

Effect of lactic fermentation on the antioxidant capacity of Malaysian herbal teas

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Abstract

This study evaluated and compared the antioxidant capacity between freshly prepared and lactic fermented Malaysian herbal teas. Herbal teas are rich in antioxidants. Fermentation has been known to be the oldest and cost effective method with the ability to preserve or improve food nutritional qualities. Information on the antioxidant capacity of lactic fermented food or beverage is still lacking. Hence, the objective of this study is to determine the changes in the antioxidant properties of Malaysian herbal teas after being subjected to lactic fermentation. Commercially available local herbal teas were used for this study. Herbal teas such as “Allspice”, “Scaphium”, “Gora” and “Cinnamon” were purchased from the local store in Malaysia and were subjected to 24-hour lactic fermentation. Lactic fermented herbal teas were analyzed for their total phenolic, total flavonoid and antioxidant properties via DPPH, FRAP, and β -carotene linoleate bleaching assay. All lactic fermented herbal teas exhibited higher phenolic contents, flavonoid contents and antioxidant properties compared to the freshly-prepared herbal teas with majority showing significant changes ($p < 0.05$) in FRAP and β -carotene bleaching assay. Lactic fermented herbal teas also showed an increase in antioxidant capacity in DPPH assay, however non-significant changes were observed.

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Introduction

In normal aerobic metabolism, reactive oxygen or nitrogen species (ROS or RNS) is being produced in the living systems. These intermediates are involved in several metabolic processes in our body such as the production of energy, cell signaling, gene transcription, followed by immune defense and many more (Seifried, 2007). Nonetheless, it is the deterioration of antioxidant defense mechanism that leads to the increasing ROS or RNS production, causing oxidative stress. Oxidative stress has been associated with a number of diseases involving cancer, diabetes, heart disease, neurological disorder and ageing (Valko, 2007). With the presence of antioxidant, deleterious effects of oxidant reactions could be restricted. Antioxidant is a substance that could delay or prevent oxidation of a particular substrate when available at lower concentrations than that oxidizable substrate (Swaran, 2009). Antioxidants can be classified into two categories according to their mechanism of action; primary antioxidants which are also known as the chain breaking antioxidants and secondary antioxidants which are the preventive antioxidants. Primary antioxidants which are referred to as type 1, has the ability to directly scavenge free radicals.

Secondary antioxidants on the other hand thwart the development of free radicals indirectly via Fenton's reaction (Oh, 2013). Hence, it is unquestionable concerning the high demand for products fuelled with antioxidants. Considering the popularity and frequent intake of herbal tea worldwide, the interest in its nutritional benefits have also heightened. Herbal tea is rich in antioxidant and can be a part of a healthy diet (Halliwell, 1999). Herbal preparations are believed to have great therapeutic values such as antioxidant, anti-carcinogenic, neuroprotective, and cardio-protective (Nainthani, 2006). Several individuals opt for herbal remedies to prevent or treat diseases in view that natural remedies are safer, and more effective than pharmaceutically derived medicines (Murphy, 1999). Fermentation is the oldest and most economical method to preserve or enhance food nutritive values. Fermentation of herbs with lactic acid bacteria has been reported to increase the antioxidative value. It was also suggested that tea which undergoes microbial fermentation process exhibits a unique phytochemical profile than unfermented tea. For example, fuzhuan tea showed the presence of catechins following fermentation process. Furthermore, beneficial organic acids such as acetic, lactic, and ascorbic acid increased with fuzhuan tea

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fermentation (Wu, 2010). Nevertheless, no reports have been found on the effect of lactic fermentation on the antioxidant capacity of Malaysian herbal teas. The aim of the present work was to study the changes in the antioxidant capacity of Malaysian herbal teas after being subjected to lactic fermentation.

Materials and Methods

Herbal teas

Commercially available herbal teas namely "Allspice", "Scaphium", "Gora", and "Cinnamon" were purchased from the local store in Malaysia on 1st January 2013. Extensive research has been carried out on the antioxidant properties of black and green tea however, no literature review has been found on the effect of lactic fermentation on antioxidant activity of these 4 commercial herbal teas.

Extraction of herbal teas

The extraction of herbal teas was based on the method by (Chan, 2010) with slight modifications. In herbal tea extraction, 1 g of tea in powder form was extracted with 100 ml boiling distilled water. Infusion was left to steep for 3 minutes with continuous stirring. Next, extracts were filtered with Whatman filter paper 150 mm and stored at -20°C for 6 months for further analysis. Samples were stored in -20°C to avoid the enzyme activity which could alter their biochemical properties (More, 1995).

Bacterial strains and isolation

Lactobacillus casei Shirota strain was isolated from commercial cultured milk drink, 'Yakult'.

Fermentation of Malaysian herbal teas

This method was based on the method by Yoon (2006). Fermentation experiments were carried out in 1 L Schott bottle, each containing 750 ml of different herbal teas with concentrations of 0.8 mg/ml. Each sample was inoculated with a 24 hour culture ($>10^5$ CFU/ml) and incubated at 37°C for 24 hours.

Total phenolic contents

Total phenolic content (TPC) was estimated using the Folin - Ciocalteu assay (Kahkonen *et al.*, 1999). Samples (300 mL, in triplicate) were pipetted into test tubes. Then 1.5 mL of Folin-Ciocalteu's reagent (10 times dilution) and 1.2 mL of sodium carbonate (7.5%, w/v) were added. The tubes were incubated at room temperature for 30 min before absorbance at 765 nm was measured. TPC was expressed as gallic acid equivalent (GAE) in mg per 100 g material.

Total flavonoid contents

The $AlCl_3$ method (Amitabye, 2005) was applied to determine the total flavonoid content of the herbal teas. Firstly, 1.5 ml of samples were added to 1.5 ml of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 ml methanol). The mixture was mixed and incubated for 10 min in room temperature. Absorbance was read at 367.5 nm. Data were expressed in mg quercetin equivalents/ 100 g material.

Antioxidant properties

DPPH assay

Radical-scavenging activity (RSA) was determined using the 2,2- diphenyl-1 picrylhydrazyl (DPPH) assay (Miliauskas *et al.*, 2004). Different samples (1 mL) were added to 2 mL of DPPH (5.9 mg per 100 mL methanol). After 30 min, absorbance was measured at 517 nm. Scavenging effect was calculated using the equation below:

$$1 - (\text{absorbance of sample at 517 nm} / \text{absorbance of control at 517 nm}) \times 100 \%$$

FRAP assay

FRAP value was assessed based on the reduction of Fe^{3+} -TPTZ to a blue coloured Fe^{2+} TPTZ (Benzie and Strain, 1996). The FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ and 20 mmol/L $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 at room temperature. FRAP reagent (3 ml) was added into test tubes. Then, 100 μ l of sample and 300 μ l of distilled water was added to the same test tubes, and incubated at room temperature for 4 min. Each sample was done in triplicate. Absorbance was measured at 593 nm. The FRAP value was calculated using the equation described by Benzie and Strain (1996). In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using $FeSO_4 \cdot 7H_2O$ at concentrations ranging from 0.2 mmol/L to 1 mmol/L.

β - carotene linoleate bleaching assay

The antioxidant activity of herbal teas were assessed by β -carotene bleaching method developed by Velioglu *et al.* (1998). BHT was used as a standard. β -carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were pipetted into a round bottomed flask. The mixture was then added to 0.2 ml of samples or standard. Chloroform was evaporated at room temperature under vacuum using a rotary evaporator (Unimax1010, Heidolph, Germany). Then, 50 ml of distilled water was added

to the mixture. The mixture was shaken vigorously to form an emulsion. In addition, 2 ml aliquots of the emulsion were transferred into test tubes and directly incubated in a water bath (Techne, Duxford Cambridge, UK) at 45°C. The absorbance was read at 15 min intervals for 1 h at 470 nm, using a SECOMAM Anthelie Advanced 5 spectrophotometer. Degradation rate (DR) was calculated according to first order kinetics, using the below equation based on Al-Saikhan, Howard, and Miller (1995):

$$\ln(a/b) \times 1/t = DR_{\text{sample}} \text{ or } DR_{\text{standard}}$$

Antioxidant activity (AA) was expressed using the following formula:

$$AA = [(DR_{\text{control}} - DR_{\text{sample}} \text{ or } DR_{\text{standard}}) / DR_{\text{control}}] \times 100 \%$$

Statistical analysis

All data were expressed as mean \pm standard deviation. Data were analyzed using one-way ANOVA using SPSS. Duncan's new multiple-range test was used to assess differences between means. A significant difference was considered at the level of $p < 0.05$. Pearson correlation coefficient was calculated using SPSS.

Results and Discussions

Total phenolic contents (TPC)

Major constituents of plants and herbs are believed to be polyphenols. Polyphenol contents in plants and herbs somehow link to their antioxidant capacity. Thus, it is essential to discover the effect of lactic fermentation on TPC in herbal teas. It was discovered that lactic fermentation has a positive effect on the polyphenols contents. Based on successive overnight lactic fermentation, from Figure 1, TPC of fermented Allspice (4375 mg/100 g), Scaphium (2308.33 mg/100 g), Gora (1970.83 mg/100 g) and Cinnamon (2187.5 mg/100 g) were found to be significantly higher ($p < 0.05$) than freshly prepared Allspice (4091.31 mg/100 g), Scaphium (1992.05 mg/100 g), Gora (1482.2 mg/100 g) and Cinnamon (984.11 mg/100 g). These findings are in accordance with previous work for instance, it was reported that fermented *Echinacea* spp, a medicinal herb which is often consumed as herbal drink in the Western countries showed a rise in polyphenols content following lactic fermentation (Rizzello, 2013). The increase in TPC of lactic fermented herbal teas is possibly due to the enzymatic reaction within the substrates that secretes more phenolic compounds as the end product. As

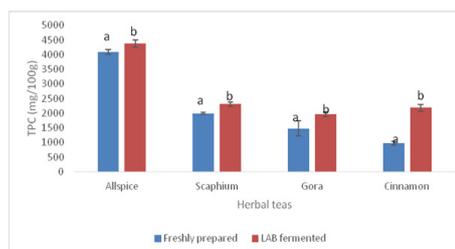


Figure 1. Effect of fermentation on TPC. Concentration sample was 0.8 mg/ml.

Values are expressed as mean \pm standard deviation ($n = 3$). Means with different letters were significantly different at the level ($p < 0.05$).

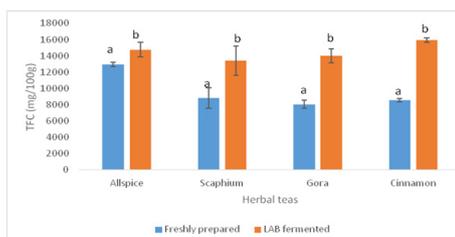


Figure 2. Effect of fermentation on TFC. Concentration sample was 0.8 mg/ml.

Values are expressed as mean \pm standard deviation ($n = 3$). Means with different letters were significantly different at the level ($p < 0.05$).

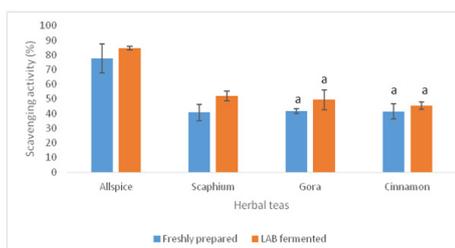


Figure 3. Effect of fermentation on scavenging activity. Scavenging effect of freshly prepared and LAB fermented herbal teas on DPPH radicals.

Concentration sample was 0.2 mg/ml. Values are expressed as mean \pm standard deviation ($n = 3$). Vitamin C was used as a standard.

fermentation with natural fermenting microorganisms trigger a pH reduction, some enzymes which are involved in hydrolyzing complex polyphenols are activated, producing a much simpler and active polyphenols (Dueñas, 2005).

Total flavonoid contents (TFC)

Figure 2 showed the contents of flavonoids in freshly prepared and lactic fermented herbal teas expressed as quercetin respectively per 100 g of extract. Total flavonoid content exhibited in lactic fermented Allspice (14766.67 mg/100 g), Scaphium (13416.67 mg/100 g), Gora (14000 mg/100 g) and Cinnamon (15958.33 mg/100 g) were significantly higher ($p < 0.05$) than in the freshly prepared herbal teas. The information obtained from this study indicated that bioactive compounds of these herbal teas were probably modified during lactic fermentation, indirectly increasing the flavonoid contents. There is a high possibility that lactic fermentation may induce structural breakdown of substrates cell wall leading

to the release of several bioactive compounds in plant based functional foods. It was reported that amylases, proteases and xylanases derived from microbes and grains during fermentation have modified grain composition, releasing phenolics via the enzymes treatment (Katina, 2007).

DPPH assay

DPPH assay was applied to determine the scavenging capability of free radicals by freshly prepared and lactic fermented herbal teas. A comparison was made and it was found that lactic fermented herbal teas exhibited higher antioxidant activities than freshly prepared herbal teas (Figure 3). All lactic fermented herbal teas showed non-significant differences except lactic fermented Scaphium which displayed significantly ($p < 0.05$) higher antioxidant effect than freshly prepared Scaphium. The antioxidant activity of lactic fermented herbal teas ranged from 45% to 85%. These findings are in agreement with previous study as Taiwanese medicinal plant, *Anoectochilus formosanus Hayata* which has been used as a nutraceutical herbal tea in Taiwan and other parts of Asia (Wang, 2002) was reported to show higher scavenging effect following lactic fermentation (Ng, 2011). In this study correlation between total phenolic contents and scavenging activity of herbal teas was also executed. There was a significantly ($p < 0.05$) positive correlation between scavenging activity and phenolic contents for both lactic fermented ($r = 0.961$) and freshly prepared herbal teas ($r = 0.894$). There was also a positive correlation between flavonoid contents and scavenging effect for freshly prepared herbal teas ($r = 0.901$) however no correlation was observed for lactic fermented herbal teas. Thus, compared to phenolic compounds, the role of flavonoid in scavenging activity was not prominent. Hence, present study suggested that scavenging ability of herbal teas in DPPH assay could be due to the presence of phenolic compounds.

FRAP assay

Based on Figure 4, all lactic fermented herbal teas have higher FRAP value compared to freshly prepared herbal teas. The differences in antioxidant potential following fermentation are significant ($p < 0.05$) except for Cinnamon where the difference was non-significant. Ferric reducing antioxidant power (FRAP) assay as the name suggests is a measure to determine the reducing capacity of antioxidant to produce blue colored ferrous tripyridyltriazine (Fe^{2+} -TPTZ) from ferric tripyridyltriazine (Fe^{3+} -

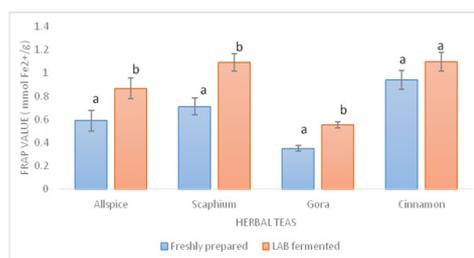


Figure 4. Effect of fermentation on antioxidant potential assayed by FRAP assay.

Concentration sample was 0.8 mg/ml. Values are expressed as mean \pm standard deviation ($n = 3$). Ferrous sulphate was used as a standard.

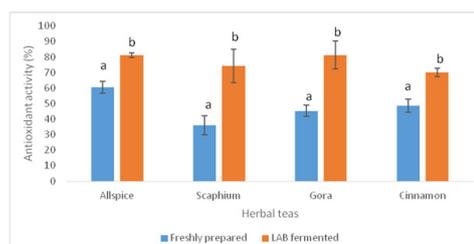


Figure 5. Effect of fermentation on antioxidant potential assayed by β -carotene linoleate bleaching assay.

Concentration sample was 0.8 mg/ml. Values are expressed as mean \pm standard deviation ($n = 3$). BHT was used as a standard.

TPTZ) complex (Othman, 2007). The reducing properties are related to the presence of compounds that involves in the breaking of the free radical chain via a hydrogen atom (Duh, 1999). Furthermore, it was reported that blue color forms at low pH (Benzie and Strain, 1996). It was known that herbal teas are acidic and have low pH (Brunton, 2001). The freshly prepared herbal teas may have low pH but lactic fermented herbal teas have even lower pH due to the liberation of lactic acid as fermentation product. In short, the higher antioxidant potential in lactic fermented herbal teas compared to freshly prepared herbal teas are probably due to their very acidic nature (low pH) which has a strong influence on the assay medium (Othman, 2007). Pearson correlation was conducted and it was observed that TPC and TFC had no correlation with FRAP value in freshly prepared and lactic fermented herbal teas. TPC was expected to have a strong positive correlation with FRAP value but previous studies have also reported on different relations between antioxidant activity and TPC. Othman, (2007) reported a strong correlation of TPC with FRAP assay but no correlation with DPPH assay. Girish, (2011) also reported that phenolic contents are not correlated with antioxidant activity and Ramkisson, (2012) showed no correlation between TPC and FRAP assay. Therefore, from this assay, it was suggested that antioxidant potential in lactic fermented herbal teas and freshly prepared herbal teas are not contributed by their phenolic and flavonoid compounds but could possibly be due to other factors such as the pH and chemical structures

of herbal teas.

β-carotene bleaching assay

In β -carotene linoleate bleaching assay, hydroperoxides as free radicals were produced by linoleic acid during incubation between 45-50°C. The oxidation of β -carotene by hydroperoxides was minimized with the presence of antioxidants (Othman, 2007). In short, hydroperoxides in this system will be neutralized by the antioxidants from the sample extracts. All lactic fermented herbal teas showed significantly higher ($p < 0.05$) antioxidant activity compared to freshly prepared herbal teas with values ranging between 70% and 80%. Lactic fermented herbal teas seemed to inhibit the oxidation of β -carotene better than freshly prepared herbal teas. Pearson correlation was carried out to determine the correlation between phenolic contents and antioxidant activity assayed by β -carotene linoleate bleaching assay and it was found that there was no correlation between those two for freshly prepared and lactic fermented herbal teas. The same pattern was observed for TFC. This finding is supported by previous study which revealed poor correlation between TPC and antioxidant activity in green tea extracts (Kodama, 2010). Therefore, it can be simplified that phenolic and flavonoid contents are not directly involved in inhibiting the oxidation of β -carotene. The reason for the stronger antioxidant activity in lactic fermented herbal teas compared to the freshly prepared herbal teas is probably due to other factor for instance the bacteria itself. Previous report has claimed that lactic acid bacteria is believed to have a specific effect on oxidative stress. It was suggested in a study by Choi (2006) that lactobacilli strain may constitute anticancer and antioxidative agent.

Conclusion

Lactic fermentation of Malaysian herbal teas with *Lactobacillus casei* caused considerable positive changes in the antioxidant capacity with majority showing significant changes ($p < 0.05$) in FRAP and β -carotene linoleate bleaching assay. For recommendation, it is essential to apply different assays instead of depending on a single assay to observe the antioxidant activity as each method has different mechanisms and methodological limitations.

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