

## Effect of drying temperatures on antioxidant properties of Napier grass (*Pennisetum purpureum*)

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### Abstract

This study was carried out to determine the effect of oven drying temperatures on antioxidant properties of Napier grass. Fresh samples of Napier grass were oven dried at 50, 60, 70, 80 and 90 °C for 7 h. Fresh and dried samples were extracted with water (95 °C, 30 min) and the extracts were analysed for 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. The data show significant changes in TPC and TFC of Napier grass. DPPH radical scavenging activity of all the samples were significantly increased after drying. Ferric reducing potential of fresh Napier grass extract ( $71.31 \pm 1.30$ ) and samples dried at 50 °C ( $66.62 \pm 2.77$ ) and 90 °C ( $65.58 \pm 5.98$ ) were not significant. Sample dried at 50 °C showed no significant difference with that of fresh Napier grass extract in FTC and TBA assay indicating it is a recommended drying temperature in preserving antioxidants. The results suggested that Napier grass extract possess high antioxidant properties and it can be potent natural antioxidants. It also shows that sample dried at 50 °C have the greatest antioxidant properties.

**Keywords:** Antioxidant activity, Oven drying, *Pennisetum purpureum*

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## Introduction

*Pennisetum purpureum*, also known as Napier grass, is a tall, fast growing perennial grass. It is originated from Africa in 1913 and can be found throughout the wet tropics of the world (Rao et al., 2007; Reddy, 2017). In Malaysia, it is considered as a popular weed that commonly found along the roadsides, wastelands and housing areas (Norhafizah et al., 2012). Napier grass is widely used as silage for animal feeds because

of its drought tolerance and high yield potential as it can withstand repeated cutting and rapidly regenerates (Lowe et al., 2003; Nyambati et al., 2011). Studies show that high flavonoids content is found in Napier grass and it is a potential source of minerals, vitamins and dietary protein (Akah and Ani, 2014; Akah and Onweluzo, 2014; Ukpabi et al., 2015). Therefore, this plant was selected for this study.

Natural antioxidant from phytochemical of plants may inhibit the production of free radicals (Moukette et al.,



2015). The compounds contribute to the antioxidant activity in plants are phenolic compounds (such as flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids (Dubey et al., 2014). Phenolic compounds could be a major determinant of antioxidant potentials of foods (Balasundram et al., 2006). Flavonoids are secondary plant metabolites. Based on their variations in the heterocyclic C-ring, flavonoids can be classified into six subclasses which are flavones, flavonols, flavanones, flavanols, isoflavones and anthocyanidins (Hollman, 2004). Adequate consumption of dietary antioxidants could help to increase the protection against free radicals, prevent diseases and promote health benefits (Alam et al., 2013). Previous studies have reported thermal processing such as drying could affect the antioxidant properties of plant materials (Katsube et al., 2009; Jihène et al., 2013; Mediani et al., 2013; Sagrin and Chong, 2013).

Drying is one of the food nutrients preserving methods, by removing the moisture content of food that is needed by bacteria, yeast and mould growth. Freshly harvested raw foods contain the highest amount of nutrients. However, it begins to drop during the handling process. Therefore, it is important to dry the food right after it is harvested (Clement et al., 2017). Besides preserving foodstuffs by reducing the moisture content, drying is done to increase the macronutrient content, decrease the weight and volume, reduce packaging requirements, storage and transportation costs (Reis et al., 2013; Youssef and Mokhtar, 2014; Pham et al., 2015). Oven drying is an effective way to dehydrate food as it inactivates enzyme rapidly (Bernard et al., 2014). Antioxidant capacity, stability of bioactive compounds and sensory quality were significantly affected by drying (Pham et al., 2015). Moreover, no previous study had reported the optimum drying temperature for Napier grass. Therefore, it is interesting to study the optimum drying temperature of Napier grass for further processing steps. The objective of this study was to determine the effect of temperature on antioxidant properties such as TPC, TFC, DPPH radical scavenging activity, ferric reducing potential and inhibition of peroxide of Napier grass.

## Material and Methods

Fresh Napier grass was purchased from Kuala Berang, Terengganu. The leaves were cleaned with tap water to clean the soil and dust. After that, cleaned Napier

grass were blotted with tissue towels and allowed to dry for 10 min at room temperature ( $25 \pm 1$  °C).

### 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 min 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. After cooling down to room temperature, tea infusions was stored at -20 °C until analysis.

### Preparation of dried samples

Fifty grams of the cut Napier grass (0.5 cm) was oven dried at different temperatures, namely 50, 60, 70, 80 and 90 °C for 7 h. Seven hours of drying time was used because more than 7 h of drying time can increase the loss of antioxidant activity (Sagrin and Chong, 2013).

### Preparation of dried 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. The larger particles that could not pass through the sieve was 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 min 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. After cooling down to room temperature, tea infusions were stored at -20 °C until analysis.

## Antioxidant properties

### Determination of total phenolic content (TPC)

Folin Ciocalteu's method was used to measure the TPC. One millilitre of aliquots and gallic acid with different concentrations (10, 20, 40, 60, 80, 100 µg/ml) was 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 min, 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 h 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).

#### **Determination of total flavonoid content (TFC)**

Aluminium chloride colorimetric assay was used to measure the TFC. One milligram of aliquots and 1ml 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 were added into each test tubes. After 5 min, 0.3 ml of 10 % (m/v) aluminium chloride was added. At 6<sup>th</sup> min, 2 ml of 1 M sodium hydroxide was added. Finally, volume was made up to 10 ml with distilled water. The absorbance was measured at 510 nm using UV-visible spectrophotometer (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).

#### **Free radical scavenging ability by the use of stable DPPH radical (2,2,-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 min. 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  $A_t$  stand for absorption of the blank sample and absorption of tested extract solution, respectively.

#### **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 min 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 min. 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).

#### **Ferric thiocyanate assay**

The inhibitory effect of the plant against oxidation by peroxides was evaluated by modified method of Udaya Prakash (2014). Two milliliters 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 FeCl<sub>3</sub> in 3.5 % (v/v) HCl were added. The absorbance of the solution was measured at 500 nm, after 3 min. 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.

#### **Thiobarbituric acid assay**

Thiobarbituric acid assay was evaluated by modified method of Udaya Prakash (2014). Two milliliters 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 min. 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  $A_t$  stand for absorption of the blank sample and absorption of tested extract solution, respectively.

#### **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  $P < 0.05$ .

## Results and Discussion

### Determination of total phenolic content (TPC)

Table 1 shows the average TPC of oven dried Napier grass. TPC of Napier grass was significantly affected by the drying temperature. All the dried Napier grass samples were found to have lower TPC than that of fresh Napier grass ( $16.71 \pm 0.15$  mg of GAE/200ml infusion), it ranged from  $13.72 \pm 2.01$  to  $16.66 \pm 0.55$  mg of GAE/200 ml infusion. This result was in accordance to numerous previous studies which reported that drying temperature could affect the TPC in plant materials (Katsube et al., 2009; Jihène et al., 2013; Sagrin and Chong, 2013). [Table 1]

Napier grass dried at  $50^{\circ}\text{C}$  ( $15.37 \pm 0.11$  mg of GAE/200 ml infusion) shows slightly but not significantly lower TPC than that of raw sample. However, there is a drastic decrease of TPC in sample dried at  $60^{\circ}\text{C}$  ( $13.72 \pm 2.01$  mg of GAE/200 ml infusion). The reduction of TPC might be caused by the formation of complexes or reaction of phenolics to other components in the sample such as metals and proteins (Jihène et al., 2013; López et al., 2013; Sagrin and Chong, 2013; Sharma et al., 2015; Barimah et al., 2017). The alteration of chemical structure caused by drying could also result in the loss phenolic content (Sagrin and Chong, 2013).

In contrast, TPC of sample was increased significantly at  $90^{\circ}\text{C}$ . High temperature could cause the conversion or increased the release of glucosides or bound phenolics into free phenolic derivatives and thus increase the TPC (Sharma et al., 2015). Phenolic acids may present in plants as glycosides or esters with other natural components such as sterols, alcohols, glucosides and hydroxyfatty acids (Ghasemzadeh and Ghasemzadeh, 2011). Level of phenolic compound of sample dried at  $90^{\circ}\text{C}$  was not significantly different with that of fresh sample indicating high TPC. High phytochemical content of the dried plant materials can be attributed to the inactivation of enzymes (Bernard et al., 2014). Enzymatic oxidation of phenolic compounds is caused by the damage or injury of cell structure of plants. Damaged cell membrane liberates and activates enzymes which oxidise the phenolic compounds to quinones (Reis et al., 2013). Besides, in the study by Planinić et al. (2015) assumed that

prolonged drying of grape pomace regardless of the drying temperature ( $60^{\circ}\text{C}$  to  $80^{\circ}\text{C}$ ) may result in the formation of new compounds such as Maillard products which could react with Folin-Ciocalteu reagent and cause the inaccuracy of the result. The overall finding is in accordance with a study by López et al. (2013) which revealed that TPC of golden berries was dropped at  $50^{\circ}\text{C}$  but increased at  $70^{\circ}\text{C}$  to  $90^{\circ}\text{C}$  of drying temperature. They reported that highest TPC was found in the sample dried at  $90^{\circ}\text{C}$ . Increase in TPC at  $90^{\circ}\text{C}$  might be caused by the non-enzymatic interconversion between phenolic molecules which increased the availability of phenolic precursor molecules (López et al., 2013).

However, this result was in contrast with the study by Pham et al. (2015) who revealed that increasing temperature from  $80^{\circ}\text{C}$  to  $90^{\circ}\text{C}$  caused a significant decline in phenolic contents in *Helicteres hirsute* Lour. leaves due to the degradation of antioxidant components. Plant species and cell wall stability were also the factors that influence the effect of drying on TPC (Barimah et al., 2017). A research by Jihène et al. (2013) demonstrated that drying ginger at different temperatures ( $40$ ,  $50$ ,  $60$ , and  $70^{\circ}\text{C}$ ) lead to loss of TPC at higher temperature due to thermal degradation.

### Determination of total flavonoid content (TFC)

Table 1 shows the average of TFC of oven dried Napier grass. TFC of oven dried Napier grass varied from  $49.16 \pm 8.23$  to  $97.82 \pm 13.00$  mg of QE/200 ml infusion. This result shows that TFC was significantly affected by drying temperature. It is in agreement with the study by Kessy et al. (2016) which revealed that litchi pericarps undergone hot air drying at temperatures exceeding  $60^{\circ}\text{C}$  had a significant loss of TFC caused by thermal degradation. [Table 1]

Napier grass undergone oven drying at  $50^{\circ}\text{C}$  showed the highest TFC. A study by Pham et al. (2015) reported that greatest yield of TFC was retained in the mulberry leaf vacuum dried at  $50^{\circ}\text{C}$  compared to other drying conditions which range between  $30^{\circ}\text{C}$  and  $90^{\circ}\text{C}$  with drying time in the range of 2 h to 19 h. The decline of TFC at  $80^{\circ}\text{C}$  ( $49.46 \pm 8.23$  mg of QE/200 ml infusion) was in agreement with the previous study which reported that certain temperature caused damage to some flavonoids depends on their structure (Sharma et al., 2015). The reduction of TFC at higher temperature was caused by the degradation of flavonoids (Sharma et al., 2015). However, there is a slight increase of TFC at  $90^{\circ}\text{C}$  ( $59.16 \pm 8.95$  mg of QE/200 ml infusion).



**Table 1. Effect of oven drying at different temperature on the antioxidant properties of Napier grass (n = 3 ± S.D)**

Samples	Raw	50°C	60°C	70°C	80°C	90°C
<b>TPC (mg of GAE/200 ml infusion)<sup>A</sup></b>	16.71 ± 0.15 <sup>a</sup>	15.37 ± 0.11 <sup>ab</sup>	13.72 ± 2.01 <sup>b</sup>	14.28 ± 0.80 <sup>ab</sup>	14.22 ± 0.37 <sup>ab</sup>	16.66 ± 0.55 <sup>a</sup>
<b>TFC (mg of QE/200 ml infusion)<sup>B</sup></b>	81.38 ± 3.85 <sup>ab</sup>	97.82 ± 13.00 <sup>a</sup>	83.16 ± 9.44 <sup>ab</sup>	78.27 ± 8.11 <sup>ab</sup>	49.16 ± 8.23 <sup>c</sup>	59.16 ± 8.95 <sup>bc</sup>

\*Letters followed the same letter are not statistically significant from each other at P > 0.05; means ± standard deviation; <sup>A</sup> Gallic acid equivalents; <sup>B</sup> Quercetin equivalents.

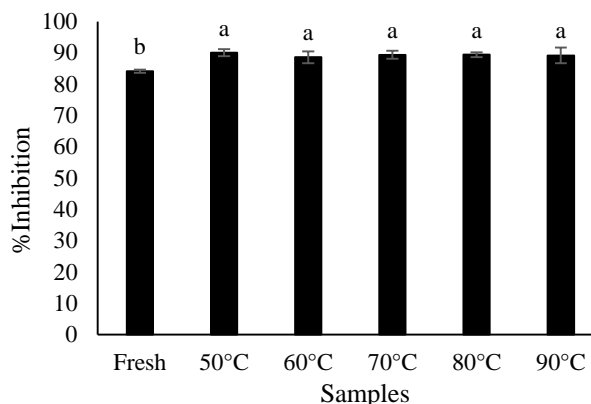
Increase of TFC of goldenberry dehydrated at 90 °C was also observed in the study by López et al. (2013). The results obtained from TFC assay was different from that of TPC assay. This is supported by a previous study by Madrau et al. (2009) who reported that although flavonoids are naturally occurring phenolic compounds, the degradation mechanisms of flavonoids and phenolic acids are different. Different from hydrocinnamic acids which are non-flavonoid polyphenols and catechins which are present as aglycones and oligomers in diet, most of the flavonoids are in the form of β-glycosides, the degradation of flavonols is not directly correlated to polyphenol oxidase (PPO) activity as PPO does not act directly on the glycosides (Hollman, 2004; Madrau et al., 2009). The flavonols compounds degrade with increasing temperature (Madrau et al., 2009). The data obtained was also supported by Sharma et al. (2015) who reported that although TFC of onion varieties decreased after heating at a certain temperature indicating some flavonoids were destroyed, TPC of onion varieties were increased. Formation of monomers by hydrolysis of C-glycosides bonds of flavonoid dimers or oligomers might increase the TPC (Sharma et al., 2015).

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

Figure 1 shows that there is no significant difference between the samples undergone oven drying at different temperatures in DPPH free radical scavenging activity. This result is in contrast with that of FRAP which showed that antioxidant activity of Napier grass is significantly affected by oven drying temperature. However, the inhibition of DPPH radical scavenging activity of all the dried samples (88.58 ± 1.90 to 90.02 ± 1.11) were significantly higher than that of raw Napier grass extract (84.11 ± 0.49). [Figure 1].

All the antioxidant activity of dried samples were significantly higher than that of fresh Napier grass. This is in agreement with a research by Sharma et al.

(2015) who reported that antioxidant activity of onion was increased at higher temperatures.



**Fig. 1. Percentage of inhibition of DPPH free radical scavenging activity of infusions of fresh Napier grass and Napier grass undergone different drying temperatures**

Result from triplicate analysis, a-b means sample with the same letters for each histogram are not statistically significant different at p > 0.05.

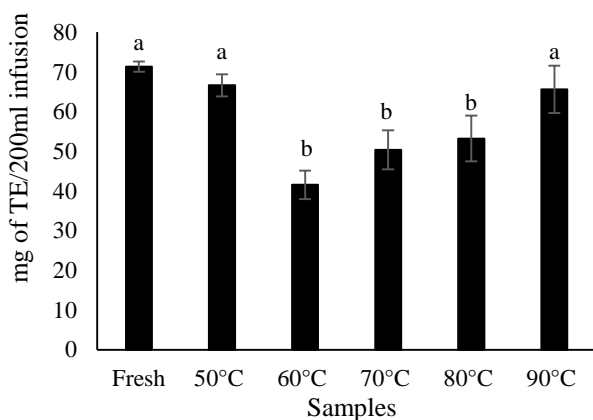
Previous findings revealed that heating improves antioxidant activity of plant materials as the antioxidant properties were enhanced by the naturally occurring compounds or the development of novel compounds such as the products of Maillard reaction that have antioxidant properties (Dewanto et al., 2002a; Dewanto et al., 2002b; Kusznierevicz et al., 2008; Chumyam et al., 2013; Damian and Oroian, 2013; Sharma et al., 2015).

The increase of antioxidant activity of Napier grass after drying at different temperature was in accordance with the previous studies (Bernard et al., 2014; Sharma et al., 2015). Sharma et al. (2015) reported that heating enhances initial antioxidant status which is caused by alteration of the antioxidant structure and the formation of novel antioxidant components that have antioxidant activity. This result was also supported by a study by Bernard et al. (2014) who revealed that percentage of DPPH inhibition of

cinnamon was increased by oven drying due to the inactivation of oxidative enzymes. A study by Madrau et al. (2009) had stated a few reasons of the simultaneous decline in polyphenol and increase in antioxidant activity such as increased antioxidant activity of polyphenols at an intermediate state of oxidation, increased level of reducing sugar and formation of Maillard reaction products.

**Ferric reducing antioxidant potential (FRAP) assay**

Figure 2 shows the average of mg of TE/200 ml infusion of oven dried Napier grass. There is a significant difference between the dried samples which varied from  $41.53 \pm 3.62$  to  $66.62 \pm 2.77$  mg of TE/200 ml infusion. Ferric reducing antioxidant potential of all the samples were lower than that of raw Napier grass infusion ( $71.31 \pm 1.30$ ). However, no significant difference was observed between fresh Napier grass infusion and samples dried at  $50^\circ\text{C}$  ( $66.62 \pm 2.77$ ) and  $90^\circ\text{C}$  ( $65.58 \pm 5.98$ ), respectively. High antioxidant activity was observed in the samples undergone drying at  $50^\circ\text{C}$  which coincides with the higher TPC and TFC. Antioxidant activity of Napier grass was dropped at  $60^\circ\text{C}$ . The drastic loss of antioxidant activity at  $60^\circ\text{C}$  was in accordance with the result obtained from TPC assay. Decrease of antioxidant activity of Napier grass was closely related to the loss of TPC. [Figure 2]



**Fig. 2. Ferric reducing antioxidant potential of infusions of fresh Napier grass and Napier grass undergone different drying temperatures**

Result from triplicate analysis, a-b means sample with the same letters for each histogram are not statistically significant different at  $p > 0.05$ .

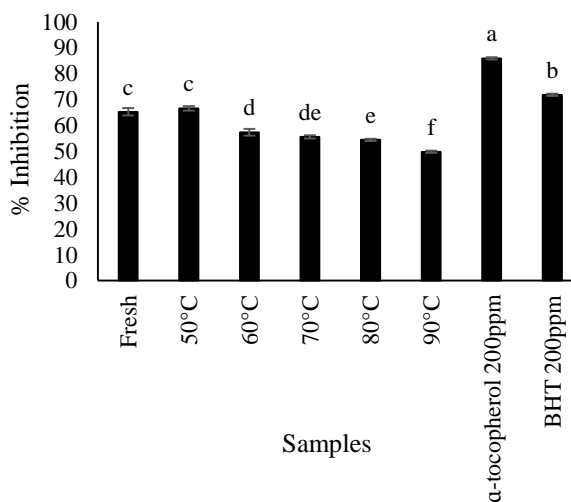
A gradual increase of antioxidant activity was observed at  $70^\circ\text{C}$  to  $90^\circ\text{C}$ . Increase in antioxidant

properties following thermal treatment has been reported in plants such as cabbage (Kusznierewicz et al., 2008), tomato (Dewanto et al., 2002a), carrot (Damian and Oroian, 2013), sweet corn (Dewanto et al., 2002b) and eggplant (Chumyam et al., 2013). Increase in antioxidant activity at increasing temperatures caused the breakdown of cellular constituents which subsequently enhanced the release of bound phenolic compounds (Dewanto et al., 2002b). It also caused the increase of TPC as the result of the formation of monomers by the hydrolysis of C-glycosides bonds of flavonoid dimers or oligomers (Sharma et al., 2015).

**Ferric thiocyanate (FTC) assay**

The FTC assay was used to determine the level of peroxide during the initial stage of lipid peroxidation (Zahin et al., 2009; Inbathamizh et al., 2013). During the initial stage of lipid peroxidation, reaction of peroxide and ferrous chloride forms ferric ions which then combines with ammonium thiocyanate and leads to the formation of thiocyanate which is red in colour (Emynur Shafekh et al., 2012).

The results (Figure 3) showed that percentage of inhibition of positive controls which were butylated hydroxytoluene (BHT) ( $71.82 \pm 0.46$ ) and  $\alpha$ -tocopherol ( $85.91 \pm 0.46$ ) were significantly higher than that of the samples. BHT and  $\alpha$ -tocopherol were used as positive controls in this analysis. BHT is a common synthetic antioxidant that is widely used in food processing whereas  $\alpha$ -tocopherol is a natural antioxidant (Haworth, 2003; Balasundram et al., 2006). [Figure 3]



**Fig. 3. Lipid peroxidation inhibition of infusions of fresh Napier grass and Napier grass undergone different drying temperatures by FTC assay**



Result from triplicate analysis, a-f means sample with the same letters for each histogram are not statistically significant different at  $p > 0.05$ .

Antioxidant activity of sample dried at 50 °C ( $66.57 \pm 0.82$ ) showed the highest antioxidant activity and it had no significant difference with that of fresh Napier grass infusion ( $65.25 \pm 1.39$ ) indicating antioxidant activity was not destroyed by this temperature. Antioxidant activity was significantly decreased at 60 °C ( $57.32 \pm 1.33$ ). Sample dried at 90 °C showed the least antioxidant activity which was  $49.80 \pm 0.46$ . This result was in agreement with the previous study which reported that high temperature can cause decrease of antioxidant activity in plant materials (Chan et al., 2009). Flavonoids are closely related to the antioxidant activity (Karagöz et al., 2015). The result obtained from FTC assay was supported by the findings of TFC assay which indicated antioxidant activity of the samples decrease with the increasing drying temperature. This phenomenon was caused by the degradation of flavonoids at higher temperature (Sharma et al., 2015).

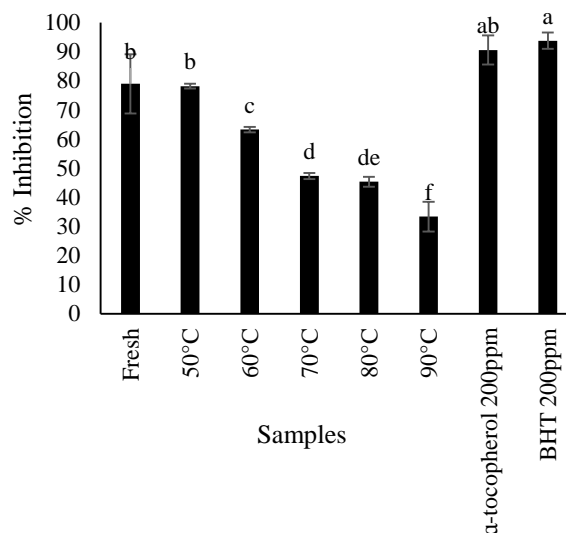
#### Thiobarbituric acid (TBA) assay

TBA assay (Figure 4) shows similar results obtained from FTC assay (Figure 3), antioxidant activity of all the samples were lower than that of positive controls which were  $\alpha$ -tocopherol ( $90.60 \pm 5.00$ ) and BHT ( $93.80 \pm 2.81$ ). Low antioxidant activity of the infusions compared to that of positive controls could be explained by their preparation in water. This is because BHT and  $\alpha$ -tocopherol are lipophilic antioxidants which exhibit stronger antioxidant activity in emulsions as they concentrate at the lipid/air surface (Kulišić et al., 2006). [Figure 4]

TBA assay was carried out to measure the level of carbonyl compounds formed by the decomposition of peroxides during the secondary stage of lipid oxidation (Zahin et al., 2009; Emynur Shafekh et al., 2012). A lower molecular weight compounds, Malondialdehyde were formed from the decomposition of peroxides and this compounds were used as an index of lipid peroxidation (Emynur Shafekh et al., 2012; Sengupta et al., 2015).

Fresh Napier grass infusion ( $78.98 \pm 10.14$ ) and sample dried at 50 °C ( $78.16 \pm 0.83$ ) had no significant difference. Antioxidant activity was significantly decreased at 60 °C ( $63.24 \pm 0.89$ ). Sample dried at 90 °C showed the least antioxidant activity which was  $33.33 \pm 5.11$ . This results were also in accordance

with that of FTC assay which revealed that antioxidant activity of samples decreased with increasing drying temperature.



**Fig. 4. Lipid peroxidation inhibition of infusions of fresh Napier grass and Napier grass undergone different drying temperatures by TBA assay**

Result from triplicate analysis, a-f means sample with the same letters for each histogram are not statistically significant different at  $p > 0.05$ .

#### Conclusion

Temperature of oven drying showed significant effect on the total phenolic content, total flavonoid content and antioxidant activity of Napier grass based on DPPH, FRAP, FTC and TBA assays. Napier grass dried at 50 °C showed high phenolic and flavonoid content in both analysis. It also showed the highest antioxidant activity in DPPH, FRAP, FTC and TBA assays. The overall results showed that 50 °C was a recommended oven drying temperature for Napier grass. Besides, the data obtained from this study revealed that antioxidant properties of Napier grass were not necessarily decreased with increasing drying temperature.

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## Contribution of Authors

Shin NGK: Conducted the study, analysed the data, evaluated the results, wrote the Manuscript

Zin ZM: Plan the experimental design of the study, reviews the manuscript

Maidin NM: Plan the experimental design of the study, reviews the manuscript

Abdullah MAA: Plan the experimental design of the Study, reviews the manuscript

Zainol MK: Planned the experimental design of the study, analysed the data, approved the final manuscript

**Disclaimer:** None.

**Conflict of Interest:** None.

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