase inhibition assay and 25 minutes for the sucrose inhibition assay. Proportion of sample, enzyme, and reagents are as shown in Table 5.

Afterwards, 750 µl of Tris-HCl solution was added in each tube. Then the mixtures were run through an aluminium oxide column made from a shortened Pasteur pipette, cotton, and 1 cm of aluminium oxide (Merck, Germany). After that, 200 µl of glucose test-kit (Wako Pure Chem. Co., Japan) solution was mixed with 20 µl of filtered mixture for maltase inhibition assay and 30 µl for sucrose inhibition assay in a 96 well plate. The samples were incubated at 37°C for 5 minutes. Lastly, the sample absorbance was read at 505 nm with a microplate reader

The activity was determined using Equation 1 below:

$$\% \text{Inhibition} = 1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{control}} - A_{\text{control blank}})} \times 100\% \quad (1)$$

In the formulation above, 'Asample' and 'Acontrol' represent absorbances of sample and positive control, respectively.

## 2.6. Sensory Evaluation

Focus group discussion (n=7) was conducted using untrained panelists in order to assess the perceived sensorial properties of coffee samples with two different concentrations of sucralose as sweetener and CMC as thickener. The purpose of conducting focus group discussion was to obtain the most suitable concentration of sucralose and CMC in milk coffee. The sample descriptions assessed were product description, aroma, appearance, taste, aftertaste, leafy aroma, thickness rating, sweetness rating, and sample preference.

In order to conduct the sensory evaluation, the beverage was chilled in the refrigerator until it was cool enough. The purpose was because the sample served later would be in a chilled condition. The samples presented were coded with 3-digits of numeric and the order of the samples was different between each panelist. Hedonic test (n=53) was conducted in order to assess the overall acceptance of the product samples. The panelists were untrained panelists. The test used a 9-point hedonic scale that ranged from like extremely (=1) to dislike extremely (=9). The evaluated attributes were the overall likeness.

In order to assess the most preferred sample among the given samples, a forced ranking test (n=53) was conducted and the panelists were untrained. They were asked to rank the samples from 1 to 3, where 1



represented the most preferable and 3 the least preferable samples. The sensory test results were analyzed using Friedman's test and Wilcoxon test, with the probability of 0.05.

### 3. RESULTS AND DISCUSSION

#### 3.1. Effect of *S. palustris* Addition to Black Tea and Coffee Beverages

Extraction of dried *kelakai* powder with water at 88°C for 1 hour yielded a clear dark brown solution as can be seen in Figure 1. The *kelakai* extract was observed to have a putrid aroma and it can be unpleasant for most people. Thus, it might be impossible to make beverages with *kelakai* extract alone. On the other hand, with the popularity of black tea and coffee beverages also for their potential in controlling blood sugar level, it was decided that *kelakai* extract was added to the black tea and coffee.



Figure 1. Aqueous *kelakai* extract

With the addition of *kelakai* in black tea and coffee, hopefully it could help improve the sensorial characteristics. Additionally, the *kelakai* extract was also observed to have a leafy aroma. The samples of black tea and coffee beverages added with *kelakai* were prepared according to formulations as in Table 1. For preliminary observation, the sensory characteristics of the beverage samples were assessed in terms of taste, aroma, trigeminal sensation and color. Due to the current pandemic situation, the observation was conducted by 1 trained panelist.

The results of *kelakai* addition in black tea and coffee beverages on the color intensity is shown in Table 6 and 7. Based on Table 6 and 7, it was found that the addition of *kelakai* slightly influenced the color intensity. Black tea samples added with *kelakai* were shown to have a slightly darker brown color compared to the control samples, in this case the black tea samples. Additionally, the slight difference in color intensity was more visible in the sample with ratio of water : black tea = 3:1 (v/v) compared with K3T1 (ratio of *kelakai* : black tea = 3:1 (v/v)). In black tea with *kelakai* samples, it showed that the increasing concentration of *kelakai* resulted in lighter color.

Table 6. Effect of *kelakai* and milk addition in black tea and coffee beverages on the visual appearances

		Samples		
Black tea				
	Water : black tea = 1:1 (v/v)	Water : black tea = 2:1 (v/v)	Water : black tea = 3:1 (v/v)	
Black tea with <i>kelakai</i>				
	<i>Kelakai</i> : black tea = 1:1 (v/v) (K1T1)	<i>Kelakai</i> : black tea = 2:1 (v/v) (K2T1)	<i>Kelakai</i> : black tea = 3:1 (v/v) (K3T1)	

Black tea with <i>kelakai</i> and milk			
	<b>11</b> <i>Kelakai</i> : black tea = 1:1 (v/v) + 60 mL milk (KIT1M)	<b>11</b> <i>Kelakai</i> : black tea = 2:1 (v/v) + 60 mL milk (K2T1M)	<i>Kelakai</i> : black tea = 3:1 (v/v) + 60 mL milk (K3T1M)

Table 7. Effect of *kelakai* and milk addition in black tea and coffee beverages on the visual appearances (cont.)

Coffee			
	Water : coffee = 1:1 (v/v)	Water : coffee = 2:1 (v/v)	Water : coffee = 3:1 (v/v)
	Coffee with <i>kelakai</i>		
<i>Kelakai</i> : coffee = 1:1 (v/v) (K1C1)		<i>Kelakai</i> : coffee = 2:1 (v/v) (K2C1)	<i>Kelakai</i> : coffee = 3:1 (v/v) (K3C1)
Coffee with <i>kelakai</i> and milk			
	<i>Kelakai</i> : coffee = 1:1 (v/v) + 60 mL milk (K1C1M)	<i>Kelakai</i> : coffee = 2:1 (v/v) + 60 mL milk (K2C1M)	<i>Kelakai</i> : coffee = 3:1 (v/v) + 60 mL milk (K3C1M)

Apart from the slight changes in the visual appearances, the other effects on the sensorial characteristics of the samples were also assessed as can be seen in Table 8. Since *kelakai* extract used in this experiment gave a putrid aroma, thus the aroma was detected in black tea with *kelakai* samples and increased respectively with higher concentration of *kelakai* extract added to the beverage.

In this experiment, it was found that black tea could not mask the putrid aroma from *kelakai*. Aside from putrid aroma, the *kelakai* extract was also observed to have a slight leafy aroma. Since black tea also exhibits leafy aroma, as a result, the leafy aroma was detected in both samples. However, the leafy aroma in black tea samples was perceived to be less compared to the black tea with *kelakai* samples. In terms of taste, both samples were observed to have the same sweetness and bitterness

level. Thus, it could be stated that the addition of *kelakai* did not affect the taste of the black tea beverages.

**Table 8.** Sensorial characteristics comparison of black tea added with *kelakai*

Sensorial Characteristics	Black Tea	Black Tea with <i>Kelakai</i>
Color	Clear dark brown	Clear, darker brown
Aroma	No putrid aroma Slight leafy aroma	High putrid aroma Moderate leafy aroma
Taste	Same sweetness Same bitterness	Same sweetness Same bitterness
Trigeminal Sensation	Same astringency	Same astringency

Furthermore, both samples were also observed to have the same astringency level. Dry mouthfeel is a term used to describe astringency [37] and is often perceived together with bitterness, since many astringent molecules are also bitter [38]. This sensation might give negative impacts and influence the overall liking of the product.

To sum up, when *kelakai* was added in black tea, it slightly increased the color intensity, slightly increased the existing leafy aroma in black tea, and gave a putrid aroma in the beverage, which increased respectively with higher *kelakai* concentration.

Subsequently, the addition of *kelakai* in coffee also slightly increased the color intensity, although it was less visible compared to the black tea samples as can be seen in Table 6. It was due to coffee extract having a very dark brown to black color and more intense color compared to black tea. In coffee with *kelakai* samples, it was observed that with the increasing concentration of *kelakai* in the samples, it slightly decreased the color intensity if observed in detail. The slight color intensity difference was slightly visible when comparing the sample with ratio of water : coffee = 3:1 (v/v) with K3C1 (ratio of *kelakai* : coffee = 3:1 (v/v)). Besides that, the addition of *kelakai* in the coffee beverages were also observed to affect other sensorial characteristics of the coffee and the result is listed in Table 9.

Unlike the black tea with *kelakai* samples, putrid aroma was not detected in all of the coffee with *kelakai* samples. However, the leafy aroma in coffee with *kelakai* samples were detected when higher concentration of *kelakai* was used, namely in K2C1 (ratio of *kelakai* : coffee = 2:1 (v/v)) and K3C1 (ratio of *kelakai* : coffee = 3:1 (v/v)). The leafy aroma perceived in the samples slightly increased when *kelakai* concentration also increased. Thus, it could be said that coffee has the ability in masking putrid and slight leafy aroma from the *kelakai* extract. Besides that, all the samples had the same sweetness, bitterness, and astringency level.

**Table 9.** Sensorial characteristics comparison of coffee added with *kelakai*

Sensorial Characteristics	Coffee	Coffee with <i>Kelakai</i>
Color	Clear dark brown-black	Clear, darker brown-black
Aroma	No putrid aroma No leafy aroma	No putrid aroma Slightly leafy aroma
Taste	Same sweetness Same bitterness	Same sweetness Same bitterness
Trigeminal Sensation	Same astringency	Same astringency

When comparing both black tea and coffee, the effect of *kelakai* addition in coffee was not really significant compared to black tea. In coffee, the putrid aroma emitted by *kelakai* was masked by the strong aroma compounds in coffee. The ability of coffee in masking the undesirable aroma due to the nitrogen in the caffeine can boost the ability of the carbon in adsorbing sulfur from the air [39], which is why coffee is usually used to neutralize lingering odor.

To conclude, the addition of *kelakai* in coffee beverages gave a slightly darker color and slightly leafy aroma when using higher *kelakai* concentration. However, the putrid aroma resulting from *kelakai* extract was not detected in the coffee beverages due to the ability of coffee in neutralizing undesirable aroma.

All in all, the *kelakai* extract used in this experiment gave a putrid aroma to the beverage. This finding was also supported by the study conducted by Rahmah (2017), which pointed out that fresh *kelakai* had a putrid aroma. In addition, *kelakai* extract was also found to give a slightly leafy aroma in the beverages. However, there is a need for further investigation to clarify which aroma compounds in *kelakai* plants are responsible for the putrid and leafy aroma.

### 3.2. Effect of Milk Addition to Black Tea and Coffee Beverages with *S. palustris*

Nowadays, there are a lot of black tea and coffee beverages using an additional ingredient such as milk to improve the sensorial characteristics of the beverages. It has found that milk can lower bitterness, astringency, and give higher body mouthfeel. Besides that, milk and milk proteins were also found to be beneficial for people with sugar concern [41]–[45]. With the increasing popularity of milk tea and milk coffee beverages, it might also add more value to the utilization of *kelakai* in common beverages.

In order to assess the effect of milk addition to black tea and coffee beverages with *kelakai* extract, samples were prepared following the formulations in Table 2. The same as previously, during the preliminary observation,



the sensorial characteristics of the samples evaluated were color, taste, aroma, and trigeminal sensation. The effect of milk addition in black tea and coffee beverages with *kelakai* extract on the visual appearances can be found in Table 6 and 7.

Firstly, when comparing black tea with *kelakai* and black tea with *kelakai* and milk samples, it was found that the addition of milk changed the beverage color from clear dark brown to cloudy light brown. Same as the black tea with *kelakai* samples, with the increasing concentration of *kelakai* in the black tea with *kelakai* and milk samples, it also decreased the color intensity. Thus, K3T1M (ratios of *kelakai* : black tea = 3:1 (v/v) with 60 mL milk) gave a lighter cloudy brown color compared to K1T1M (ratios of *kelakai* : black tea = 1:1 (v/v) with 60 mL milk), which gave a slightly darker cloudy brown. Furthermore, the milk addition in black tea with *kelakai* samples also slightly affected other sensorial characteristics and the results can be seen in the Table 10.

**Table 10.** Sensorial characteristics comparison of black tea with *kelakai* and additional milk

Sensorial Characteristics	Black Tea with <i>Kelakai</i>	Black Tea with <i>Kelakai</i> and Milk
<b>Color</b>	Clear dark brown	Cloudy light brown
<b>Aroma</b>	High putrid aroma Moderate leafy aroma No milky aroma	No putrid aroma Slight leafy aroma High milky aroma
<b>Taste</b>	Same sweetness Slight bitterness	Same sweetness No bitterness
<b>Trigeminal Sensation</b>	Slight astringency	Less astringency

The addition of milk was observed to have the advantages in masking the putrid aroma and slightly reduced the leafy aroma emitted by *kelakai* extract. The putrid aroma was not detected in all black tea with *kelakai* and milk samples. Whereas, the leafy aroma was still perceived but lesser compared to black tea with *kelakai* samples. Moreover, when black tea with *kelakai* was added with milk, it gave a high milky aroma in the sample.

Unlike the black tea with *kelakai* samples, the bitter taste in black tea with *kelakai* and milk was not perceived. Thus, the addition of milk was found to be helpful in masking the bitterness in black tea with *kelakai* beverages. Furthermore, the astringency perceived in black tea with *kelakai* and milk samples was less compared to black tea with *kelakai* samples. It was observed when comparing K1T1M (ratio of *kelakai* : black tea = 1:1 (v/v) with 60 mL milk) with sample K1T1 (ratio of *kelakai* to black tea 1:1 (v/v)). With the addition

of milk, it was found that the bitterness and astringency could be masked. These findings were also supported by a study conducted by Ares *et al.* (2009). The authors found that milk and sucrose were the most efficient alternatives in reducing bitterness, astringency, and distinct flavor in antioxidant extracts from two Uruguayan natural plants. It was reported that milk proteins formed complex compounds with polyphenols found in the plants which resulted in less polyphenols.

Therefore, it could be suggested that the addition of milk in black tea beverages with *kelakai* helped to mask the bitterness, putrid aroma, slightly reduce the astringency, and leafy aroma. Besides that, with the addition of milk also gave a high milky aroma to the beverage and changed the color into cloudy light brown.

Additionally, the milk addition in coffee beverages with *kelakai* also affected the color of the beverages. The addition of milk changed the color of coffee with *kelakai* from a very dark brown-black color to a cloudy dark brown. Identically with the comparison of coffee and coffee with *kelakai* samples, the increasing *kelakai* concentration in the coffee with *kelakai* and milk samples gave a lighter color. Previously, when comparing coffee and coffee with *kelakai*, the color differences were less visible. However with the addition of milk, it helped to clarify the color differences. The difference in color intensity was visible when comparing K1C1M (ratio of *kelakai* : coffee = 1:1 (v/v) with 60 mL milk) to K3C1M (ratio of *kelakai* : coffee = 3:1 (v/v) with 60 mL milk), where the prior showed a darker color and the latter showed a lighter color. Furthermore, the other effect of milk addition in coffee with *kelakai* on the sensorial characteristics can be seen in the Table 11.

**Table 11.** Sensorial characteristics comparison of coffee with *kelakai* and additional milk

Sensorial Characteristics	Coffee with <i>Kelakai</i>	Coffee with <i>Kelakai</i> and Milk
<b>Color</b>	Very dark brown to black	Cloudy dark brown
<b>Aroma</b>	No putrid aroma Slight leafy aroma Intense coffee aroma	No putrid aroma Less leafy aroma Moderate coffee aroma
<b>Taste</b>	Same sweetness Moderate bitterness	Same sweetness Slight bitterness
<b>Trigeminal Sensation</b>	Same astringency	Same astringency

Since in coffee with *kelakai*, there was already no putrid aroma detected, thus the addition of milk was found to slightly reduce the strong intensity of coffee aroma. Besides that, the addition of milk in coffee with

*kelakai* samples also helped to slightly reduce the leafy aroma since the aroma could only be perceived when the sample used the highest concentration of *kelakai*, namely in K3C1M (ratios of *kelakai* : coffee : milk = 3:1 (v/v) with 60 mL milk). The bitterness perceived in coffee with *kelakai* and milk was less compared to coffee with *kelakai* without the addition of milk. In addition, the sweetness and astringency of both of the samples were perceived as the same. Thus, it could be said that the addition of milk helped to reduce the strong intensity of coffee aroma, slightly reduced leafy aroma, and slightly reduced the bitter taste coming from coffee with *kelakai* samples.

As the crucial elements in determining the product quality, aroma and visual appearance plays an important role and may influence how the sample is perceived by the panelists. It can lead to whether the sample will be accepted or rejected before it is analyzed. Thus, when comparing all the samples, it was found that coffee gave a more promising future to be developed with *kelakai*. Apart from its ability in masking the putrid aroma, the coffee samples were observed to have a more balanced flavor compared to black tea with *kelakai* and milk samples, thus eliminating all the black tea samples.

Subsequently, between coffee with *kelakai* and coffee with *kelakai* and milk samples, it was found that coffee with *kelakai* and milk samples were better due to its better ability in masking the leafy aroma from *kelakai* extract. The leafy aroma was perceived at higher concentration compared to coffee with *kelakai* only. As a result, milk coffee was chosen to be optimized for its attributes. Additionally, among the three samples made in coffee with *kelakai* and milk, the selected formula to be optimized was K3C1M (ratios of *kelakai* : coffee : milk = 3:1:1 (v/v/v)) since the leafy aroma observed was not too significant.

### 3.3. Formula Optimization for Sweetness and Thickness

There were three commercialized milk coffee beverages used for attribute observation and it was found that all the commercialized products had higher

sweetness levels compared to the samples made by the author. Most commercial beverages are known to have high sugar content. However, the common primary problem in reduced-sugar beverages is the loss of body mouthfeel of the beverages. Sucrose is known not only to enhance sweet taste but also creates body mouthfeel. Therefore, in this study the sweetness and thickness levels of the samples were adjusted.

The thickener that was used in this experiment was carboxymethylcellulose (CMC). CMC is characterized as odorless, tasteless white powder. Moreover, it is soluble in water at any temperature, which indicates its high solubility. CMC gives a clear solution when dissolved in water, and is commonly used in beverages [47]. Another advantage of using CMC is because it can give a liquid beverage a smoother and rounded taste [48]. However, vigorous mixing is needed when CMC is incorporated into beverages to prevent lumps. Further advantage of CMC is that it is commercially available and can be purchased from many supermarkets.

Before determining the concentration of CMC to be evaluated in FGD, the author conducted preliminary observation by using several concentrations of CMC (0.1%, 0.15%, and 0.2%) in the coffee with *kelakai* and milk beverage. Two most-likely suitable concentrations were chosen to be used in FGD, which are 0.1% (w/v) and 0.15% (w/v). This observation also applied to determine the sweetener concentration and the chosen concentrations of sucralose were 0.015% (w/v) and 0.02% (w/v). Afterwards, there were four formulations made with varying concentrations of sucralose and CMC, which are T1S1, T2S1, T1S2, and T2S2. Sucralose with concentration of 0.015% was indicated with "S1", whereas 0.02% sucralose concentration was indicated with "S2". On the other hand, "T1" indicated 0.1% CMC and "T2" indicated 0.15% CMC. These varying concentrations were applied in the chosen formula which was K3C1M (ratios of *kelakai* : coffee : milk = 3:1:1 (v/v/v) and sodium benzoate 0.04% (w/v)). Then, focus group discussion with untrained panelists (n=7) was conducted in order to obtain the most suitable concentration of sucralose and CMC (Table 12).

**Table 12.** Focus group discussion for descriptive and preference evaluation of selected samples

Sample	T1S1	T1S2	T2S1	T2S2
<b>Product Description</b>	Watery milk coffee	Milk coffee	Plain watery-based milk coffee	Milk coffee
<b>Aroma</b>	Strong coffee aroma compared to milky aroma	Stronger milky aroma compared to coffee aroma	Less coffee aroma, less milky aroma	Slightly more milky aroma compared to coffee aroma
<b>Appearance</b>	Less dark milk coffee-like color	Milk coffee-like color	Milk coffee-like color	Milk coffee-like color
<b>Taste</b>	Slight sweet, slight bitter	Very sweet, slight bitter	Slight sweet, slight bitter	Sweet, slight bitter

Aftertaste	Slight bitter	Slight bitter and sweet	Slight bitter	Slight bitter
Leafy Aroma	Slight leafy	Not detected, strong milky aroma	Slight leafy	Slight leafy
Thickness Rating	Slightly thin	Slightly thin	Slightly thin	Enough
Sweetness Rating	Slightly less sweet	Enough	Very less sweet	Enough
Preference (%)	42.90	42.90	0	100



**Figure 2.** Coffee with *kelakai* and milk samples. From left to right: K2C1M (ratio of coffee : *kelakai* = 2:1 (v/v) with 60 mL milk), K3C1M (ratio of coffee : *kelakai* = 3:1 (v/v) with 60 mL milk), and K4C1M (ratio of coffee : *kelakai* = 4:1 (v/v) with 60 mL milk)

From the result obtained, most of the participants chose formula T2S2, with sucralose (0.02%) and CMC (0.15%) concentration, as the most preferable formula. With the lowest concentration of sucralose and CMC (T1S1), the participants perceived the product as watery milk coffee. However, when concentration of sucralose was increased with the same concentration of CMC as before (T1S2), it was perceived as milk coffee. The participants described the samples to be more milky compared to coffee. Furthermore, when the concentration of CMC was increased and used the lowest concentration of sucralose (T2S1), the sample was perceived as plain watery-based milk coffee. Sample with the highest concentration of CMC and sucralose (T2S2) was perceived again as milk coffee.

Some researchers studied the interaction between viscosity and perceived flavour [49]–[53]. A research conducted by He *et al.* (2016) found that the score of overall sweetness of the product increased with the increase of viscosity. However, the result contradicted the findings from other studies mentioned. The study conducted by Christensen (1980) found that higher viscosity led to a decreased intensity of the sweetness and saltiness. Furthermore, other studies also agreed that higher viscosity can suppress the flavor and aroma intensity of a product [50–52]. It was concluded that increasing thickness concentrations could lower the

quantity of free water available to transport the taste stimuli to receptors [51].

As shown on Table 11, it could be concluded that increasing viscosity with the same sucralose concentration could decrease the sweetness intensity, which aligned with the results from previous studies. Increasing CMC concentration slightly affected the perceived aroma. In samples with higher CMC but same sucralose concentration (T2S1 compared to T1S1), both coffee and milky aroma were found to be less intense. A similar observation was found in samples T1S2 and T2S2, where the milky aroma intensity was found to be slightly reduced. However, the panelists did not perceive a reduced coffee aroma with increasing CMC concentration. On the other hand, higher sucralose concentrations with the same CMC concentrations enhanced the sweetness intensity of the sample. Although the participants agreed to choose T2S2 as the most suitable concentration, there was a suggestion in increasing the coffee concentration in the selected sample.

### 3.4. Sensory Acceptance of Coffee Beverages with *S. palustris* and Milk

From the FGD results, there was a suggestion that from the chosen formula, the coffee concentration in the sample should be increased to have a stronger coffee taste. Therefore, three formulas were used to assess the



maximum *kelakai* concentration preferred by the consumers.

The samples applied three varying ratios of *kelakai* to coffee, i.e. 2:1 (K2C1M), 3:1 (K3C1M), 4:1 (K4C1M), with same concentrations of sodium benzoate (0.04%), sucralose (0.02%), CMC (0.15%), and addition of 60 mL milk. The detailed formula can be seen in Table 4. The reason for choosing K2C1M was to increase the coffee concentration as suggested in the FGD, whereas, K4C1M was chosen to evaluate whether a higher *kelakai* concentration was still acceptable. As can be seen in Figure 2, samples with higher concentration of *kelakai* were shown to have a lighter color compared with other samples.

Sensory test (n=53) was conducted using the 9-point hedonic scales test and forced ranking test on untrained panelists. In the hedonic test, the panelists were asked to evaluate the product based on the overall acceptance using scales ranging from like extremely (=1) to dislike extremely (=9). Whereas in the forced ranking test, the panelists were asked to rank the samples based on their preference from rank 1 representing the most preferable sample to rank 3 representing the least preferable sample. The results showed that K2C1M (*kelakai* : coffee = 2:1 (v/v), sucralose 0.02% (w/v), sodium benzoate 0.04% (w/v), CMC 0.15% (w/v), and 60 mL milk) was chosen to be the most preferred formula, with a mean value of  $3.43 \pm 1.47$  as can be seen in Table 13. This value indicated that the sample was between slightly to moderately liked. The second most preferred formula was shown with the sample formula of K4C1M (*kelakai* : coffee = 4:1 (v/v), sucralose 0.02% (w/v), sodium benzoate 0.04% (w/v), CMC 0.15% (w/v), and 60 mL milk) which had a mean value of  $4.23 \pm 1.7$ , followed by sample formula K3C1M (*kelakai* : coffee = 3:1 (v/v), sucralose 0.02% (w/v), sodium benzoate 0.04% (w/v), CMC 0.15% (w/v), and 60 mL milk) with mean value of  $4.40 \pm 1.90$ . Those both samples were shown to be in a range between slightly liked to neither liked nor disliked.

Table 13. Sensory evaluation results

Formula	Overall Acceptance Mean	Forced Ranking Test Mean
K2C1M	$3.43 \pm 1.47^a$	$1.62 \pm 0.84^c$
K3C1M	$4.40 \pm 1.90^b$	$2.19 \pm 0.81^d$
K4C1M	$4.23 \pm 1.71^b$	$2.19 \pm 0.68^d$

Based on Friedman's test, the value for K2C1M was significantly different ( $p < 0.05$ ). However samples K4C1M and K3C1M showed no significant differences ( $p > 0.05$ ) based on the Wilcoxon test. Therefore, it can be concluded that the sample formula of K2C1M was chosen to be the most favorite one. This analysis also

applied to the forced ranking test (n=53), which proved that K2C1M was the most preferable sample among the three samples. Thus, it could be said that samples with higher coffee concentration were more preferred by the panelists.

A research conducted by Ariefandi & Rizki (2015) found that the greater content of cardamom in herbal coffee, thus lowering the panelists' acceptance. This was due to the alteration of panelists' present opinion of common coffee. The authors suggested that the distinctive aroma of cardamom altered the original coffee aroma, thus resulting in an unusual perception of a regular coffee product. Same with this study, where the higher concentration of *kelakai* in milk coffee reduced the panelist's acceptance and it might be due to unusual perception of the panelists.

Subsequently, K2C1M as the most preferred sample was further analyzed to check whether the sample has AGI activity. In order to compare their AGI activities, *kelakai* extract and milk coffee without *kelakai* were analyzed as well. However, the results obtained from AGI assay showed that a of the samples had inhibition activity below 20%. The activity was very small, thus it can be concluded that the sample did not inhibit alpha-glucosidase enzyme.

The low inhibition activity can be caused due to low concentration of the extract. Besides that, long storage time and heat treatment processing might have also reduced the activity of the alpha-glucosidase. The same *kelakai* sample was also used by Teji (2019), however the extraction method was slightly different. The author extracted the *kelakai* using water at room temperature for 24 hours and it resulted in 15.27% sucrose inhibition activity at concentration of 5 mg/mL and maltose inhibition activity of 11.77%. Both of the results also showed inhibition activity below 20%.

#### 4. CONCLUSION

Unlike other common natural resources which have been properly developed into food and/or beverage products, application of *kelakai* could give a promising future to be developed since it has a good survivability, low economical value, and has various potential health benefits. In this study, it was found that the effect of *kelakai* addition in black tea was more significant compared to coffee beverages. When *kelakai* was added to black tea beverages, it gave a putrid aroma, increased the existing leafy aroma, and the color changes were more visible. On the other hand, the putrid aroma was not detected in *kelakai* coffee and the color changes were observed to be less visible. However, the leafy aroma in coffee was still detected at higher concentration of *kelakai*.



When milk was added to the beverages, not only it could reduce the bitterness in both black tea and coffee, but also reduced the leafy aroma. In black tea with *kelakai* samples, the addition of milk helped to mask the putrid aroma and slightly reduced the astringency, but it gave a high milky aroma in the beverage. Whereas, the milk addition in coffee with *kelakai* samples was found to slightly reduce the strong coffee aroma.

The selected sample to be optimized was coffee with *kelakai* and milk, where the sweetness level and body mouthfeel needed to be adjusted. From the FGD result, it was found that the highest sucralose (0.02%) and CMC (0.15%) concentration was the most suitable for fixing the attributes of coffee with *kelakai* and milk. It appeared that addition of CMC in the beverage could reduce the flavor and aroma perception due to less free water available to transport those taste stimuli. There were three samples evaluated with ratio of *kelakai* to coffee 2:1 (v/v), 3:1 (v/v), and 4:1 (v/v), with the same concentration of milk (60 mL), sucralose 0.02% (w/v), sodium benzoate 0.04% (w/v), and CMC 0.15% (w/v). Those three samples were evaluated using hedonic test (n=53) and forced ranking test (n=53) on untrained panelists. As a result, the maximum *kelakai* concentration that was still acceptable was found in sample K2C1M with ratios *kelakai*, coffee to milk 8:4:3 (v/v/v), with the overall acceptance mean of  $3.43 \pm 1.47$ . This score represented slight to moderate likeness. The forced ranking test results were in line with the result from hedonic test, where sample K2C1M was ranked highest. Through AGI assay, it was found that the chosen formula had AGI inhibition activity very low (<20%), thus it can be concluded that the sample showed no inhibition activity. For the future research, there is a need for improvement in the formula to achieve higher acceptability and formula optimization to achieve formula that can give the best AGI activity.

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