Food Research 4 (2) : 349 - 357 (April 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

The ramification of fermentation time on antioxidant properties of Napier grass herbal tea by black tea processing method

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Article history:
Received: 27 July 2019
Received in revised form: 26 September 2019
Accepted: 30 September 2019
Available Online: 9 October 2019

Keywords:
Napier grass,
Antioxidant properties,
Herbal black tea,
Fermentation

DOI:
https://doi.org/10.26656/fr.2017.4(2).265

Abstract

Tea fermentation relates to the enzymatic oxidation of the leaves of the tea and the formation of dark pigments. This method is usually conducted in a setting where temperature and humidity can be regulated. However, the impact of fermentation time on the antioxidant characteristics of Napier grass herbal tea is still unanswered. This study aimed to determine the effect of fermentation time on antioxidant properties of Napier grass black tea. Napier grass was subjected to fermentation for 3, 6, 9, 12 and 24 hrs. Fresh and dried samples were extracted in water (95°C, 30 mins) and the extracts were then analysed by total phenolic content (TPC) assay, total flavonoid content (TFC) assay, diphenyl-picryl-hydrazyl (DPPH) assay, Ferric reducing antioxidant potential (FRAP) assay, ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) method. Sample fermented for 9 hrs showed the highest results in TPC (18.32±0.38) and FRAP assay (91.00±2.78). Whereas, the highest flavonoids and antioxidant activity were found in dried sample without fermentation (control) in TFC (97.82±13.00) and DPPH (90.02±1.11). However, samples fermented for 3 to 9 hrs showed no significant difference with that of the fresh sample in TFC and DPPH indicating 3 to 9 hrs fermentation time did not affect the antioxidant properties of Napier grass. In FTC and TBA, all the fermented samples showed lower antioxidant activity than that of fresh samples and control. The results suggested that 9 h of fermentation time is suitable for the preparation of Napier grass black tea as it exhibited high antioxidant properties in TPC, TFC, DPPH and FRAP assays.

1. Introduction

Black tea is produced by the leaves of *Camellia sinensis* that have undergone fermentation. During fermentation, the significant flavonoids that are catechins in tea leaves are oxidized and dimerized to theaflavins or polymerized to thearubugins that are complicated condensation products (Leung *et al*., 2011). Tea fermentation includes enzymatic oxidation (oxidative fermentation process). When injury to the cell structure of the plant happens, the cell sap of the ruptured leaves allows response between the chemical constituents and the enzyme (PPO and PO) in the presence of atmospheric oxygen (Jolvis Pou, 2016). PPO and PO act on catechins in the presence of oxygen and oxidized polyphenolic compounds that are usually in dimer or polymer form. These polyphenolic molecules (theaflavins and thearubigins) add to the bitter taste and dark colour of black tea (Almajano *et al*., 2008; Jolvis Pou, 2016).

Herbal teas are popular globally owing to their antioxidant characteristics and distinctive fragrance, which are thought to have a calming impact on the mind (Ariffin *et al*., 2011). Previous studies indicated that the consumption of herbs or herbal teas could prevent disease and encourage excellent health (Korrapati *et al*., 2016; Magcwebeba *et al*., 2016). The health benefits of herbal tea consumption can be explained by their high content of phytochemical compounds which contributed to the antioxidant activity (Mukherjee *et al*., 2011; Fazeelath Banu, 2014; Im *et al*., 2014; Magcwebeba *et al*., 2016; Hardoko *et al*., 2018).

In Malaysia, there are numerous plant species with potential for commercial growth and those for business growth that has been used for food, industrial, cultural and medicinal reasons. However, there are still many

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eISSN: 2550-2166

underutilised plants (Arora, 2014). One of the underutilised plants is Napier grass (*Pennisetum purpureum*). In Malaysia, it is commonly used as silage for animal feeds due to its high yield potential and drought tolerance (Lowe *et al*., 2003; Nyambati *et al*., 2011). Napier grass is edible and is used as food in Nigeria (Akah and Ani, 2014; Akah and Onweluzo, 2014). High flavonoids content is found in Napier grass and it is also a potential source of various nutrients such as minerals, vitamins and dietary protein (Akah and Ani, 2014; Akah and Onweluzo, 2014; Ukpabi *et al*., 2015).

There are many studies on the impact of tea fermentation on different plant products such as *Clicanthus nutans* (Lee *et al*., 2016), *Cantella asiatica* (Ariffin *et al*., 2011), and *Ocimum tenuiflorum* (Rabeta and Lai, 2013). However, there is a lack of data on the impact of fermentation time on the antioxidant characteristics of Napier grass herbal tea. This research, therefore, seeks to explore the impact of fermentation time on the antioxidant characteristics of Napier grass herbal tea.

2. Materials and methods

Fresh Napier grass was obtained from Kuala Berang, Terengganu. The leaves were cleaned with tap water and then blotted with tissue towels to absorb the water. After that, the leaves were allowed to dry for 10 min in room temperature (25±1°C).

2.1 Preparation of Napier grass tea by black tea processing method with different fermentation time

Cleaned fresh Napier grass was subjected to the withering process at 30°C for 30 mins until 70% moisture content was obtained. A total of 50 g of the cut Napier grass (0.5 cm) was sprinkled with distilled water in 1:1 (w/v) ratio before undergoing oxidation-fermentation process (3, 6, 9, 12 and 24 hrs) at room temperature. Then, the sample was dried at the determined optimum temperature (50°C) for 7 hrs (< 6% moisture content). Dried Napier grass was ground and was sieved through a 1 mm metal sieve (Hafezi *et al*., 2006; Heong *et al*., 2011; Ma *et al*., 2014; Lusia Barek *et al*., 2015 with modifications).

2.2 Preparation of fermented Napier grass infusion (hot water extraction)

Dried Napier grass was ground using a waring blender (Waring Commercial, Torrington. CT, U.S.A). The ground material was sieved through a 1 mm metal sieve in order to achieve a standard size of particles. Larger particles that could not pass through the sieve were further ground. Ground samples were stored at -18° C before any further treatments. Extracts were prepared in the usual way of preparing hot tea beverage using the method from Lusia Barek *et al*. (2015) with some modifications. Two grams of powdered Napier grass was infused in 200 mL of heated distilled water (95°C) and continuously stirred for 2 mins using a magnetic stirrer. The infusion was left to cool for 30 mins of infusion time before filtration through a Whatman No. 1 filter paper. After cooling down to room temperature, tea infusions were stored at -20°C until analysis.

2.3 Preparation of fresh Napier grass infusion (hot water extraction)

Infusion of fresh Napier grass was used as a control in all experiments. Five grams of fresh Napier grass was ground using a waring blender (Waring Commercial, Torrington. CT, U.S.A). Then it was infused in 200 mL of hot distilled water (95°C) and stirred continuously for 2 mins using a magnetic stirrer. The infusion was left to cool for 30 min of infusion time before filtration through a Whatman No.1 filter paper twice, in order to further remove the debris. After cooling down to room temperature, tea infusions were stored at -20°C until analysis.

2.4 Antioxidant properties assays

2.4.1 Determination of total phenolic content (TPC)

Folin Ciocalteu's method was used to measure the TPC. The aliquots (1 mL) and gallic acid with different concentrations (10, 20, 40, 60, 80, 100 µg/mL) were transferred into the test tubes. Then, 5 mL of distilled water and 0.5 ml of Folin Ciocalteu's reagent were added into each test tube and shaken. After 5 mins, 1.5 mL of 20% (m/v) sodium carbonate was added and the volume was made up to 10 mL with distilled water. The samples were incubated for 2 hrs at room temperature. After incubation, absorbance was measured at 750 nm using UV-visible spectrophotometer (SHIMADZU, Kyoto, Japan). The data for TPC of tea infusion was expressed as mg of gallic acid equivalent weight (GAE)/200 ml infusion (Kamtekar *et al.*, 2014).

2.4.2 Determination of total flavonoid content (TFC)

Aluminium chloride colorimetric assay was used to measure the TFC. One milligram of aliquots and 1 mL quercetin with different concentrations (100, 200, 400, 600, 800, 1000 µg/mL) were added into test tubes. Then, 4 mL of distilled water and 0.3 mL of 5% (m/v) sodium nitrite solution was added into each test tubes. After 5 mins, 0.3 mL of 10% (m/v) aluminium chloride was added. A total of 2 mL of 1 M sodium hydroxide was added at 6th min. Finally, with distilled water, volume was produced up to 10 mL. The absorbance was measured at 510 nm using UV-visible spectrophotometer

eISSN: 2550-2166

(SHIMADZU, Kyoto, Japan). The data of TFC of tea infusion was expressed as mg of quercetin equivalents (QE)/200 mL infusion (Kamtekar *et al*., 2014).

2.4.3 Free radical scavenging ability of DPPH radical (1,1-diphenyl-2-picryl hydrazyl)

DPPH test was performed using the method of Barku *et al.* (2013) with some modifications. An aliquot of 2 mL of 0.004% (m/v) DPPH solution in methanol and 1 ml of plant extract were incubated at 25°C for 45 mins. The absorbance of the test mixture was read at 517 nm using a UV-visible spectrophotometer (SHIMADZU, Kyoto, Japan) against a DPPH control containing only 1 mL of distilled water in place of the extract. Percent inhibition was calculated using the following expression:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

Where A_0 and A_t is the absorption of the blank sample and absorption of tested extract solution, respectively.

2.4.4 Ferric reducing antioxidant potential (FRAP) assay

The FRAP reagent was prepared by 300 mM sodium acetate buffer (pH 3.6), 20 mM iron chloride and 10 mM 2,4,6-tripyridyl-s-triazine dissolved in 40 mM hydrochloric acid at a ratio of 10:1:1 (v:v:v). After mixing, the reagent was allowed to incubate at 37°C for 5 mins before use. The initial reading of the reagent was measured at 593 nm using a UV-visible spectrophotometer (SHIMADZU, Kyoto, Japan). An aliquot of 0.1 mL of tea infusion was then added to 2.9 mL of FRAP reagent and kept in the dark for 30 mins. Trolox solution was used to create the calibration curves. Results were expressed as µmol Trolox equivalents (TE)/200 mL infusion (Benzie and Strain, 1996; Heong *et al*., 2011).

2.4.5 Ferric thiocyanate (FTC) assay

The inhibitory effect of the plant against oxidation by peroxides was evaluated by the modified method of Udaya Prakash *et al*. (2014). A total of 2 mL of 2.51% (v/v) linoleic acid in ethanol, 120 µL of 98% ethanol and 9 mL of 40 mM phosphate buffer (pH 7) were added to 100 µL of the plant extract. The mixture was incubated in dark, at 40°C. To 100 µL of the mixture, 9.7 mL of 75% (v/v) ethanol, 100 µL of 30% (m/v) ammonium thiocyanate and 100 µL of 20 mM FeCl3 in 3.5% (v/v) HCl were added. The absorbance of the solution was measured at 500 nm, after 3 mins. Butylated hydroxytoluene (BHT) (200 ppm) and α-tocopherol (200 ppm) were used as standards. The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

Where A_0 and A_t stand for absorption of the blank sample and absorption of tested extract solution, respectively.

2.4.6 Thiobarbituric acid (TBA) assay

Thiobarbituric acid assay was evaluated by modified method of Udaya Prakash *et al*. (2014). Two millilitres each of 20% (m/v) trichloroacetic acid (TCA) and 0.67% (m/v) thiobarbituric acid (TBA) were mixed with 1 mL sample. The solution was heated in boiling water bath for 10 mins. After cooling, the solution was centrifuged at 3000 rpm. The absorbance of the supernatant was measured at 532 nm. Butylated hydroxytoluene (BHT) (200 ppm) and α-tocopherol (200 ppm) were used as standards. The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

Where A_0 and A_t is the absorption of the blank sample and absorption of tested extract solution, respectively.

2.5 Statistical analysis

Data were expressed as means ± standard deviation (SD) of triplicates. All data were submitted to one-way analysis of variance (ANOVA) using SPSS (version 20) software. The values were considered to be significantly different when $P<0.05$. The correlations among antioxidant assays were calculated using the Pearson's correlation coefficient.

3. Results and discussion

3.1 Determination of total phenolic content (TPC)

The findings in Figure 1 show that TPC of Napier grass was significantly affected by fermentation time. The highest TPC was found in Napier grass undergone 9 hrs fermentation which was 18.32±0.38 whereas the lowest TPC, 14.12±0.32 was found in Napier grass fermented for 24 hrs. Sample fermented for 9 hrs was also significantly higher than that of fresh Napier grass (16.71±0.15) and Napier grass without undergoing fermentation (15.37±0.11).

The increase of TPC of fermented Napier grass at 9 hrs fermentation might be caused by the structural change of the phenolic compounds. During tea fermentation, galloyl groups of EGCG and ECG were cleaved and resulted in increase of free gallic acid (Kim *et al*., 2011). Besides flavonoids, there are other phenolic compounds in plants such as phenolic acids, tannins, stillbenes and lignans (Dai and Mumper, 2010). These phenolic compounds might also contribute to the result of TPC of Napier grass.

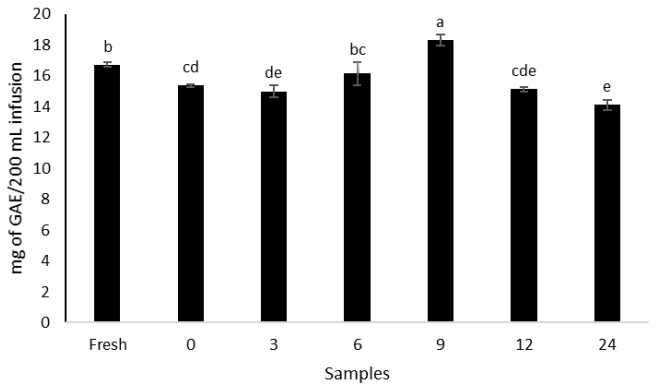

Figure 1. TPC of infusions of Napier grass undergone different fermentation times. The results are presented as mean ± SD. Values with similar letters are insignificantly different at $p<0.05$, (n = 3).

TPC of the plant sample increased at a certain extent of fermentation was also shown in the study by Lee *et al*. (2016). The study also revealed that the time of tea fermentation could affect the TPC of *C. nutans*. In their study, the optimum fermentation time of *C. nutans* was 12 hrs as higher TPC was found in 12 hrs fermented sample compared to that of other samples which had undergone 6, 24, 48 and 72 hrs fermentation, respectively.

In contrast, TPC of Napier grass was significantly reduced at 12 hrs and 24 hrs fermentation which were 15.13±0.13 and 14.12±0.32, respectively. Low TPC of fermented samples might be caused by the formation of colour and flavour compounds during fermentation process that decreased the concentration of polyphenol (Rabeta and Lai, 2013). There are studies showed that fermentation causes decrease in TPC of plant samples. TPC of *Centella asiatica* herbal tea undergone 24 h fermentation was found to be lower than that of non-fermented one (Ariffin *et al*., 2011). The fermented *C. nutans* sample was found to have lower TPC compared to that of *C. nutan* sample which undergone drying only indicates that certain phenolic compounds might be converted or degraded during fermentation process (Lee *et al*., 2016).

3.2 Determination of total flavonoid content (TFC)

The result (Figure 2) shows that the TFC of Napier grass was significantly affected by fermentation time. The highest TFC was found in dried Napier grass without fermentation which was 97.82±13.00 mg of QE/200 mL infusion. The dried sample without fermentation was dried within the shortest period possible after washing and cutting. Immediate drying of the sample terminate the enzymatic reaction and helps to retain the flavonoid compounds of the sample. Drying reduces the water activity of the sample and therefore halt the enzymatic activity (Mediani *et al*., 2014).

There is no significant difference between fresh Napier grass and samples undergone 3 hrs (80.27±1.16), 6 hrs (73.38±4.91) and 9 hrs (70.93±7.57) fermentation indicating 3 to 9 hrs fermentation did not affect the TFC of Napier grass. These findings were supported by Ariffin *et al*. (2011) who reported that although TPC of *C. asiatica* herbal tea was significantly reduced after fermentation, TFC of *C. asiatica* was not significantly affected by the fermentation time (0, 2 and 24 h).

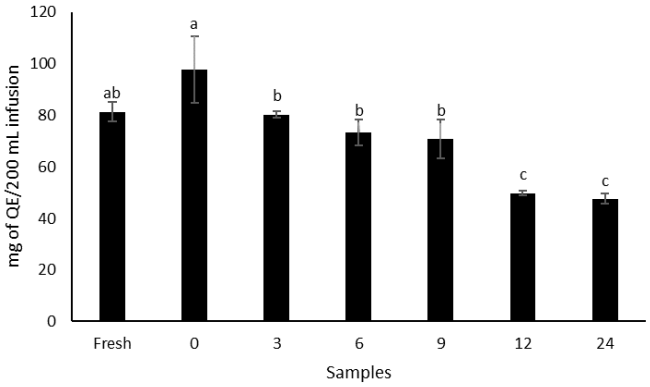

Figure 2. TFC of infusions of Napier grass undergone different fermentation times. The results are presented as mean ± SD. Values with similar letters are insignificantly different at $p<0.05$, (n = 3).

TFC of Napier grass was significantly reduced at 12 hrs and 24 hrs fermentation which were 49.82±1.02 and 47.60±2.00, respectively. The reduction of TFC might be caused by the conversion of catechins into theaflavins and thearubigin (Yuniartini *et al*., 2015). TFC was found to decrease along with fermentation time was also reported previously by Kim *et al*. (2011).

3.3 Free radical scavenging ability of DPPH radical (1,1 -diphenyl-2-picryl hydrazyl)

The results (Figure 3) showed that there were significant changes in the percentage of inhibition of DPPH free radical scavenging activity of Napier grass undergone different fermentation time. Similar to the result of the TFC, dried sample without fermentation was found to have the highest antioxidant activity which was 90.02±1.11. There is no significant change between fresh Napier grass (84.11±0.49) and samples undergone 3 hrs (84.07±2.24), 6 hrs (83.58±2.37) and 9 hrs (82.29±2.72) fermentation indicating that 3 to 9 hrs fermentation did not affect the antioxidant activity of Napier grass. The similarity of the results of TFC and DPPH assay indicates the contribution of flavonoids as free radical scavengers. The phenolic compounds in plants play an important role in redox reaction as reducing agents, hydrogen donors and singlet oxygen scavengers (Lee *et al*., 2016). The hydroxyl groups attached to aromatic ring allow the flavonoids to involve in a redox reaction,

which in turn, enables them to scavenge the free radicals (Lee *et al*., 2016).

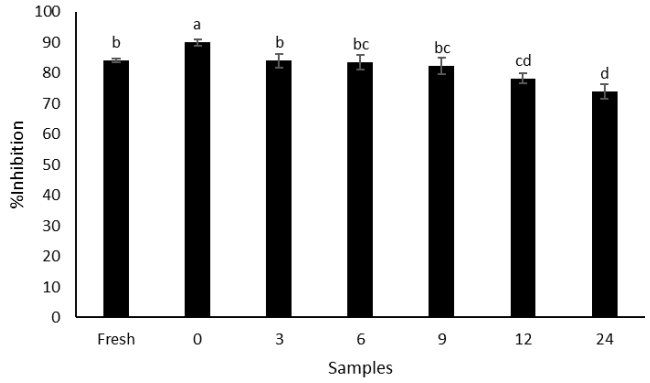

Figure 3. Percentage of inhibition of DPPH free radical scavenging activity of infusions of Napier grass undergone different fermentation times. The results are presented as mean ± SD. Values with similar letters are insignificantly different at $p<0.05$, (n = 3).

However, the antioxidant activity was significantly reduced at 12 hrs and 24 hrs fermentation which was 78.24±1.61 and 73.92±2.36, respectively. The reduction of antioxidant activity might be caused by the degradation of flavonoids as a result of long hours of fermentation. Previous studies had reported the effect of fermentation on antioxidant activity of various plant samples. Lee *et al*. (2016) reported that the longer the fermentation time of *C. nutans* the lower is the antioxidant activity. Fermented *C. asiatica* was reported to have significantly lower antioxidant activity than that of non-fermented sample (Ariffin *et al*., 2011). The antioxidant activity of guava leaves undergone 100 min fermentation was significantly lower than that of samples prepared by green tea processing method which undergone steaming process (Yuniartini *et al*., 2015).

3.4 Ferric reducing antioxidant potential (FRAP) assay

The findings (Figure 4) showed that ferric reducing potential of Napier grass was significantly affected by fermentation time. The highest antioxidant activity was obtained by sample underwent 9 hrs fermentation which was 91.00±2.78, whereas the lowest antioxidant activity was found in sample fermented for 24 hrs which was 54.84±2.67. Sample fermented for 9 hrs was also significantly higher than that of fresh Napier grass (71.31±1.30) and dried Napier grass without fermentation (66.62±2.77).

The significant increase of antioxidant activity of Napier grass fermented for 9 hrs might be caused by the high TPC of the sample. This is because the result of FRAP assay may be related partially to phenolic and flavonoid compounds of the sample. However, FRAP assay is not able to detect compounds such as thiols and proteins that exercise their effect by radical quenching (Rabeta and Lai, 2013). Moreover, although phenolic compounds contribute to the antioxidant activity of plant, besides the level of antioxidants, total antioxidant capacity of plant also depends on the possible synergistic interactions between the different constituents in plant (Rabeta and Lai, 2013).

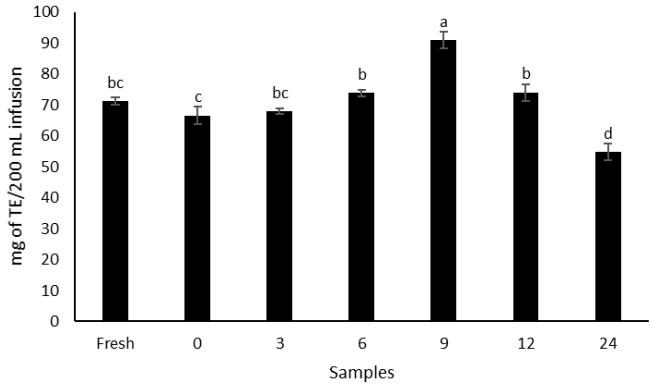

Figure 4. Ferric reducing the antioxidant potential of infusions of Napier grass undergone different steaming time. The results are presented as mean ± SD. Values with similar letters are insignificantly different at $p<0.05$, (n = 3).

The drastic reduction of antioxidant activity of Napier grass at 24 hrs fermentation indicated that long hours of fermentation causes adverse effects on the antioxidants of Napier grass. This assumption was supported by Ariffin *et al*. (2011) who reported that the antioxidant activity of *C. asiatica* was significantly reduced after 2 hrs and 24 hrs fermentation.

The contrary to the results of DPPH and FRAP assay can be explained by the differences in what each method measures in evaluating antioxidant activity (Rabeta and Lai, 2013).

3.5 Ferric thiocyanate (FTC) assay

FTC assay was used to measure the level of peroxide formed at the beginning of lipid peroxidation. The reaction of peroxides with ferrous chloride causes the formation of ferric ion. Then, the ferric ion combines with ammonium thiocyanate and form red coloured ferric thiocyanate (Zahin *et al*., 2009). The result of the FTC assay (Figure 5) shows that lipid peroxidation inhibition of Napier grass was significantly affected by fermentation time.

Among the Napier grass samples, fresh Napier grass (65.25±1.61) and dried Napier grass without fermentation (66.57±0.61) obtained significantly higher antioxidant activity than that of the samples fermented for 3 to 24 hrs (48.45±2.43 to 25.53±3.75). Drastic decrease of antioxidant activity was found in the samples fermented for 12 hrs (29.78±1.38) and 24 hrs (25.53±3.75). Low antioxidant activity of fermented Napier grass indicates the degradation of antioxidants as

a result of fermentation (Ariffin *et al*., 2011; Lee *et al*., 2016).

All the samples obtained significantly lower antioxidant activity than that of positive controls which were α-tocopherol (77.60±2.20) and BHT (78.70±0.23), respectively. α-tocopherol and BHT are lipophilic and are effective in preventing lipid oxidation in foods (Brewer, 2011). Therefore, the result of FTC assay might also be affected by the lipophilicity of phenolic compounds of the plant extract, whereas the lipophilicity of phenolic compounds was affected by the extraction method. According to the study by Materska (2010), ethanolic plant extracts were found to have high amount of less polar phenolic compounds. In our study, water was used as extraction media because chemicals such as methanol and ethanol which are popular media for phenolic compounds extraction are not suitable for food preparation due to the fact that tea is consumed as a water infusion (Ariffin *et al*., 2011). Therefore, there might be lesser lipophilic phenolic compounds were extracted from the Napier grass infusion and resulted in less efficiency of protection on the lipid from oxidation.

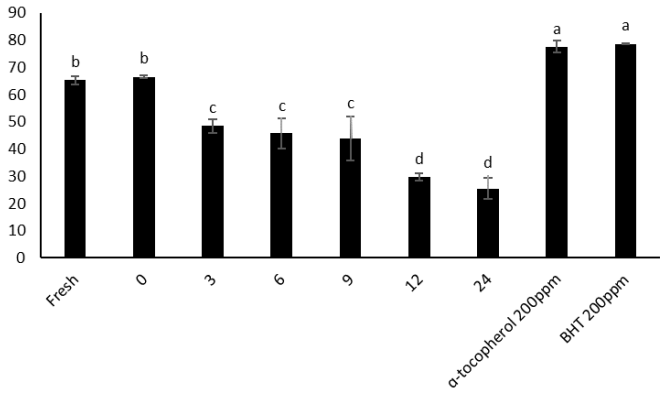

Figure 5. Lipid peroxidation inhibition of infusions of Napier grass undergone different fermentation times by FTC assay. The results are presented as mean ± SD. Values with similar letters are insignificantly different at $p<0.05$, (n = 3).

3.6 Thiobarbituric acid (TBA) assay

TBA assay was used to measure the secondary product, malondialdehyde (MDA) produced from the decomposition of peroxide (Kikuzaki and Nakatani, 1993; Zahin *et al*., 2009). Figure 6 shows the lipid peroxidation inhibition of samples against the secondary stage of lipid peroxidation. The findings show that the lipid peroxidation inhibition of Napier grass was significantly affected by the fermentation time.

Fresh Napier grass (78.98±10.14), dried sample without fermentation (78.16±0.96) and positive controls which were α-tocopherol (89.20±1.34) and BTH (90.07±1.41) were found to have no significant difference. However, all the samples fermented for 3 to 24 hrs (65.27±3.59 to 42.39±2.74) had significantly lower antioxidant activity compared to that of positive controls. The low inhibition might be caused by the degradation of antioxidants such as flavonoids during fermentation. TFC was reduced with increased fermentation time (Kim *et al*., 2011). Besides, the lipophilicity of the phenolic compounds might also affect the peroxide inhibition of the samples. As discussed in FTC assay, α-tocopherol and BHT are lipophilic and exhibit stronger antioxidant activity in emulsions as the sample concentrate at the lipid/air surface (Kulišić *et al*., 2006; Brewer, 2011).

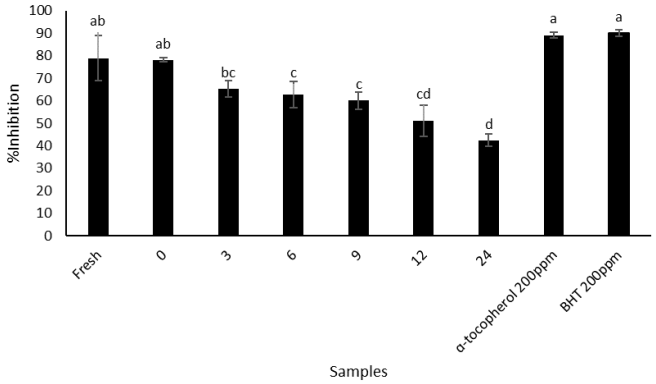

Figure 6. Lipid peroxidation inhibition of infusions of Napier grass undergone different steaming times by TBA assay. The results are presented as mean ± SD. Values with similar letters are insignificantly different at $p<0.05$, (n = 3).

In general, the results of peroxide inhibition of TBA was higher than that of FTC assay. Similar results were found by Zahin *et al*. (2009) indicating the peroxide level in the initial stage of lipid peroxidation is lesser than that of the secondary stage. In addition, besides MDA, the sensitivity of TBA assay was also affected by other lipid oxidation products such as alkenals, alkadienals, other aldehydes and ketones. These compounds cause the overestimation of MDA values in mechanically debone chicken meat and frankfurter samples by more than 25% and 27%, respectively, compared to RP-HPLC method (Reitznerová *et al*., 2017).

In contrast, Aris *et al*. (2009) reported that the peroxide inhibition of the hexane, chloroform and methanol extracts of fruits of *Ficus deltoidea* in FTC assay was higher than that of TBA assay.

4. Conclusion

Fermentation time showed a significant effect on antioxidant properties of Napier grass based on TPC, TFC, DPPH, FRAP, FTC and TBA assays. TPC and FRAP of Napier grass were significantly increased at 9 hrs fermentation. All the fermented samples showed significantly lower TFC than that of dried unfermented Napier grass. However, the TFC of 3, 6 and 9 hrs

fermented samples showed no significant change with that of fresh sample indicating 3 to 9 hrs fermentation time did not affect the flavonoid content of Napier grass. Similarly, all the fermented samples (73.92±2.36 to 84.07±2.24) showed significantly lower antioxidant activity than that of unfermented Napier grass (90.02±1.11) in DPPH assay but there is no significant difference between samples fermented for 3 hrs (84.07±2.24) to 9 hrs (82.29±2.72) and fresh sample (84.11±0.49). The findings of TFC and DPPH showed that 3 to 9 h fermentation time did not affect the flavonoid content and radical scavenging activity of Napier grass. All the fermented samples showed low antioxidant activity in FTC (25.53±3.75 to 48.45±2.43) and TBA (42.39±2.74 to 65.27±3.59) assays. The overall results showed that 9 hrs fermentation time was suitable for the preparation of Napier grass black tea as it exhibited high antioxidant properties in TPC, TFC, DPPH and FRAP assays.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This research was supported by Universiti Malaysia Terengganu. The authors would like to thank the UMT's central lab and PPSTM for the facilities provided to conduct the study.

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eISSN: 2550-2166