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Quality assessment of Non-fermented, partially-fermented, and fully-fermented Betel (*Piper betle* L.) Leaves Infusion

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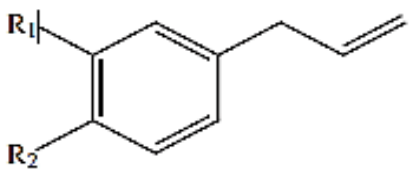
The objectives of the present study were to determine the total phenolic content (TPC); total flavonoid content (TFC); antioxidant properties (i.e., 2.2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing assays (FRAP)); physical properties (i.e., color and turbidity); and sensory evaluation of infusions prepared from non-fermented, partially-fermented, and fully-fermented betel leaves. The infusions of non-fermented, partially-fermented, and fully-fermented betel leaves were prepared at the concentration of 0.75%. All infusions were brewed at 100 ºC for 5 min prior to the analysis. The infusion of non-fermented betel leaves presented a significantly higher value ($p<0.05$) of TPC (4413.77 mg GAE /100mL), TFC (4034.65 mg QE /100mL), DPPH (4721.32 µmol TE /100mL), and FRAP (3570.7 µmol $FeCl_3$ /100mL) compared to those made from partially-fermented and fully-fermented betel leaves. However, the turbidity results revealed the infusion of fully-fermented betel leaves having a higher turbidity value (12.97%) than those made of the non-fermented (9.00%) and partially-fermented (8.77%) betel leaves. Moreover, the sensory evaluation results indicated that the infusion of non-fermented betel leaves received the highest score in overall acceptability. This information could be disseminated to interested industry partners for the commercialization of betel leaves-based herbal tea. Such product, if developed, is expected to offer health benefits for its consumers.

Keywords: betel leaves, fermentation, chemical properties, physical properties, sensory evaluation

INTRODUCTION

The current trend of research aims at developing new food products that have functional properties. This is due to the increasing awareness of beneficial functional food among consumers. According to Patel and Mohan (2017), more than 80% of the world population depends on herbal medicines as a major source of meeting their health care needs. *Piper betle* ('*sirih*') vines are a common plant cultivated in Malaysia for their health benefits. The leaf is recognized to contain an abundance of chemical, phytochemicals, and nutritional compositions. Chauhan et al. (2016) have reported that betel leaves exhibit antioxidant and anti-diabetic properties, as well as a broad spectrum of antibacterial effect. This is due to the presence of major constituents such as polyphenols, especially chavibetol (53.1%) and chavibetol acetate (15.5%), while the presence of other compounds such as allypyrocatechol diacetate (0.71%), campene (0.48%), chavibetol methyl ester (0.48%), eugenol (0.32%), α-pinene (0.21%), β-pinene (0.21%), a-limonene (0.14%), safrole (0.11%), and 1, 8-cineole (0.04%) also contributed to the therapeutic values of betel leaves (Figure 1) (Suhaimi, 2020).



chavicol	R_1=H, R_2=OH
chavibetol	R_1=OH, R_2=OCH_3
chavibetol acetate	R_1=OAc, R_2=OCH_3
allylpyrocatechol	R_1=OH, R_2=OH
allylpyrocatechol diacetate	R_1=OAc, R_2=OAc

Figure1: The chemical structures of several compounds found in *Piper betle* leaves

However, previous studies on betel leaves for phenolic compounds and antioxidant activity have mainly focused on using organic solvents as the extraction medium, such as methanol and ethanol (Putri and Farida, 2013; Jaiswal et al. 2014; Kumari and Rao, 2015; Muruganandam et al. 2017; Purba and Paengkoum, 2019). The extraction media utilized are irrelevant for food production due to the fact that they are carcinogenic to humans.

Fresh betel leaves contain moisture content of 85.4%, fat (0.8%), protein (3.1%), fiber (2.3%), carbohydrate (6.1%), and various minerals, such as calcium (230 mg/100 g), phosphorous (40 mg/100 g), iron (7 mg/100 g), ionize-able iron (3.5 mg/100 g), and iodine (3.4 µg/100 g) (Periyanayagam et al. 2012). However, the high moisture content of fresh betel leaves renders them highly perishable and susceptible to deterioration due to their fresh state (Sewald and DeVries, 2015; Ho et al. 2018). As such, the leaves are always subject to wastage from quick spoilage due to dehydration, fungal infection, and dechlorophyllation. In addition, betel vine grows abundantly during the rainy season, which causes wastage from unsold or spoiled betel leaves. These surplus leaves are always utilized as animal feed, while at times buried in the ground to avoid environmental pollution. Current research on betel leaves has shown that these leaves possess many beneficial bioactivities and the extracts have a good potential to be utilized in developing both edible and non-edible products.

Herbal teas have gained popularity globally, with them being the second most-consumed beverage worldwide after water (Muktar and Ahmad, 2000). This is due to herbal teas containing a variety of active phytochemicals with biological properties that promote human health and may reduce the risk of certain chronic diseases (i.e., insomnia, headaches, anxiety, intestinal disorders, depression, and high blood pressure) (Craig, 1999). According to Joubert et al. (2017), herbal tea made from dried plant parts infused in warm or hot water is gaining popularity owing to scientific evidence of health benefits from its consumption. The parts used include the leaves, flowers, seeds, fruits, and roots of plant species (Aoshima et al. 2007). Most of the reported studies have focused on tea produced from *Camellia sinensis* and other popular tropical herbal teas, such as *misai kucing* (*Orthosiphon aristatus*), lemongrass (*Cymbopogon citratus*), lemon myrtle (*Backhousia citriodora*), ginger (*Zingiber officinale*), *mas cotek* (*Ficus deltoidea*), and *pegaga* (*Centella asiatica*) (Chan et al. 2010; Suhaimi, 2020). However, studies regarding the functional health properties of herbal tea processed from betel leaves have yet to be reported. Therefore, there is a need to look for new sources for herbal tea processing such as betel leaves in order to expand the tea market at the local and international levels alike.

Generally, there are three categories of tea differentiated according to the processing methods in reference to the degree of fermentation, namely non-fermentation, partial fermentation, and full fermentation (Engelharrt, 2010). Green tea is a non-fermented tea used as the main beverage in Japan and China, while black tea is more popular in North America and Europe. In contrast, oolong tea is an intermediate variant between green and black tea (Nor Qhairul Izzreen and Mohd Fadzelly, 2013). In particular, green tea is manufactured from fresh *C. sinensis* leaves wherein significant oxidation of the major leaf polyphenols, otherwise known as catechins, is prevented. In terms of oolong tea, it is partially oxidized and retains a significant amount of catechins, whereas the production of black tea leaves involves extensive enzymatic oxidation of the leaf polyphenols to yield dark products (i.e., theaflavins and thearubigins) (Ho et al. 1994).

Tea manufacturers try to produce tea products with high micro- and macro-nutrient contents and other beneficial chemical compounds, such as polyphenols. However, the content and stability of nutrients and polyphenols during tea manufacturing are dependent on several factors, such as processing methods during the infusion preparation. To date, there is a limited amount of published studies on herbal tea processed from betel leaves via different fermentation processes. Therefore, the objective of this study is to produce non-fermented,

partially-fermented, and fully-fermented herbal teas with a high content of chemical composition and good physical quality in response to the increasing market for functional food offering potential health benefits.

MATERIALS AND METHODS

Material

Fresh betel (*Piper betle* L.) leaves were purchased from a local wet market in Besut, Terengganu, Malaysia. The leaves were cleaned with water to remove dirt and then subjected to different fermentation processes, namely non-fermentation, partial fermentation, and full fermentation.

Processing of non-fermented betel leaves

The cleaned betel leaves were withered in a forced air oven at 30 °C for 2 h and then steam-blanched for 10 min to inactivate the enzymes in the leaves (Nor Qhairul Izzreen and Mohd Fadzelly, 2013). Then, the treated leaves were dried in a hot air oven at 100 °C to seal their inherent flavors until the moisture content was less than 6.5% (Chen and Mujumdar, 2015). Next, the dried leaves were ground into smaller particles and kept in an airtight container prior to usage.

Processing of partially-fermented and fully-fermented betel leaves

In the partial fermentation process, the cleaned betel leaves were withered in a forced air oven at 30 °C for 2 h. Then, the twisting and tearing processes were undertaken for 20 min to ensure all leaves were fully twisted and torn. The crumpled leaves were subjected to partial fermentation for 120 min in an incubator at 30°C. For the full fermentation process, similar procedures were repeated but at a prolonged fermentation time of 24 h. Then, the leaves were dried in a hot air oven at 100 °C to seal the leaf flavors until the moisture content was less than 6.5% (Chen and Mujumdar, 2015). Next, the dried leaves were ground into smaller particles and then kept in an airtight container prior to usage.

Infusion preparation

Approximately 0.75 g of non-fermented, partially-fermented, and fully-fermented betel leaves were infused separately in 100 mL distilled water at 100 ºC for 5 min and cooled immediately using an ice bath to stop the enzymatic reaction. All of the prepared infusions were further analyzed with regard to their chemical and physical properties and sensory evaluation.

Total phenolic content determination

The Folin-Ciocalteau method served as the reference for the determination of infusion total phenolic content (TPC). A 3 mL infusion was added to 3 mL of Folin-Ciocalteau reagent (pre-diluted 10 times with distilled water) and kept at rest for 5 min at room temperature. After that, 4 mL of Na_2CO_3 (7.5%, w/v) solution was added into the mixture. The solution was vortex-mixed and allowed to stand for 1 h at room temperature. Next, the absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu Model 1601, Kyoto, Japan) against a blank of distilled water (Ho et al. 2018). The result was expressed as grams of gallic acid equivalents per hundred milliliters of infusion (mg GAE/100 mL infusion).

Total flavonoid content determination

The infusion total flavonoid content (TFC) was determined according to the method described by Ho et al. (2018). The infusion (1 mL) was mixed with 4 mL of distilled water and 0.3 mL of $AlCl_3$ (10%). At the 6^{th} min, 2 mL of NaOH (1 M) and 2.4 mL of distilled water were added into the mixture. The solution was mixed well and the absorbance was measured against a prepared reagent blank (distilled water) at 510 nm using a UV-visible spectrophotometer. The infusion TFC was expressed as milligrams of quercetin equivalents per hundred milliliters of infusion (mg QE/100 mL of infusion).

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay

The DPPH free radical scavenging assay of the infusions was carried out according to the method proposed by Mensor et al. (2001). A 1 mL of DPPH methanol solution (0.3 mM) was added to 2.5 mL of infusion (positive control) or methanol-distilled water (negative control), which were then allowed to react at room temperature for 30 min. After that, the absorbance values were measured at 518 nm using a UV-visible spectrophotometer. The results were expressed as micromoles of Trolox equivalents per hundred milliliters of the infusion (µmol TE/100 mL infusion).

Ferric-reducing antioxidant potential (FRAP) assay

Ferric-reducing assay was conducted according to the method described by Benzie and Strain (1996). A 100 µL of infusion was added to 3 mL of freshly prepared FRAP reagent (300 mM sodium acetate buffer at pH 3.6, 20 mM iron chloride, and 10 mM 2,4,6-tripyridyl-s-triazine dissolved in 40 mM HCl at a ratio of 10:1:1). The solution was mixed thoroughly and incubated at 37 °C for 4 min. The reagent blank was a mixture of 100 µL distilled water and 3 mL of FRAP reagent incubated at 37 ^{0}C for 1 h. The absorbance of the infusion and reagent blank was measured against the blank (distilled water) at 593 nm by using a UV-VIS spectrophotometer. The results were expressed as micromoles of ferrous equivalent Fe (II) per hundred milliliters of infusion (µmol Fe (II)/100 mL of infusion).

Colour measurement

The color of the prepared infusion was determined according to L* [Lightness (L=100, white and L=0, black)], Chroma a* [green chromaticity (-60) to red (+60)], and Chroma b* [blue chromaticity (-60) to yellow (+60)] space value using Konica Minolta Chroma Meter CR-400. The chromameter was calibrated prior to analysis using a white calibration plate.

Turbidity measurement

The turbidity of infusion was measured according to the method described by Morton and Murray (2001) using a spectrophotometer at 800 nm. The percentage transmittance (%T) was recorded and the turbidity was calculated by subtracting %T from 100 (100 - %T).

Sensory evaluation

All prepared infusions were evaluated by 40 semi-trained panelists from the School of Food Industry, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus. Before the sensory analysis began, the non-fermented, partially-fermented, and fully-fermented betel leave infusions were left to cool to room temperature. The encoded and equal quantities of each infusion (sample) were poured into transparent cups. The panelists were provided with a questionnaire in the form of an evaluation form and they were asked to taste one sample at a time and record their response in order to determine the acceptance of the product. A 7-point Hedonic scale (1=dislike very much, 7=like very much) (Watts et al. 1989) was used to evaluate the color, turbidity, aroma, taste, aftertaste, and overall acceptability of the infusions. Plain water was provided to rinse the palate between samples.

Statistical analysis

All measurements were performed by at least three replications. The results were reported as the mean value ± standard deviation (n=3 ± s.d.). The data were subjected to analysis of variance (ANOVA) and the significant differences among the mean were tested using the SPSS (Version 22.0 for Windows, SPSS Inc., Chicago, IL). The significant differences between mean values were determined by Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Total phenolic content, total flavonoid content, and antioxidant activity of infusions

The results of the total phenolic content, total flavonoid content, and antioxidant activity (i.e., DPPH free radical scavenging and FRAP) of non-fermented, partially-fermented, and fully-fermented betel leaves infusions are shown in Table 1. The TPC of processed betel leaves infusions ranged from 2426.36 to 4034.65 mg GAE/100 mL of infusion. The TPC in food is generally linked to beneficial health effects of food that can be considered a major source of antioxidants (Lantano et al. 2015). Pradhan et al. (2013) have previously reported that cadinene, 1,8-cineole, camphene, caryophyllene, limonene, pinene, chavicol, carvacrol, safrole, chavibetol, ally pyrocatechol, and eugenol are the major compounds found in betel leaves that are possibly contributory towards the TPC in the infusion. Non-fermented betel leaves infusion (4034.65 mg GAE/ 100 mL of infusion) displayed a higher TPC than the partially-fermented (2460.26 mg GAE/ 100 mL of infusion) and fully-fermented (2426.36 mg GAE/100 mL of infusion) betel leaves infusions. Such outcome is directly proportional with the fermentation time increment. According to Bae et al. (2011), non-fermented betel leaves are generally very rich in catechins because catechin oxidation by polyphenol oxidase is prevented by the steaming or panning process. In general, this process is essential in maintaining the polyphenols as per their monomeric forms. Therefore, it proves that a lengthened fermentation time in the processing of partially-fermented and fully-fermented betel leaves has resulted in broken down polyphenols that are

Table1: Total phenolic content, total flavonoid content, and antioxidant activity of infusion prepared from non-fermented, partially-fermented, and fully-fermented betel leaves[1].

Composition[2]	Processing		
	Non-fermented	Partially-fermented	Fully-fermented
TPC (mg GAE/100 mL of infusion)	4034.65 ± 5.53[a]	2460.26 ± 2.51[b]	2426.36 ± 1.45[b]
TFC (mg QE/100 mL of infusion)	4413.77 ± 4.04[a]	4065.50 ± 0.97[a]	2833.53 ± 1.83[b]
FRAP (µmol $FeCl_3$/100 mL infusion)	3570.72 ± 1.00[a]	2216.23 ± 0.12[b]	1824.69 ± 3.00[c]
DPPH (µmol TE/100 mL infusion)	4721.37 ± 5.80[a]	2442.17 ± 0.91[b]	1697.63 ± 1.72[c]

[1] Data are presented as mean ± standard deviation (n = 3). Mean values in the same row with different superscript letters are significantly different at $p < 0.05$.

[2] TPC: total phenolic content; TFC: total flavonoid content; FRAP: ferric reducing antioxidant potential assay; DPPH: 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging assay.

available in a plant (i.e., betel leaves) (Heong et al. 2011).

Flavonoids consist of a large group of water-soluble polyphenols that are extensively distributed in the plant kingdom as glycosides. They can inhibit metal-initiated lipid oxidation via the formation of complexes with metal ions (Kumar and Pandey, 2013). Accordingly, the total flavonoid content of the processed betel leaves infusions decreased in the following order: non-fermented betel leaves infusion > partially-fermented betel leaves infusion > fully-fermented betel leaves infusion. However, no significant difference was observed between the total flavonoid contents of non-fermented (4413.77 mg QE/ 100 mL of infusion) and partially-fermented betel leave infusions (4065.5 mg QE/ 100 mL of infusion). The results obtained in this present study are thus in agreement with those reported by Nor Qhairul Izzreen and Mohd Fadzelly (2013).

The ferric reducing antioxidant power (FRAP) assay determines the ability of antioxidants against the oxidative effect of reactive oxygen species. Therefore, the infusions were analyzed based on the reduction of the O-Phenanthroline-Fe (2+) complex and the distraction due to the chelating agent. The highest value of FRAP belongs to non-fermented betel leaves infusion (3570.72 µmol $FeCl_3$/ l00 mL of infusion), followed by partially-fermented betel leaves infusion (2216.23 µmol $FeCl_3$/ l00 mL of infusion), and lastly fully-fermented betel leaves infusion (1824.69 µmol $FeCl_3$/ l00 mL of infusion) (Table 1). According to Ndife et al. (2019), the high FRAP values signify a product's ability to scavenge free radicals. This indicates that all betel leaves infusions have the ability to reduce the Fe^{3+} ion to Fe^{2+} ion. The concentration of Fe^{2+} still present in all three infusions is indicative of the antioxidant capacity offered by the herbal concoction (Benzi and Szeto, 1999). However, the antioxidant activity of plants may vary due to the influence by several factors, such as climate conditions, soil nutrient of plant growth, harvest seasons, processing techniques, storage conditions and also the phenolic compound presence in the plants (Azli et al. 2018; Muhamad et al. 2018).

The results of the DPPH free radical scavenging assay showed a similar trend with the results of the FRAP assay. A very high antioxidant activity was found in the non-fermented betel leaves infusion with a DPPH free radical scavenging value of 4721.37 µmol TE/100 mL. This was followed by partially-fermented betel leaves infusion (2442.17 µmol TE/100 mL) and fully-fermented betel leaves infusion (1697.63 µmol TE /100 mL) accordingly. Moreover, prolonged betel leaves heating process during the processing of the partially-fermented and fully-fermented leaves resulted in a reduction of bioactive compounds contained in them. According to Ayu et al. (2018), the decreasing antioxidant activity can be caused by the enzymatic polyphenols through the process of oxidation, which will then retard these compounds. In particular, gallocatechins such as epigallocatechin gallate and epigallocatechin are the antioxidant compounds with less resistance to heat, thereby showing the tendency to be oxidized first via polyphenol oxidase action (Xie et al. 1993). This is in comparison to their further oxidation in forming theaflavins and thearubigins, which are the major phenolic compounds of black tea, as a result of prolonged fermentation duration (Robertson, 1992). In this case, the partially-fermented betel leaf infusion was intermediate in terms of theaflavin and thearubigin composition and valued between non-fermented and fully-fermented betel leaves infusions. Moreover, a positive correlation can be found between the numbers of DPPH free radicals and available hydroxyl groups; the higher the number of

available hydroxyl groups present in the compounds, the higher the radical scavenging capability shown. Therefore, the unpaired electron of the free radical becomes paired in the presence of a hydrogen donor (Nasir et al. 2019). Mensor et al. (2001) have also reported that a very good activity of the polar extracts may be due to the presence of bioactive compounds with high hydroxyl groups, an example being the phenolics.

Physical properties of infusions

The results obtained regarding the physical quality (i.e., color, and turbidity) shown by non-fermented, partially-fermented, and fully-fermented betel leaves infusions are tabulated in Table 2. In general, the lightness (L*) values indicated that the infusions for all samples were brown to dark brown in color, whereby the L* value for the fully-fermented betel leaves infusion exhibited the darkest color among them. The value was reduced from 38.45 to 35.61 parallel to the intensity of the fermentation period on betel leaves. Thus, this shows a complementary trend alongside the work by Heong et al. (2011). In contrast, the non-fermented betel leaves infusion had the highest value of L* (38.45). According to Lin et al. (2014) and Azli et al. (2018), the dark color of fermented tea leaves can be attributed to the formation of larger polyphenolic molecules in the form of polymers or dimers (i.e., aflavins, theaflavins, and thearubigins). This may be obtained through the condensation and oxidative polymerization of catechins as the degree of fermentation increases.

The a* value represents the greenness (negative value) for all prepared infusions, which were in a range of -0.41 to -0.54. The infusion prepared without the fermentation process had a significantly and the highest negative a* value (-0.54), followed by partially-fermented (-0.45), and fully-fermented (-0.41) betel leaves infusions. This was indicated by greenish color of the non-fermented betel leaves infusion in comparison to the partially- and fully-fermented betel leaves infusions. Such outcome was due to the non-fermented betel leaves non-exposure to hot air during processing, causing more chlorophyll retained within the leaf cells compared to partially- and fully-fermented betel leaves. Accordingly, the longer the senescing time during fermentation, the more chlorophyll content is diminished within the plants (Patra et al. 2016). In addition, Bae et al. (2011) have stated that fermentation significantly increases the redness of an infusion. Therefore, compounds such as flavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin 3,3'-digallate were produced through the oxidation of catechins, epicatechins, and their gallates during the fermentation. These compounds greatly contributed to the color of the brewed infusion.

On the other hand, the b* value of all prepared betel leaves infusions ranged from 4.34-4.46, which were not significantly different from each other. The positive value of b* indicated a yellowish color instead of a blue color. The result obtained showed a higher b* value than the green tea from *Camellia sinensis* (3.17) and mulberry leaf teas (1.45-2.57) as reported by Bae et al. (2011), but lower than the b* value of *sukun* leaf (20.66) (Azli et al. 2018).

Besides, the turbidity values of the fully-fermented betel leaves infusion (12.97%) were significantly higher than those of the non-fermented (8.77%) and partially-fermented (9.00%) betel leaves infusions, whereby turbidity increased with fermentation time. The results presented that turbidity values in both non-fermented and partially-fermented betel leaves infusions were generally below 10%, indicating a low level of turbidity (Heong et al. 2011). Moreover, the fully-fermented betel leaves infusion showed the highest level of turbidity (12.97%), thereby suggesting that compounds linked with macromolecules (i.e., soluble proteins and metal ions that bind with oxidized polyphenolics such as theaflavin and thearubigin) came off the leaves during fermentation. This allows their diffusion to occur readily and thus contribute to a more cloudy or smoky infusion (Bae et al. 2011).

Sensory evaluation of infusions

The mean score for the sensory evaluation of non-fermented, partially-fermented, and fully-fermented betel leaves infusions is presented in Table 3. In general, the mean scores for color, turbidity, aroma, taste, aftertaste, and overall acceptability showed significant differences between non-fermented betel leaves infusion and other infusions (i.e., partially- and fully-fermented infusions). Furthermore, the results revealed that the non-fermented betel leaves infusion had the highest mean score (6.17-6.20) in all sensory attributes.

Table 2: Colour properties and turbidity of infusion prepared from non-fermented, partially-fermented, and fully-fermented betel leaves[1].

Parameter	Processing		
	Non-fermented	Partially-fermented	Fully-fermented
*L**	38.45 ± 0.91[a]	38.31 ± 1.05[a]	35.61 ± 0.77[b]
*a**	-0.54 ± 0.02[b]	-0.45 ± 0.04[a]	-0.41 ± 0.06[a]
*b**	4.34 ± 0.11[a]	4.34 ± 0.11[a]	4.46 ± 0.03[a]
Turbidity (%)	8.77 ± 0.06[b]	9.00 ± 0.10[b]	12.97 ± 1.79[a]

[1] Data are presented as mean ± standard deviation (n = 3). Mean values in the same row with different superscript letters are significantly different at $p < 0.05$.

For the color, the non-fermented betel leaves infusion yields significantly higher scores (6.17) than the other two infusions (4.63-4.77) as shown in Table 3. This was attributed to the green color of the raw non-fermented betel leaves, which impacted the overall color of the formulation. When brewed separately, this particular infusion showed a light and clear green color, while partially- and fully-fermented betel leaves infusions had a hazy dark brown color and was less preferred by the panelists. A similar trend was also observed in the turbidity score for all evaluated infusions. This indicates that the higher the turbidity value (less clearness) of infusions (Table 2), the less the acceptability level by the panelists.

The mean scores for the aroma of infusion samples were 6.17 (like moderately) and 4.67-4.73 (like slightly) for non-fermented and partially- and fully-fermented betel leaves infusions, respectively. This was due to the non-fermented betel leaves infusion giving off green tea-like aroma, which was the perceived aroma stated by the panelists. However, they also commented that both partially- and fully-fermented betel leaves infusions gave the burning smell and bitter taste. According to Prakash and Gupta (2009), the aromatic metabolites widely found in plants are the polyphenols, which can be correlated to antioxidant activity and thus affecting the sensory acceptability (i.e., aroma) of food. Moreover, the burning smell may be derived from the pyrazines, furans, pyrroles, and ionone-associated compounds formed during the fermentation (Ho et al. 2015). Therefore, both partially- and fully-fermented betel leaves infusions were unpleasant compared to the non-fermented betel leaves infusion.

The scores were low for taste (4.60-4.73) and aftertaste (4.67-4.73) attributes of the partially- and fully-fermented betel leaves infusions compared to non-fermented betel leaves infusion (6.17 and 6.17, respectively). This was due to the bitter taste of the infusions. According to Ayu et al. (2018), the bitter taste and aftertaste of infusions made from plant-based materials are probably due to the presence of high polyphenols and flavonoid contents. This result is in agreement with the total phenolic and flavonoids contents as recorded in Table 1. Moreover, various compounds derived through enzymatic oxidations that underwent the condensation reaction resulted in a sequence of compounds. They include phenol and terpene (i.e., terpenoids, cadinene, camphene, caryophyllene, limonene, pinene, chavicol, ally pyrocatechol, carvacrol, safrole, eugenol, and chavibetol), which are commonly found in betel leaf (Pradhan et al. 2013). These compounds produced during the condensation process may impart the taste and coloration evidence of partially- and fully-fermented infusions prepared from betel leaves, which leads to them receiving similar scores for taste and aftertaste attributes. In addition, the scores were low for taste and aftertaste attributes possibly due to the astringency of the partially- and fully fermented betel leaves infusions, thereby influencing their overall taste and aftertaste.

In terms of the overall acceptability, non-fermented betel leaves infusion scored significantly higher than the partially- and fully-fermented betel leaves infusions. This was due to the higher scores received by the former infusion and in comparison with the latter infusions for all evaluated attributes. However, all betel leaves infusions were markedly acceptable by the panelists as they received a score higher than 4.00 ('neither like nor dislike-like').

Table 3: Sensory attributes of infusion prepared from non-fermented, partially-fermented, and fully-fermented betel leaves[1].

Attribute	Processing		
	Non-fermented	Partially-fermented	Fully-fermented
Colour	6.17 ± 0.75[a]	4.77 ± 1.17[b]	4.63 ± 1.27[b]
Turbidity	6.20 ± 0.76[a]	4.77 ± 1.19[b]	4.60 ± 1.28[b]
Aroma	6.17 ± 0.75[a]	4.73 ± 1.26[b]	4.67 ± 1.27[b]
Taste	6.17 ± 0.75[a]	4.73 ± 1.14[b]	4.60 ± 1.25[b]
Aftertaste	6.17 ± 0.75[a]	4.73 ± 1.26[b]	4.67 ± 1.27[b]
Overall acceptability	6.17 ± 0.75[a]	4.73 ± 1.14[b]	4.60 ± 1.25[b]

[1] Data are presented as mean ± standard deviation (n = 3). Mean values in the same row with different superscript letters are significantly different at $p < 0.05$.

CONCLUSION

In conclusion, the obtained results showed that the TPC, TFC, and antioxidant activity were directly proportional to the fermentation time, whereby the antioxidant activity was greater for the infusion made of non-fermented betel leaves. Moreover, during a longer fermentation period, the infusion obtained a less-clear and hazy brown color. Similarly, the results of the sensory evaluation revealed that non-fermented betel leaves infusion received the highest scores for all sensory attributes evaluated. Consequently, non-fermented betel leaves could be used to produce herbal tea of better quality in order to enhance one's bodily health due to their robust antioxidant activities.

CONFLICT OF INTEREST

All the authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Ho, L.-H. and Suhaimi, MA: wrote and reviewed the manuscript. Sangar, M: performed the experiment and wrote the manuscript.

REFERENCES

Aoshima H, Hirata S Ayabe S, 2007. Antioxidative and anti-hydrogen peroxide activities of various herbal teas. Food Chem 103(2): 617e622.

Ayu DF, Yamin M, Hamzah F, 2018. Antioxidant activity and quality of ketepeng cina (*Cassia alata* l.) herbal tea. Appl Sci Technol 2(1): 57–63.

Azli SNS, Bakar MFA, Sanusi SB, Awang-Kanak F, 2018. Nutritional, phytochemical, antioxidant activity and sensory attributes of herbal infusion from sukun (*Artocarpus altilis*) leaf. Proceedings, 3rd International Conference on Applied Science and Technology, 10–12 April 2018, Georgetown, Penang, Malaysia, pp 1–6.

Bae HA, Baek H, Park HI, Choung MG, Sohn EH, Kim SH, Kim DS, Chung IM, Seong ES, Yu CY, Lim JD, 2011. Effect of fermentation time on the chemical composition of mulberry (*Morus alba* L.) leaf teas. Korean J Med Crop Sci 19(4): 276–286.

Benzie IFF, Strain JJ, 1996. The ferric reducing ability of plasma (FRAP) as measure of 'antioxidant power': The FRAP assay. Anal Biochem 239:70–76.

Benzie IFF, Szeto YT, 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J Agric Food Chem 47(2): 633–636.

Chan EWC, Lim YY, Chong KL, Tan JBL, Wong SK, 2010. Antioxidant properties of tropical and temperate herbal teas. J Food Compos Anal 23: 185–189.

Chauhan ES, Aishwarya J, Singh A, Tiwari A, 2016. A review: nutraceuticals properties

of *Piper betel* (Paan). Ame. J Phytomed Cli Thera 49(2): 28–41.

Chen G, Mujumdar AS, 2015. Drying of herbal medicines and tea. In A. S. Mujumdar, eds, Handbook of Industrial Drying, Ed 3. CRC Press, Boca Raton, New York, pp 637–646.

Craig WJ, 1999. Health-promoting properties of common herbs. Am J. Clin Nutr 70(3): 491Se499S.

Engelharrt UH, 2010. Chemistry of Tea. In L. Mander and H. Liu, eds, Comprehensive Natural Products II: Chemistry and Biology. Elsevier Science and Technology, Oxford, United Kingdom, pp 999–1032.

Heong CS, Kaur, Bhupinder, Huda N, Karim AA, Fazilah A, 2011. Effect of Fermentation on the Composition of *Centella asiatica* Teas. Am J Food Technol 6: 581–593.

Ho CT, Osawa T, Huang MT, Rosen RT, 1994. Food phytochemicals for cancer prevention II: tea, spices & herbs. American Chemical Society, Washington, D.C.

Ho C-T, Zheng X, Li S, 2015. Tea aroma formation. Food Sci Hum Wellness. 4(1): 9–27.

Ho L-H, Paet M, Suhaimi MA, 2018. The physicochemical and sensory attributes of watermelon rind flour incorporated steamed cupcake. J Agrobiotech 9(1S): 31–42.

Ho L-H, Ramli NF, Tan T-C, Muhamad N, Haron MN, 2018. Effect of extraction solvents and drying conditions on total phenolic content and antioxidant properties of watermelon rind powder. Sains Malaysia 47(1): 99–107.

Jaiswal SG, Patel M, Saxena DK, Naik SN, 2014. Antioxidant properties of *Piper betel* (L) leaf extracts from six different geographical domain of India. J Biores Eng Technol 2(2): 12–20.

Joubert E, de Beer D, Malherbe CJ, 2017. Herbal teas—Exploring untapped potential and strengthening commercialisation. S Afr J Bot 110: 1–3.

Kumar S, Pandey AK, 2013. Chemistry and biological activities of flavonoids: an overview. Sci World J 2013: 1–16.

Kumari OS, Rao NB, 2015. Phyto chemical analysis of *Piper betel* leaf extract. World J Pharma. Pharm Sci 4(1): 699–703.

Lantano C, Rinaldi M, Cavazza A, Barbanti D, Corradini C, 2015. Effects of alternative steeping methods on composition, antioxidant property and colour of green, black and oolong tea infusions. J Food Sci Technol 52(12): 8276–8283.

Lin SD, Udompornmongkol P, Yang JH, Chen SY, Mau JL, 2014. Quality and antioxidant property of three types of tea infusions. J Food Process Preserv 38(4): 1401–1408.

Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 15: 127–130.

Morton PAJ, Murray BS, 2001. Acid beverage floc: protein-saponin interactions and an unstable emulsion model. Colloids Surf B Biointerfaces 21(1-3): 101–106.

Muhamad N, Sahadan W, Hoon HL, 2018. Effect of drying temperatures and extraction solvents on total phenolic, flavonoid contents and antioxidant properties of immature Manis Terengganu Melon (*Cucumis melo*). J Agrobiotech 9(1S): 114–121.

Muktar H, Ahmad N, 2000. Tea polyphenols: prevention of cancer and optimizing health. Am J Clin Nutr 71(6): 1698Se1702S.

Muruganandam L, Krishna A, Reddy J, Nirmala GS, 2017. Optimization studies on extraction of phytocomponents from betel leaves. Resource-Efficient Technologies 3(4): 385–393.

Nasir NM, Mohd KS, Ibrahim N, Rodi MMM, Zakaria AJ, 2019. Physicochemical analysis and antioxidant activity of honey from three Malaysian stingless bees species. J Agrobiotech 10(2): 1–11.

Ndife J, Uka NC, Ukom NA, 2019. Development and comparative evaluation of green and black tisanes using scent leaves (*Chromolaena odorata*). Food Res 3(5): 448–455.

Nor Qhairul Izzreen NQ, Mohd Fadzelly M, 2013. Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah tea plantation in Sabah, Malaysia. Int Food Res J 20(1): 307–312.

Patel N, Mohan JSS, 2017. Isolation and

characterization of potential bioactive compounds from *Piper betle* varieties Banarasi and Bengali leaf extract. Int J Herb Med 5(5): 182–191.

Patra B, Das MT, Dey SK, 2016. A review on *Piper betle* L. J Med Plants Stud 4(6): 185–192.

Periyanayagam K, Jagadeesan M, Kavimani S, Vetriselvan T, 2012. Pharmacognostical and phyto-physicochemical profile of the leaves of *Piper betle* L. Var Pachaikodi (Piperaceae) – valuable assessment of its quality. Asian Pac J Trop Biomed S506-S510.

Pradhan DK, Suri KA, Biswasroy P, 2013. Golden Heart of the Nature: *Piper betle L.* J. Pharmacogn Phytochem 1: 147–167.

Prakash D, Gupta KR, 2009. Antioxidant phytochemicals of nutraceutical importance. The Open Nutraceuticals Journal 2: 22–36.

Purba RAP, Paengkoum P, 2019. Bioanalytical HPLC method of *Piper betle* L. for quantifying phenolic compound, water-soluble vitamin, and essential oil in five different solvent extracts. J Appl Pharm Sci 9(05): 033–039.

Putri, TA, Farid Y, 2013. Total phenolic, flavonoids content and antioxidant activity of the ethanolic extract on betel leaf (*Piper betle* L.). International Conference in Nanotechnology, 26th October 2013, Jakarta.

Robertson A, 1992. The chemistry and biochemistry of black tea production–the non-volatiles. In K.C, Willson and M.N. Clifford, eds. Tea cultivation to consumption. Chapman and Hall, London, England, pp 555–602.

Sewald M, DeVries J, 2015. Food product shelf life. (http://www.medlabs.com/Downloads/food_product_shelf_life_web.pdf) Accessed 18 May 2020.

Suhaimi MA, 2020. Effects of drying conditions on physical, chemical and biological properties of betel (*Piper betle* L.) leaves powders and its application in lemongrass-scented betel tea. Master thesis. Universiti Sultan Zainal Abidin, Terengganu, Malaysia.

Watts BM, Ylimaki GL, Jeffery LE, 1989. Basic sensory method for food evaluation. The International Development Research Centre, Ottawa, Canada.

Xie M, Von Bohlen A, Klockenkaemper R, Jian X, Guenther K, 1998. Multielement analysis of Chinese tea (*Camellia sinensis*) by total-reflection X-ray fluorescence. Eur Food Res Tech 207: 31–38.